28th Young Research Fellows Meeting

Société de Chimie Thérapeutique

A web edition

February, 11th-12th

Faculté de Pharmacie – Université de Paris

PHARMACIE
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Welcome to the web edition of the 28th Young Research Fellows Meeting!

The YRFM Organizing Committee is very proud to welcome scientists from 25 countries, to celebrate the **twenty-eighth edition of this annual meeting**! Despite the pandemic and despite the forbidden travels thorough the world, we have the ambition to forget the worldwide depressive atmosphere by sharing together, in a friendly atmosphere, our common passion for science and researches in drug discovery. So, the organizing committee is happy to offer this year again both top-quality scientific and career sessions.

Therefore, we are very grateful to our senior scientists **A. Spivey** (London), **F. Rombouts** (Belgium), **J. Broggi** (Marseille), **E. Saada Bouzid** (Nice), **H. Munier-Lehmann** (Paris), **X. Morelli** (Marseille), **B. Liagre** (Limoges), and **D. Bonnet** (Strasbourg) for accepting to give plenary session lectures. Their presence is the guarantee of the scientific excellence of the conference! Many thanks to them! We also welcome a prestigious keynote lecture given by **P. Wu** (Dortmund), who is the 2020 laureate for the 2020 SCT Award for young investigator in Medicinal Chemistry. Importantly, this web edition allows us to welcome for the very first-time oral communication and flash posters form Asia (**India**), Africa (**Ivory Coast, Senegal and Morocco**) and South America (**Brazil**)!

Take the opportunity to highlight your projects in this outstanding environment, and maybe to receive awards for your work! The career session will enable you to be interviewed and to discuss with **professional recruiters** belonging to major pharmaceutical companies, biotechs and start-ups. It will be also for you an opportunity to learn from people who created successfully their own company.

The success of this meeting is recognized, as indicated by the number of sponsors issued from institutional and industrial partners, that you can discover in this book. Nevertheless, the SCT and the YRFM Organizing Committee are particularly indebted to the Dean of the Faculty of Pharmacy (**Pr. Jean-Louis Beaudeux**), and to all the members of our Faculty involved in this web-meeting (technical's, communication's and TICE's teams). Thanks to them, the Young Research Fellows Meeting has truly the feeling to be "at home"!

The pharmacist's students of the Faculty are also involved in this meeting! You will receive a special "Youtube" link where several performances of our student's associations (**Pharma Dance, PharmaZik and Pom-Pom Pharma**) have been recorded for your eyes only!

We sincerely hope you will never forget this web-meeting!
Welcome to YOUR web-YRFM 2021!

"Bienvenue à Paris!"
The organizing committee

The organizing committee of the 28th Young Research Fellows Meeting

Faculté de Pharmacie, UMR 8038 CNRS, CiTCoM
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Magali Blaud
Florent Huguenot
Philippe Belmont
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Cyril Ronco

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Erwann Guénin

We acknowledge the Dean J.-L. Beaudeux for welcoming us in the Faculty of Pharmacy of Paris and we are grateful to different teams involved in this meeting:

- **Service intérieur** *(Mélanie Martin)*
- **Service communication** *(Samantha Conti)*
- **Service TICE** *(Virginie Lasserre)*

We are indebted to our financial supports, in particular the institutional ones: "Commission Recherche - Faculté de Santé, Université Paris" and the CNAM.

Some words from Prof. Rebecca Deprez-Poulain, the S.C.T. President:

Dear Young Research Fellows, Dear Colleagues,

Société de Chimie Thérapeutique

On behalf of the French Medicinal Chemistry Society (*Société de Chimie Thérapeutique, SCT*) we are welcoming you to the Young Research Fellows Meeting 2021 (*Journées de Jeunes Chercheurs*).

28th Young Research Fellows Meeting
Journées Jeunes Chercheurs

The SCT supports its young members and acknowledges that The Young Research Fellows Meeting is a great opportunity for the future investigators in the field. Given the pandemia, t**he 28th Edition of the YRFM was thus maintained and transformed to a completely online meeting.**

I would like to thank the Organizing Committee for setting up this meeting. With this two-day symposium the Committee did an excellent work to offer a great scientific event by gathering experts with outstanding contribution in medicinal chemistry and chemical biology.

I am, on behalf of the SCT, thankful to the **international speakers from academia and industry** that accepted to share their expertise with the audience (Dr Munier-Lehmann, Dr Morelli, Dr Bouzid, Pr . Spivey, Dr Rombouts, Pr Liagre, Dr Bonnet, Dr Broggi). The conferences will tackle key up-to-date themes such as new chemical modalities like macrocycles, probes and chemical tools, precision medicine through novel mode of actions, natural compounds synthesis and protein mimicry, protein-protein interactions, compounds for photodynamic therapy, …

It is of utmost importance that Young Researchers are provided with slots for communications and poster presentations as face to peers essential experience at the beginning of the career, even in this context. Thus 18 oral presentations and 20 flash poster presentations have been allocated to participants. We are looking forward to discover the great works through these presentations and posters.

In the continuing efforts of SCT to support and accompany young talents, career sessions have been also maintained to offer YRF individual online one-to-one meetings with human resource managers from major pharmaceutical companies as well as from start-ups. We thank warmly all the contributors for their enthusiastic engagement on these sessions.

To recognize young talents and shed light on their work, the SCT is continuingly distributing prizes and awards. Young Research Fellows with the best oral communications and poster presentations will be awarded and offered a registration to an international congress.

Importantly, with your registration to this YRFM, you are automatically member of our Society for 2021. You will thus benefit from a very competitive registration fee for our main scientific events, such as our International Conference on Medicinal Chemistry (RICT 2021) to be held online in July 2021. You will have also the opportunity to have access to free webinars and support for your career (New in 2021). On all these forthcoming events and offers you can find more information on our web-site (www.sct-asso.fr) or social networks (Twitter LinkedIn).

Finally, the SCT especially acknowledge our industrial and academic sponsors for their financial support.

The SCT-YRFM is a decades-long success. This year edition will gather about 250 participants and show the continuing international attraction of this event. We wish you all an excellent symposium with good scientific discussions and we hope to see you soon at another event of the SCT.

Pr Rebecca Deprez-Poulain,
SCT President.

SCT Société de Chimie Thérapeutique

International Conference on Medicinal Chemistry

The date is July 7 - 9, 2021

The decision has been made to switch RICT 2021 into a fully virtual event. The programme will be reorganised to meet the needs of this Virtual Conference, but the dates will remain the same.

***Registration*: https://www.rict2021.org/**

The French Medicinal Chemistry Society (Société de Chimie Thérapeutique - SCT) is pleased to invite you to virtually attend the 56th edition of the International Conference on Medicinal Chemistry.

The congress will be devoted to "Interfacing Chemical Biology and Drug Discovery" and the main topics will be:

Chemistry in Living Systems & Chemical Biology for Targets Identification
Recent Advances in Targeted Protein-Protein Interactions Including Protein Degradation and Targeted Protein-DNA/RNA Interactions
Recent Trends in Drug Development for Neurodegenerative Diseases
Innovations in Cancer Therapeutics
Tackling New Challenges in Delivery Systems and Drug Formulation
Case Studies: Moving New Chemical Entities to the Market

As for previous editions of RICT, the scientific committee will ensure the high quality and diversity of the scientific programme together with the accessibility of the meeting to PhD/post-doc and local Master students. For the latter, an advantageous registration fee is proposed. Moreover, a special career session will be organised with industrial and academic participants.

During the meeting, you will have the opportunity to listen to:

Prof. Stuart Schreiber (HARVARD UNIVERSITY, CAMBRIDGE MA, United States), recipient of the 2020 Paul Ehrlich Award, awarded for his outstanding contributions to medicinal chemistry.

Gilles Gasser Dr Gilles Gasser (CHIMIE PARISTECH, PSL UNIVERSITY, Paris, France), recipient of the 2020 Pierre Fabre Award for Therapeutic Innovation awarded for his original scientific contributions in the field.

A poster session will provide the opportunity for all participants to present their current results. "Best poster" prizes will be attributed at the end of the meeting.

On top, opportunities to network with your peers will be offered through virtual creative solutions.

See you virtually in July!

For more information please continue to go to: **https://www.rict2021.org/**

ACKNOWLEDGMENTS

Acknowledgements

Members of the organizing committee would like to thank all the partners who have supported the organizition of this meeting.

Institution

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The Faculty of Pharmacy of Paris

In the heart of Paris, the Faculty of Pharmacy of Paris prepares its student for the Doctor of Pharmacy degree (PharmD). Created in 1803 and now part of the Faculty of Health (studies) of Paris University, it is committed to excellence in the field of pharmaceutical research and teaching and offers a wide range of professional opportunities.

The Faculty of Pharmacy of Paris is closely related to Greater Paris University hospitals (APHP, Assistance Publique Hôpitaux de Paris) and has partnerships with prestigious research institutions such as the CNRS and the IRD.

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Prof. Jean Louis Beaudeux
Dean of the Faculty of Pharmacy of Paris

THE FACULTY

The Faculty

250 teacher-researchers

140 staff members, research and teaching technicians

4500 students

120 PhD students

Six departments

Chemistry and physical chemistry of drugs

Pharmaceutical sciences

Medical and biological sciences

Public health and health products

Pharmaceutical research center of Paris

Continuing education center

RESEARCH AND INNOVATION

Productions and partnerships

More than **300** publications per year

More than **100** patent requests since 2004

International cooperation with more than **30** countries

Numerous partnerships with private companies

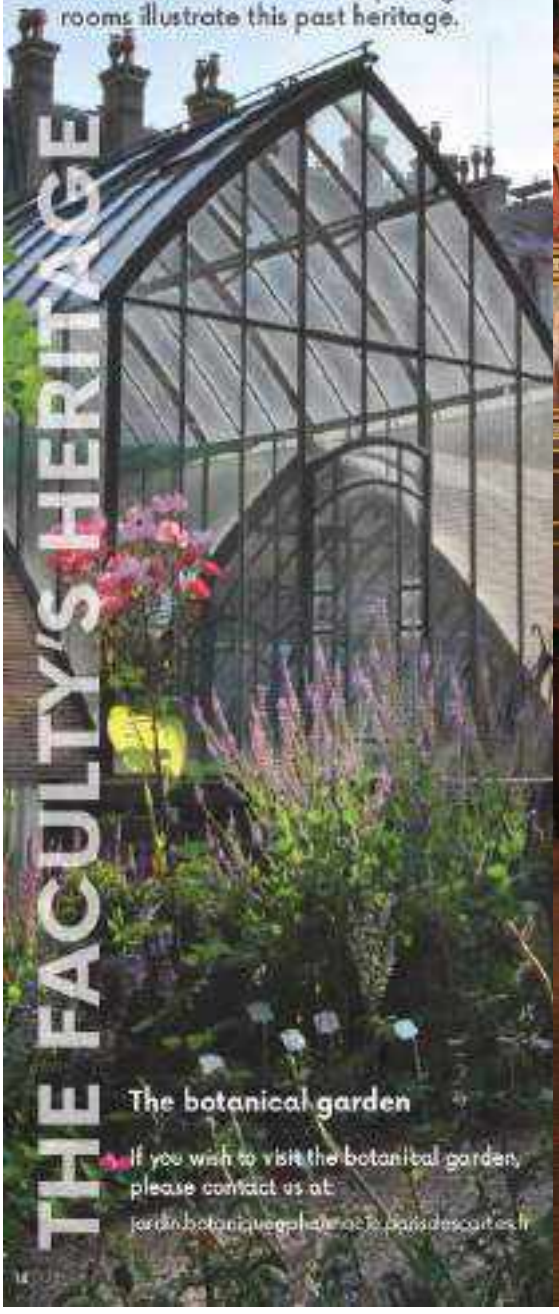
Numerous partnerships with various institutions

Research department's technical platforms

- Animal house
- Structural biology (crystallography and RMN)
- Cellular and molecular imaging
- Mass spectrometry
- Small imaging animal
- Phosphor-imaging
- Q-PCR in real time
- Metabolomics

THE FACULTY'S HERITAGE

The Faculty of Pharmacy bears witness to the history of pharmaceutical and biological sciences: paintings, busts, sculptures, stained glass windows, the botanical garden, the museum of medical matters and prestigious rooms illustrate this past heritage.



The botanical garden

If you wish to visit the botanical garden, please contact us at:

jardin.botanique@pharmacie.parisdescartes.fr

THE FACULTY'S HERITAGE

The "Salle des Actes"
A prestigious historical gallery

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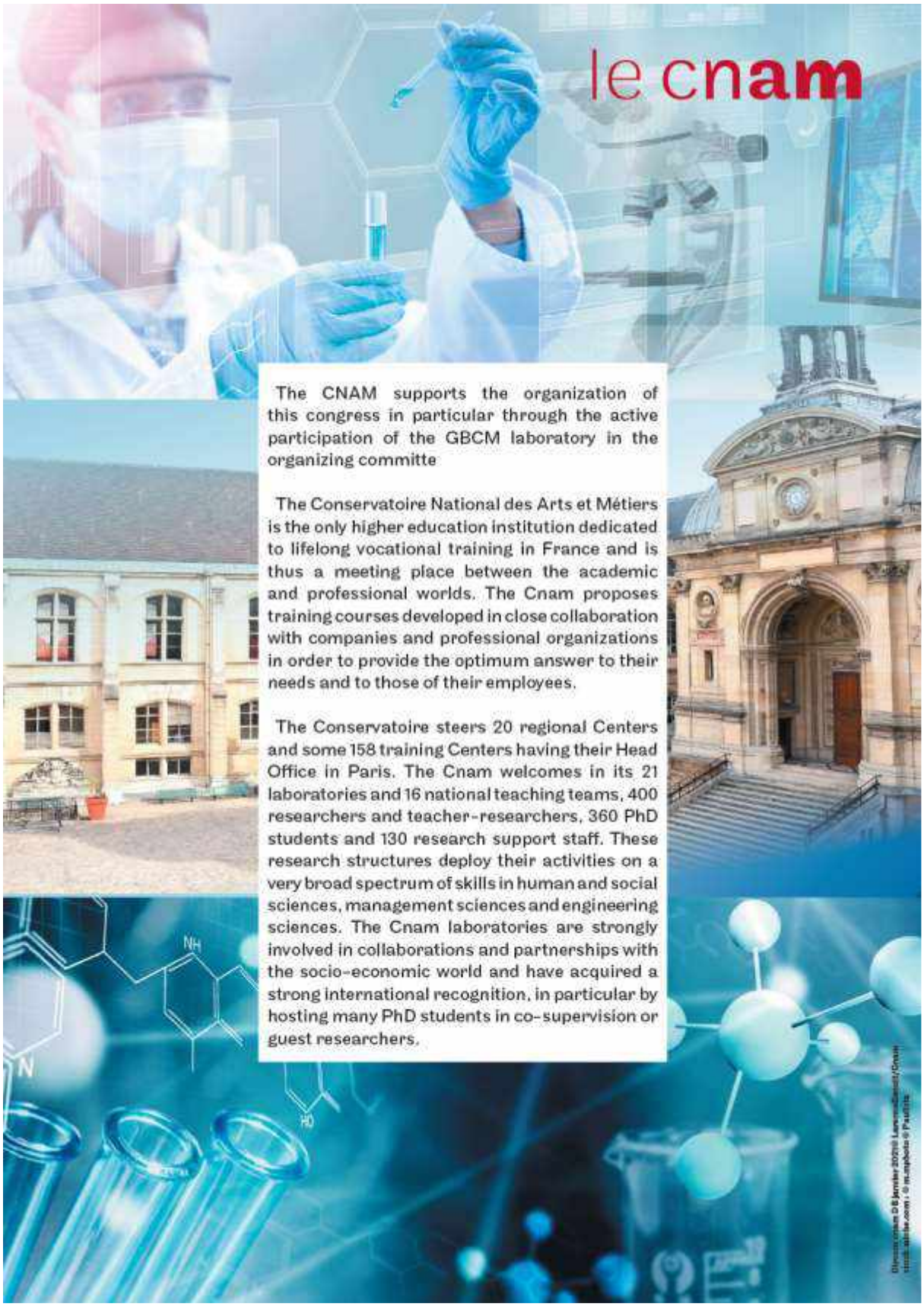

The CNAM supports the organization of this congress in particular through the active participation of the GBCM laboratory in the organizing committe

The Conservatoire National des Arts et Métiers is the only higher education institution dedicated to lifelong vocational training in France and is thus a meeting place between the academic and professional worlds. The Cnam proposes training courses developed in close collaboration with companies and professional organizations in order to provide the optimum answer to their needs and to those of their employees.

The Conservatoire steers 20 regional Centers and some 158 training Centers having their Head Office in Paris. The Cnam welcomes in its 21 laboratories and 16 national teaching teams, 400 researchers and teacher-researchers, 360 PhD students and 130 research support staff. These research structures deploy their activities on a very broad spectrum of skills in human and social sciences, management sciences and engineering sciences. The Cnam laboratories are strongly involved in collaborations and partnerships with the socio-economic world and have acquired a strong international recognition, in particular by hosting many PhD students in co-supervision or guest researchers.

28th *Journées Jeunes Chercheurs*
Young Research Fellow Meeting

PROGRAMME

Thursday, February 11th

08h50 – 09h00 : Introductive remarks

09h00 – 09h30 : Opening Lecture.
Hélène Munier-Lehmann (Institut Pasteur, Paris)
Chemical tools to get insight nucleotide metabolism.

09h40 – 11h00 : Oral Communications session 1.

09h40 – 10h00 : **OC 01 : Elizabeth Goya-Jorge, Facultat de Farmacia, U. Valencia, Spain.**
Newly synthesized triarylmethanes discovered ligands of the aryl hydrocarbon receptor.

10h00 – 10h20 : **OC 02 : Clémentine Pescheteau, ICOA, U. Orléans, France.**
Design and synthesis of dual inhibitors of DYRK1A/CLK1 kinases involved in neurodegenerative diseases..

10h20 – 10h40 : **OC 03 : Carlotta Pontremoli, Department of Chemistry, U. Torino, Italy.**
Squaraine dyes as fluorescent turn-on probes for the detection of mucin.

10h40 – 11h00 : **OC 04 : Romain Paoli-Lombardo, ICR, UMR 7273, U. Marseille, France.**
Pharmacomodulation and optimization of new 3-Nitroimidazo[1,2-a]pyridines substrates of NTR1 against kinetoplastids.

11h00 – 11h30 : Coffee break

11h30 – 11h10 : Plenary lecture.
Xavier Morelli (CRCM, Marseille, France)
Interactive drug discovery targeting protein/protein interaction: the 2P2I approach

12h10 – 12h0 : Flash Poster Presentation. Session 1
FP01 : Cosmin Butnarasu, Torino, Italy.
FP 02 : Ameni Hadj-Mohamed, Paris, France.
FP 03 : Julia Macyszyn, Warsaw, Poland.

FP 04 : Rostyslav Bardovskyi, Nice, France.
FP 05 : Gladis E H Edinsha, Nagercoil, India.
FP 06 : Lucile Brier, Lille, France.
FP 07 : Déto Ursul Jean-Paul N'Guessan, Abidjan, Ivory Coast.
FP 08 : Kévin Brugermann, ICOA, Orléans, France.
FP 09 : Ayoub El Mahmudi, Rabat, Morocco.
FP 10 : Ye Xiaoqing, Faculty of Pharmacy, Paris, France.

12h30 – 14h00 : Lunch break

14h00 – 14h40 : Plenary lecture.
Esma Saada Bouzid (CAL, Nice, France)
Precision medicine in oncology.

14h40 – 15h40 : Oral Communications session 2

14h40 – 15h00 : **OC 05 : Joanna Miszkiewicz, Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Warsaw, Poland.**
Designed RNA enzyme as a potential antibacterial.

15h00 – 15h20 : **OC 06 : Antoinette Keita, UMR IRD 261 MERIT, Faculty of Pharmacy, U. Paris, France.**
Simplified alkaloid mimics in the fight against malaria hepatic stage.

15h20 – 16h00 : Keynote lecture.
SCT award in medicinal chemistry.
Peng Wu (MPI, Dortmund, Germany)
Small molecules targeting protein–RNA interactions as chemical probes and therapeutic leads

16h00 – 16h30 : Coffee break

16h30 – 17h10 : Oral Communication session 3

16h30 – 16h50 : **OC 07 : Asma Sellami, GBCM – EA 7528, CNAM, Paris, France.**
Predicting potential EDCs binding to ERα using a pipeline combining SB and LB in silico methods.

16h50 – 17h10 : **OC 08 : Fancesco Mesiti, Net4Science, Catanzaro, Italy.**
The influence of 4-oxoquinoline tautomerism in the MAOs inhibition.

17h10 – 17h50 : Plenary Lecture.
Alan C. Spivey (Imperial College, London)
A natural product synthesis, meta-functionalisation of aryl boronates & protein structure mimicry.

18h00 – 20h00 : Pharma welcomes you!

Enjoy the artists of the Faculty of Pharmacy, music and show by our pharmacy's students.

Friday, February 12, 2019

09h00 – 09h40 : **Plenary Lecture.**
Frederik Rombouts (Janssen Pharmaceutica NV, Belgium)
Synthesis of macrocyclic MCL-1 inhibitors with optimized physicochemical properties using Petasis approach.

09h40 – 11h00 : **Oral Communication session 4**

09h40 – 10h00 : **OC 09 : Manon Mirgaux, LBS, U. Namur, Belgium.**
Crystallography and Molecular Dynamics of hIDO1 to assist structure-based drug design.

10h00 – 10h20 : **OC 10 : Alessandra Corio, LCBPT-UMR 8601, U. Paris, Paris, France.**
Design and synthesis of Red-SMU1 disruptors as new antiviral agents against Influenza A virus.

10h20 – 10h40 : **OC 11 : Florentina Geanina Lupascu, University of Medicine and Pharmacy "Grigor T. Popa" Iasi, Romania.**
Co-encapsulation of pioglitazone and curcumin into chitosan nanoparticles and in vivo antidiabetic potential.

10h40 – 11h00 : **CO 12 : Fatma Zahra Thari, Laboratory of Pharmacology and Toxicology Faculty of Medicine and Pharmacy, U. Mohamed V, Rabat, Morocco.**
Thiazolidine-2,4-dione derivatives: Synthesis, α-glucosidase and α-amylase inhibition potential and acute toxicity evaluation.

11h00 – 11h30 : **Coffee break.**

11h30 – 12h10 : **Plenary Lecture.**
Bertrand Liagre (University of Limoges, France)
Photodynamic therapy activity of a new porphyrin-xylan-coated silica nanoparticle in a human colorectal cancer in vivo model.

12h10 – 12h30 : **Flash Poster Presentation. Session 2.**
FP 11 : Marine Duplantier, Amiens, France.
FP 12 : Marco Maspero, Milano, Italy.
FP 13 : Zakaria Moutaoukil, ICN, Nice, France.
FP 14 : Diana Oliveira, Porto, Portugal.
FP 15 : Jérémy Caburet, Grenoble, France.
FP 16 : Joanna Musial, Poznan, Poland.
FP 17 : Panpan Ma, Faculty of Pharmacy, Paris, France.
FP 18 : Chaimae Ouazzani Chahdi, Fes, Morocco.
FP 19 : Monsarrat Clément, Pessac, France.
FP 20 : Duong Kim Hoang Yen, Budapest, Hungary.

12h30 – 14h00 : Lunch break.

14h00 – 14h40 : Plenary Lecture.
Julie Broggi (ICR, Marseille, France)
The application fields of enamine based organic electrons donors.

14h40 – 16h00 : Oral Communications session 5.

14h40 – 15h00 : **OC 13 : Robin Birus, Institute of Pharmaceutical and Medicinal Chemistry U. Münster, Germany.**

Analyzing Cellular Effects and Pharmacokinetic Properties of Human Protein Kinase CK2 Inhibitors with an Indeno[1,2-b]indole Scaffold.

15h00 – 15h20 : **OC 14 : Mirjana Antonijevic, CERMN, Caen, France.**

Discovery of an allosteric pocket network of the TrkB neurotrophin receptor, a promising target in the development of new ligands.

15h20 – 15h40 : **OC 15 : Davia Prischich, Institute for Bioengineering of Catalonia (IBEC), U. Barcelona, Spain.**

In vivo photopharmacology of adrenergic receptors.

15h40 – 16h00 : **OC 16 : Dinesh Dhumal, CINaM UMR 7325 CNRS, U. Marseille, France.**

An ionizable supramolecular dendrimer nanosystem for effective siRNA delivery with a favorable safety profile.

16h00 – 16h30 : Coffee break.

16h30 – 17h10 : Oral Communication session 6

16h30 – 16h50 : **CO 17 : Luana Heimfarth, Health Sciences Graduate Program, Federal University of Sergipe, Arcaju, Brazil.**

Effect of naringenin and naringenin complexed with hydroxypropyl-β-cyclodextrin on neonatal sepsis. Role of COX-2, NFκB, MAPK pathways.

16h50 – 17h10 : **CO 18 : Félix Grosjean, IBMM, UMR 5247 CNRS, U. Montpellier, France.**

Development of 5'-Nucleotidase inhibitors for cancer immunotherapy.

17h10 – 17h50 : Plenary Lecture.
Dominique Bonnet (University of Strasbourg, France)
Designing chemical tools to shed light on G-Protein-coupled receptors

LECTURES

Thursday, February 11th, 2021

Chemical tools to get insight nucleotide metabolism.

Hélène Munier-Lehmann (Institut Pasteur, Paris)

Institut Pasteur, Structural Biology and Chemistry Department, Chemogenomic and Biological Screening Platform, 28 Rue du Dr Roux - 75724 Paris Cedex 15 – France

Summary: My research interests are mainly focused on the structure-function relationships of proteins with a particular interest on enzymes belonging to nucleotide metabolism. During my talk, I will present two multidisciplinary projects at the interface betweenbiology and chemistry. In both cases, chemical compounds have been instrumental in deciphering molecular features or identifying novel biological processes linked to nucleotide metabolism. I wish to convince you that that this field, although already well documented, is still full of surprises.

Interactive drug discovery targeting protein/protein interaction: the 2P2I approach

Xavier Morelli (CRCM, Marseille)

Centre de Recherche en Cancérologie de Marseille (CRCM) ; CNRS, INSERM, Institut Paoli-Calmettes, Aix-Marseille Université, 27 Boulevard Leï Roure, 13009, Marseille, France

Over the past decades, the **identification of hits** in the pharmaceutical industry and academic research has been greatly facilitated by advances in high throughput screening (HTS) and the design of chemical libraries[1]. One of the major remaining obstacles in drug discovery is related to the **automation of the hit-to-lead optimization process**[2] (H2L). Here we present an **integrated strategy for the H2L** optimization phase and the automated design of chemical probes. Our approach "**D**iversity **O**riented **T**arget-focused **S**ynthesis" (DOTS[3] & CovaDOTS[4]) consists of a *in silico* chemical library design coupled with a *de novo* diversity-focused robotic synthesis and an automated *in vitro* High Throughput Screening (HTS) evaluation platform. Following hits identification, the binding mode of the molecule is usually determined at the atomic level using structural biology such as X-rays crystallography. In the 'DOTS' approach, the focused virtual chemical library is generated by combining an activated fragment corresponding to the binding of the substructure to the target with a collection of functionalized chemical blocks using *in silico*-encoded chemical reactions. These reactions are carefully selected from a list of reactions described as relevant in medicinal chemistry and known to give high yields, thus greatly limiting failure rates. Target-specific compounds are selected by virtual screening, coupled to *in vitro* synthesis and evaluation using our robotic platform. Proof of concept was demonstrated by optimization of bromodomain[3] and PDZ[5,6] inhibitors, leading to the validation of several compounds with several orders of magnitude improved affinity. The proposed process can be implemented in academic environments or biotechnology companies that require automation of these processes.

Selected References

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*2- Hoffer L, Muller C, Roche P, **Morelli X**. Chemistry-driven Hit-to-lead Optimization Guided by Structure-based Approaches. **Mol. Inform.** 2018 Sep;37(9-10):e1800059.*

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4- Hoffer L, Saez-Ayala M, Horvath D, Varnek A, ***Morelli X****, Roche P*. CovaDOTS: In Silico Chemistry-Driven Tool to Design Covalent Inhibitors Using a Linking Strategy.* ***J. Chem. Inf. Model.*** *2019 Apr 22;59(4):1472-1485.*

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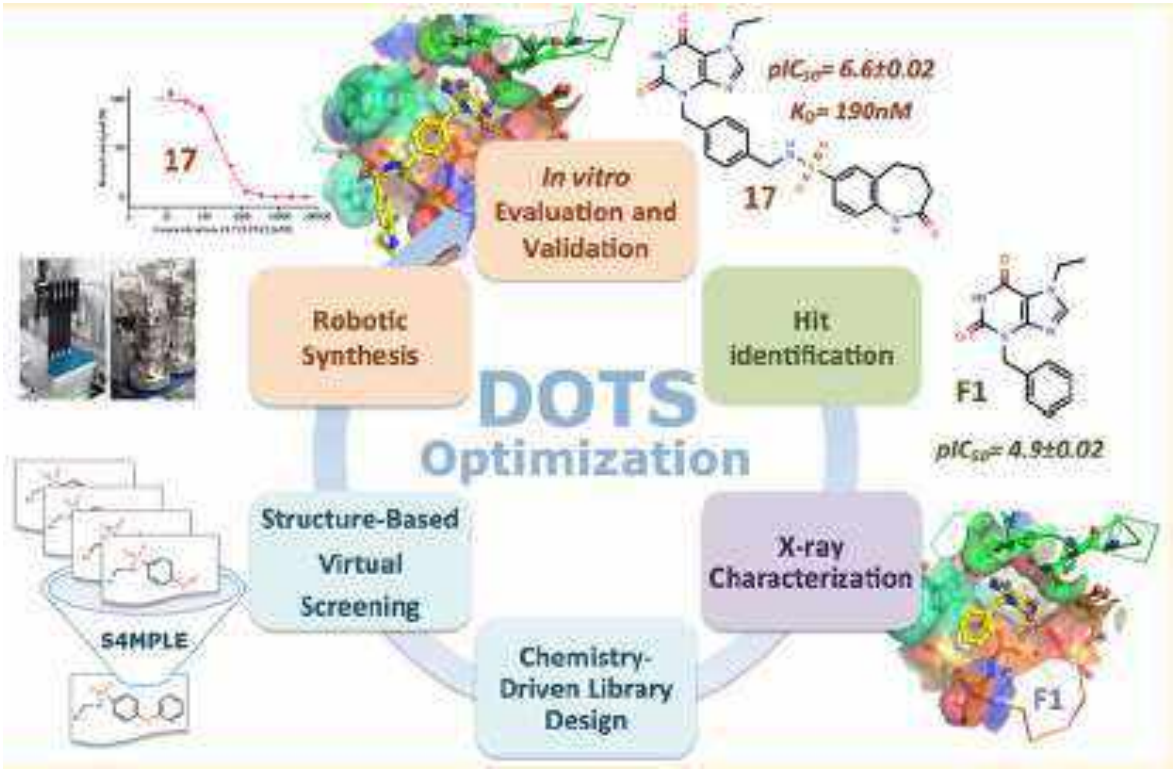
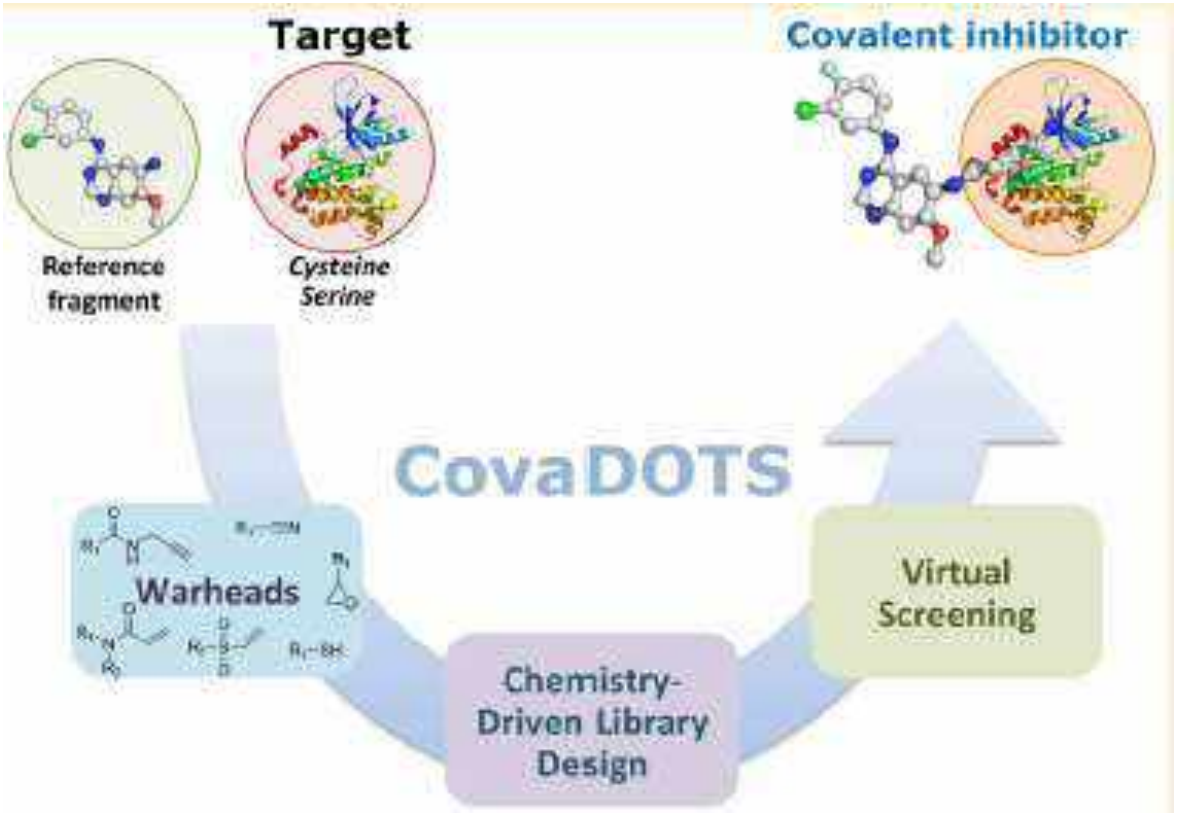
Dr. Xavier Morelli is a 'Director of Research' from the Centre National de la Recherche Scientifique (CNRS, France), group leader at the Cancer Research Center of Marseille (CRCM) and director of 'HiTS', a platform dedicated to Drug Discovery at the Hospital "Institut Paoli-Calmettes".
The main project of his team is focused on the **identification**, **understanding** and **targeting of protein-protein interaction** in **cancer signaling** using **structural** and **chemical biology** technologies. He has published >60 publications and patented 5 applications related to the inhibition of protein-protein interactions. He is an actual member of the scientific committee of the 'Cancéropôle PACA', scientific committee of the 'Fondation ARC', and has acted as secretary of the French Society of Chemoinformatics (SFCi). He is a regular consultant for pharmaceutical companies and was a member of the PhD program committee of the School of Chemistry at the Aix-Marseille University.


17
pIC50= 6.6±0.02
KD= 190nM
17
In vitro Evaluation and Validation
Robotic Synthesis
Hit identification
DOTS
Optimization
F1
pIC50= 4.9±0.02
Structure-Based Virtual Screening
S4MPLE
X-ray Characterization
Chemistry-Driven Library Design
F1


Target
Covalent inhibitor
Reference fragment
Cysteine
Serine
CovaDOTS
Warheads
Virtual Screening
Chemistry-Driven Library Design

Thursday, February 11th, 2021

Precision medicine in oncology.

Esma Saada Bouzid (CAL, Nice)

Centre Antoine Lacassagne, CHU de Nice, France

Precision medicine in oncology is a model that proposes the customization of treatments being tailored to a subgroup of patients, instead of a one-drug-fits-all model. In precision medicine, diagnostic testing is often employed for selecting appropriate and optimal therapies based on the context of a patient's genetic content or other molecular or cellular analysis. The hypothesis that is put forward is that this type of approach will improve the effectiveness of therapeutic management for cancer patients. This paradigm shift has been made possible by the better knowledge of the genomics of malignant tumors, the fall in the costs of high throughput genomic analysis technologies concomitantly with the rapid development of targeted therapies. This lecture will address the main steps in the implementation of precision medicine, with a specific focus on the successes but also the challenges that remain to be overcome.

Thursday, February 11th, 2021

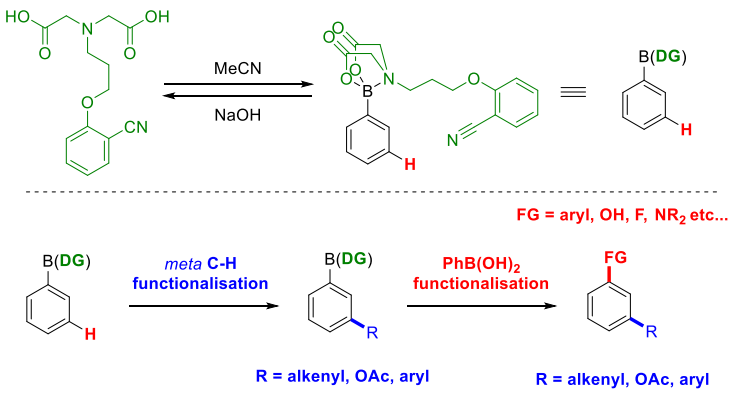
meta-Functionalisation of Aryl Boronates & Protein Structure Mimicry

Alan C. Spivey (Imperial College, London)

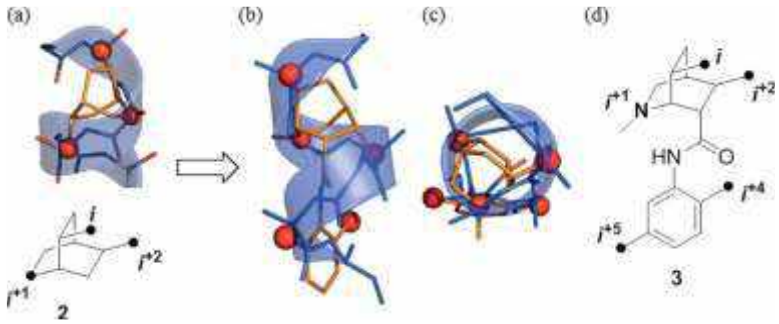
Imperial College, Department of Chemistry, Imperial College London, White City Campus, Molecular Sciences Research Hub (MSRH), 81 Wood Lane, London W12 0BZ, UK

Two areas of research will be presented.

Firstly, some work we have done to develop modified MIDA boronate ligands to allow directed *meta*-CH activation of aryl boronate derivatives will be described.



Secondly, studies directed at the development of a novel scaffold for the modular synthesis of five residue alpha-helix mimetics and their use for the development of leads for protein-protein interaction antagonists in the cancer area will be described.



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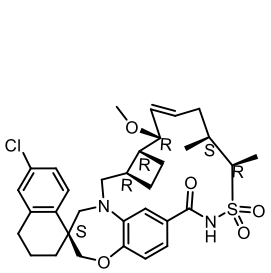
Synthesis of macrocyclic MCL-1 inhibitors with optimized physicochemical properties using Petasis approach.

Frederik Rombouts

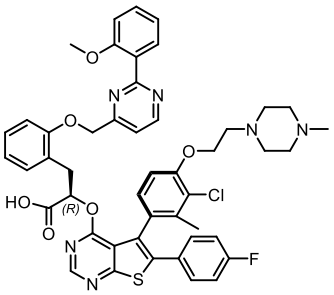
Discovery Chemistry, Janssen Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium

Soufyan Jerhaoui, Gaston Diels, Yves Van Roosbroeck, Michel Surkyn

Apoptosis is a form of programmed cell death which is tightly controlled by the anti-apoptotic BCL-2 class of proteins. BCL-2 proteins bind to the pro-apoptotic effectors BAK and BAX, which can trigger mitochondrial membrane depolarization and the apoptotic cascade. This process is initiated by death signals that activate or induce BH3-only proteins, which bind BCL-2 proteins displacing BAK and BAX. MCL-1 is the most highly expressed antiapoptotic BCL-2 family member and is genetically amplified and overexpressed in many heme and solid tumors. It is also a known intrinsic and acquired cancer therapy resistance factor. Targeting MCL-1 with BH3 mimetics hence provides an attractive strategy to treat heme malignancies and overcome treatment resistance.[1] The development of small molecule inhibitors of the MCL-1 interaction with pro-apoptotic effectors has however been a formidable challenge. Recently however, several MCL-1 inhibitors have entered phase 1 clinical trials, like AMG-176 (**1**), MIK-665 (**2**) and AZD5991 (**3**).[2] These share a large rigid chiral structure, an acid function, and high lipophilicity. Interestingly all three drugs are being administered IV in the clinic in intermittent dosing regimens, most probably to control exposure tightly maximizing therapeutic index (TI). Indeed, safety is a major concern when interfering with this key mechanism controlling cell death. The Janssen MCL-1 discovery program hence focused on maximizing TI with a novel series of macrocyclic 1-(2-fluoroprop-2-en-1-yl)azetidine inhibitors (**4**) having improved physicochemical properties and a short half-life PK profile suitable for IV infusion with tight exposure control. A synthesis approach using the Petasis reaction was developed allowing fine-tuning of properties via the macrocycle linker substitution. Here we disclose early leads from our research showing good cellular potency and in vivo target engagement.



1
AMG-176



2
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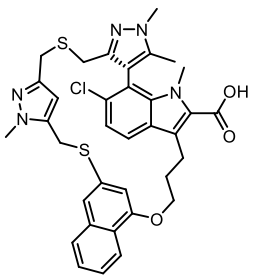
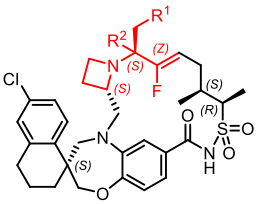


3
AZD5991



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* Correspondence: frombout@its.jnj.com

Photodynamic therapy activity of a new porphyrin-xylan-coated silica nanoparticle in a human colorectal cancer *in vivo* model.

Bertrand Liagre

University of Limoges, Laboratoire PEIRENE, EA 7500, Faculty of Pharmacie, 87000 Limoges

Ludovic BRETIN, Aline PINON, Soukaina BOURAMTANE, Frédérique BREGIER, Vincent SOL, Vincent CHALEIX, David Yannick LEGER, Bertrand LIAGRE

Colorectal cancer (CRC) is one of the most common cancer globally but above all the second leading cause of death for oncological reasons. Despite medical research advances in anti-cancer treatments, many side effects persist in patients as well as development of resistances to conventional treatments. The development of new anti-cancer therapeutic strategies is necessary in order to improve care of patients. Photodynamic therapy (PDT) using photosensitizers (PS) comes as an innovative therapeutic strategy severely restricting these undesirable side effects. PDT has been approved for treatment of some cancers due to the generation of cytotoxic reactive oxygen species only with photoactivated PS. However, low physiological solubility and lack of selectivity towards tumor sites are the main limitations of their clinical use. Indeed, targeted drug delivery is a crucial point in cancer therapy. Nanomedicine through the use of nanoparticles improves tumor-targeting because they are able to spontaneously accumulate in solid tumors through an enhanced permeability and retention effect. The purpose of this study was to prove added value of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin-xylan (TPPOH-X) vectorization by silica nanoparticles (SNPs) in order to enhance anti-cancer efficacy through better tumor-targeting. It has been demonstrated significant anti-cancer efficacy increase of TPPOH-X SNPs-PDT thanks to cellular uptake improvement relative to free TPPOH-PDT in 3 human CRC cell lines. Moreover, it has been characterized that cell death induced by TPPOH-X SNPs-PDT is conducted *via* apoptosis and autophagy acts as a resistance pathway to cell death. Furthermore, *in vivo* and without toxicity, TPPOH-X SNPs-PDT induce an elevated anti-cancer efficacy through improvement of tumor-targeting compared to free TPPOH-PDT. This study therefore highlighted the added value of PDT and nanomedicine combination in order to improve future cancer treatments.

Keywords: anticancer drugs, porphyrins, silica nanoparticles, drugs delivery, photodynamic therapy

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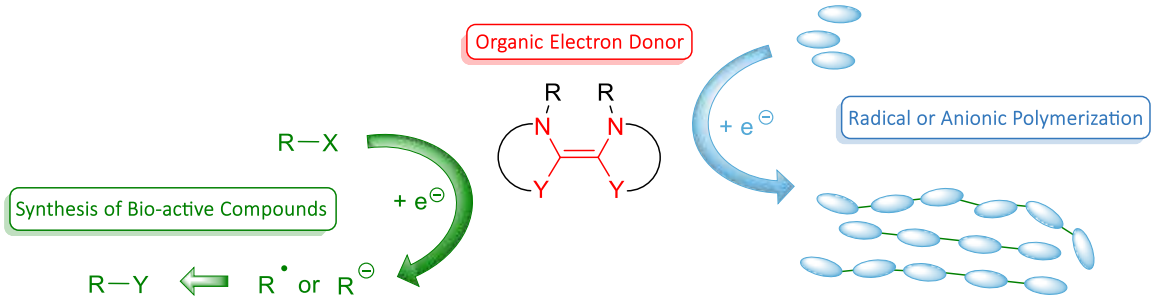
The application fields of enamine based organic electrons donors

Julie Broggi

Aix Marseille Univ, CNRS, ICR Institut de Chimie Radicalaire, Faculté de Pharmacie, Marseille, France

Enamine-based organic electron donors (OEDs) are powerful reducing agents recognized for their potential in the reduction of challenging substrates and in original applications.[1] They are capable of single- or double-electron transfer to organic substrates under mild and homogeneous conditions, promoting bond formations through the generation of radical or anionic intermediates. They thus represent serious rivals to highly aggressive metal-based reducers and emerge as an attractive novel source of reducing electrons in many aspects.

Our research focus on synthesizing new organic electron donors and establishing their fields of applications.[2-3] In this talk, we will give an overview of these fields, spanning from the synthesis of bio-active compounds to the preparation of high value-added polymers.



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julie.broggi@univ-amu.fr

Designing chemical tools to shed light on G-Protein-coupled receptors.

Dominique Bonnet

[a] *Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS/Université de Strasbourg, 67401 Illkirch, France.*
[b] *Institut du Médicament de Strasbourg, Université de Strasbourg, 67401 Illkirch, France*

G-protein-coupled receptors (GPCRs) are the largest family of transmembrane receptors in humans and the targets of more than 30% of all known drugs on the market. The ability to detect, measure and quantify the binding of ligands to these receptors and the resultant responses both *in vitro* and *in vivo* represent key elements of the drug discovery process.

Owing to their sensitivity and to their reduced environmental safety risk in comparison to isotope-labeled methods, fluorescence technics represent powerful tools to investigate the function, dynamic and location of GPCRs. In this presentation, we describe the design and the synthesis of original fluorescent GPCR ligands, combining organic synthesis,[1] medicinal chemistry and computational modeling.[2] These probes have found various applications in GPCR chemical biology and drug discovery, to set up new receptor-selective high-throughput screening assays,[3] to study the functional architecture of GPCRs, especially their ability to form heterodimers[2] and to detect those receptors in living cells[4] as well as in whole organism.[5]

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* Email: dominique.bonnet@unistra.fr

Friday January 31, 2020

SCT Award for young investigator in Medicinal Chemistry

Small molecules targeting protein–RNA interactions as chemical probes and therapeutic leads

Peng Wu

Chemical Genomics Centre and Department of Chemical Biology, Max Planck Institute of Molecular Physiology, 44227 Dortmund, Germany

Abstract:

Interactions between RNA-binding proteins and RNAs are crucial for various biological processes and cellular activities. Dysregulation of the pervasive interactions is closely associated or can lead to a wide range of human diseases including cancers, viral diseases, inflammatory and autoimmune disorders, cardiovascular diseases, and neurological disorders. My current research interests lie primarily in targeting the pervasive protein–RNA interactions using small molecule-based strategies. The aim is to utilize such strategies to discover chemical probes that will be useful to untangle the complex protein–RNA regulatory network and provide potential leads or candidates for the development of therapeutics.

In this talk, I will focus on reported small molecules that inhibit different types of RNA-binding proteins, such as the canonical microRNA-binding protein LIN28 and the noncanonical bacterial CRISPR-associated protein Cas9, using both screening-based and scaffold-based methods. The second part of the talk will discuss the potential of applying chemically induced proximity-based approaches to target RNA-binding proteins and structured RNAs. Additionally, synthetic approaches that we have used in projects to access biologically interesting small molecules covering unexplored chemical space will be summarized as the third part of the talk.

peng.wu@mpi-dortmund.mpg.de

ORAL COMMUNICATIONS

Newly synthesized triarylmethanes discovered ligands of the aryl hydrocarbon receptor	***OC 01***

Elizabeth Goya-Jorge [(1,2)*], Celine Rampal [(3)], Nicolas Loones [(3)], Stephen J. Barigye [(2)], Laureano E. Carpio [(2)], Rafael Gozalbes [(2)], Clotilde Ferroud [(3)], Rosa M. Giner [(1)], Maité Sylla-Iyarreta Veitía [(3)].

(1) Departament de Farmacologia, Facultat de Farmàcia, Universitat de València. Av. Vicente Andrés Estellés, s/n, 46100 Burjassot, Valencia, Spain.
(2) ProtoQSAR SL., CEEI (Centro Europeo de Empresas Innovadoras), Parque Tecnológico de Valencia. Av. Benjamin Franklin 12, 46980 Paterna, Valencia, Spain.
(3) Equipe de Chimie Moléculaire du Laboratoire Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Conservatoire National des Arts et Métiers (Cnam), 2 rue Conté, 75003, HESAM Université, Paris, France.

The aryl hydrocarbon receptor (AhR) is a pharmacological target in malignancies and in several inflammatory, immunological, and metabolic conditions. Recently, we have discovered a novel set of triarylmethane compounds that can act as modulators of AhR-mediated effects [(a)]. Screening in a cell-based *in vitro* bioassay, eight out of 32 newly synthesized triarylmethanes were identified as AhR agonists. The main structural determinant to activate AhR transcriptional activity was found to be the introduction of naphthol or heteroaromatic rings as one of the three aryl functions. Molecular docking simulations revealed similarities in AhR binding for the most potent triarylmethane identified (4-(pyridin-2-yl(thiophen-2-yl)methyl)phenol and the well-known AhR endogenous ligand 5,11-dihydroindolo[3,2-b]carbazole-12-carbaldehyde (FICZ). Moreover, prediction of ADME properties and a druglikeness study of the promising AhR agonists suggested they have an adequate profile as drug candidates. To our knowledge, this is the first study evaluating the AhR modulatory effects of triarylmethane class of compounds.

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* Correspondence: egoya@protoqsar.com

Design and synthesis of dual inhibitors of DYRK1A/CLK1 kinases involved in neurodegenerative diseases.

Clémentine Pescheteau[(1)]*, Sandrine Ruchaud[(2)], Pascal Bonnet[(1)], Frédéric Buron[(1)], Sylvain Routier[(1)]

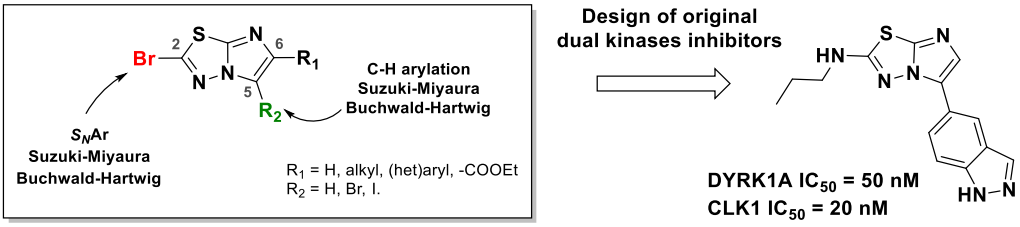
(1) Institut de Chimie Organique et Analytique, Université d'Orléans, UMR CNRS 7311, Rue de Chartres, 45067 ORLEANS, France.
(2) CNRS USR3151, Station Biologique, place G. Teissier, CS90074, 29688 ROSCOFF, France.

OC 02

Alzheimer's disease (AD) is the most common cause of dementia, and a neurodegenerative disease that affects nearly 50 million people worldwide. Despite the increasing development of solutions to fight AD symptoms, a major challenge for medicinal chemists is the development of efficient curative treatments. An innovative solution is the kinase inhibition, and it has been proven that over-expression of DYRK1A and CLK1 kinases is involved in neuronal degeneration pathway observed especially in Alzheimer's disease.[a] Our project takes part in a collaboration with European multidisciplinary teams to design new dual inhibitors of these two kinases .

Recently, our laboratory of medicinal chemistry has developed new families of heterocyclic molecules with high therapeutic interest. Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have found applications in oncology, infectiology or neurodegenerative diseases, but few functionalization methods were described.[b] Consequently, we developed several methodologies to modulate regioselectively on the *C-2*, *C-5* and *C-6* positions of this scaffold.[c] The use of various reactions as S_NAr, C-H arylation, palladium catalyzed cross coupling allowed us to increase the molecular diversity of such derivatives. By modifying not only functionalization groups but also the scaffold, replaced by bioisosters, we developed new series of molecules to test their inhibitiory activity on kinases. Thanks to SAR studies conducted with our ANR partners, we designed selective and dual inhibitors of DYRK1A and CLK1 kinases, with IC_{50} in range of nM.

The synthesis of our original compounds, the promising results of biological tests and the perspectives of the project will be presented in this communication.



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* Correspondence: clementine.pescheteau@univ-orleans.fr

Squaraine dyes as fluorescent turn-on probes for the detection of mucin

C. Pontremoli *(1), C. Butnarasu (2), M.J. Moran Plata(1), D.M. Dereje (1), C. Barolo (1), S. Visentin (2) N. Barbero(1)

(1) Department of Chemistry, NIS Interdepartmental and INSTM Reference Centre, University of Torino, Via P. Giuria 7, 10125 Torino, Italy.

(2) Department of Molecular Biotechnology and Health Sciences, University of Torino, Via G. Quarello 15A, 10135 Torino, Italy.

OC 03

Mucins are a family of long polymeric glycoconjugates and represents the principal components of mucus which cover all the mucosal surfaces of the human body. One of the main functions of mucus is to protect the underlying surfaces from environmental factors, although, in pathological conditions, mucus can act as a barrier for drug absorption or can become a concentrate of pathogens and cellular debris due to the alteration of its physic-chemical properties. Both the mucus and mucins are essential mediators of the innate immune system, and consequently their alterations or overexpression can be associated to several diseases such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis and several types of cancer [1]. In this scenario, over the last decades, mucins have been identified as an important biomarker of adverse prognosis and attractive therapeutic target [2,3]. To date, several techniques have been developed to detect mucins; among them, fluorometric assays gained increasing attention thanks to their convenience, simplicity, non-invasive monitoring capability and usability in biological samples, leading to a simultaneously increasing in developing new dyes able to non-covalently bind specific proteins for their detection.

Among the different fluorescent probes, squaraines are characterized by sharp and intense absorption and emission in the visible up to the NIR region, but in aqueous environments tend to form aggregates that lead to fluorescence quenching therefore limiting their wide applications. Despite this drawback, squaraine dyes are proved to turn on their fluorescence in response to a biological target, find promising application for living processes, medical diagnosis and biological imaging at the molecular, cellular and organism level[4,5].

In this contribution, the interaction in aqueous media between two proteins, Human Serum Albumin (HAS) and Porcine Gastric Mucin (PGM) and several squaraines with different substitutions have been investigated by using UV–Vis, circular dichroism and fluorescence spectroscopies. The aim is to understand how squaraines behave in presence of different proteins, to identify a structure-activity relationship for the design of more effective and selective fluorescent dyes.

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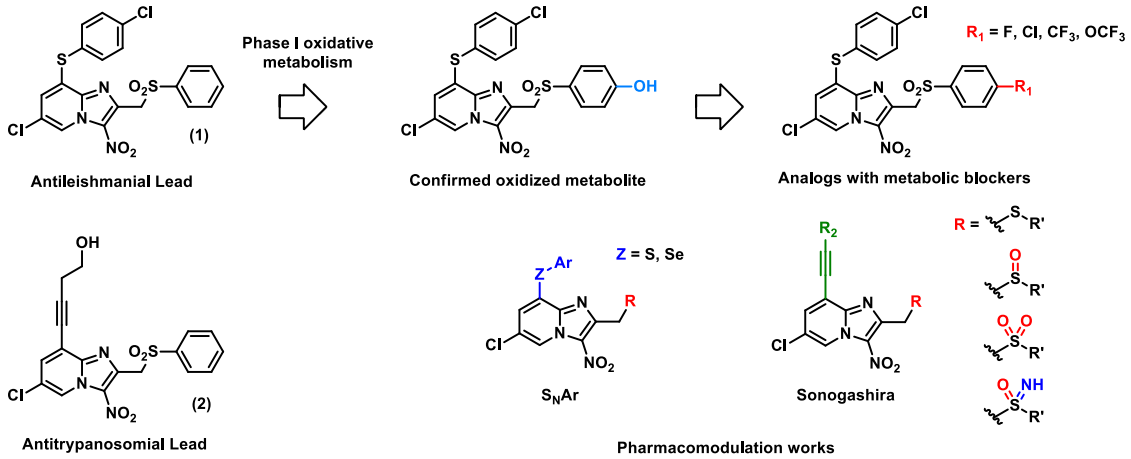
* Correspondence: carlotta.pontremoli@unito.it

Pharmacomodulation and optimization of new 3-Nitroimidazo[1,2-*a*]pyridines substrates of NTR1 against kinetoplastids	***OC 04***

Romain Paoli-Lombardo,[1] Nicolas Primas,[1]* Cyril Fersing,[1] Sébastien Hutter,[2] Clotilde Boudot,[3] Caroline Castera-Ducros,[1] Sandra Bourgeade-Delmas,[4] Nadine Azas,[2] Bertrand Courtioux,[3] Pierre Verhaeghe,[5] Pascal Rathelot,[1] and Patrice Vanelle[1].

(1) Aix Marseille Univ, CNRS, ICR UMR 7273, PCR, 13385 Marseille. (2) Aix Marseille Univ, IHU Méditerranée Infection, UMR VITROME, 13005 Marseille. (3) Université de Limoges, UMR Inserm 1094, NET, 87025 Limoges. (4) Université Paul Sabatier, UMR 152 PharmaDev, 31062 Toulouse. (5) Université Paul Sabatier, CNRS UPR 8241, LCC, 31077 Toulouse.

Kinetoplastids are a group of flagellated protozoans responsible for various parasitic diseases in mammals including *Leishmania spp* (leishmaniases), *Trypanosoma brucei* (human African trypanosomiasis) and *Trypanosoma cruzi* (Chagas disease) infections in humans. Half a billion people are at risk of contracting one of these neglected tropical diseases (NTDs), and nearly 20 million people are infected, causing up to 50,000 deaths per year. Nevertheless, currently available treatments have certain limitations due to lack of efficacy, toxicities, non-oral administration and cost. In this context, our laboratory previously described two 3-nitroimidazo[1,2-*a*]pyridine lead compounds selectively activated by the parasitic type 1 NTR: one active *in vitro* against *Leishmania* (**1**) functionalized at C8 position by a *p*-chlorophenylsulfone,[(a)] and the other against *Trypanosoma* (**2**) bearing a 4-hydroxybutyn-1-yl substituent at C8.[(b)] However, these compounds have low solubility and poor mouse microsomal stability. Among the probable metabolites, oxidized metabolites were synthetized and confirmed to be the result of the metabolism of the lead compounds. With a view to improve microsomal stability, analogs with metabolic blockers were obtained. Finally, pharmacomodulation works at C2 position were carried out and new molecules of therapeutic interest were synthetized.[(c)] Synthesis pathway and biological results of these new compounds will be presented in the communication.



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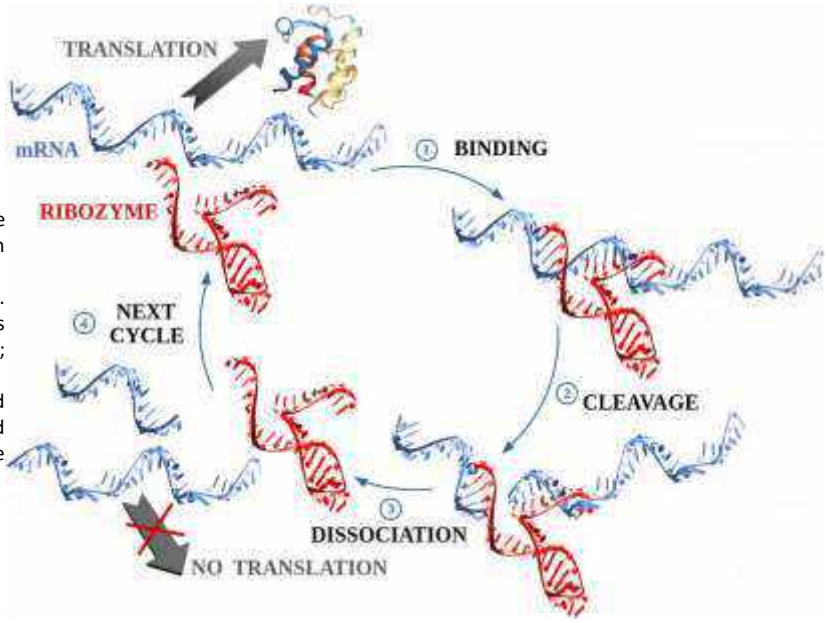
* Correspondence: nicolas.primas@univ-amu.fr

Designed RNA enzyme as a potential antibacterial	
Joanna Miszkiewicz[1,2]*, Ksenia Maximowa[2], Maciej Łukaszewicz[3], Edward Darżynkiewicz[2,3], Joanna Trylska[2] *(1) Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland.* *(2) Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland* *(3) Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Żwirki i Wigury 93, 02-089, Warsaw, Poland*	***OC 05***

The continuous misuse of antibiotics has increased bacterial resistance and led to uncured bacterial infections. Therefore, new effective ways to fight multi-drug resistant bacteria should be discovered. An innovative approach that could help solve the problem is precluding translation of an essential bacterial protein by cleaving bacterial mRNA using ribozymes. The lack of such a protein in the cell would inhibit bacterial growth.[a] Ribozymes are RNA enzymes that recognize and then cleave the target RNA sequence in a specific position in the RNA sequence. Thus, using ribozymes to recognize a specific mRNA transcript will hinder translation. The ribozyme-strategy has been successfully used in antiviral, antiprion and anticancer therapies.[b]
The goal of our study was to design a ribozyme that would efficiently cleave a fundamental bacterial mRNA fragment encoding an essential protein.

As a model system we chose the hammerhead ribozyme and mRNA fragment encoding the acyl carrier protein (acpP) in Escherichia coli K-12 MG1655.[c] First, we designed short RNA oligonucleotides mimicking $mRNA_{acpP}$ sequence, and checked their binding to the ribozyme by isothermal titration calorimetry. We showed the formation of the tight complex in a 1:1 ratio. Second, we demonstrated efficient cleavage of the $mRNA_{acpP}$ fragment by the designed ribozyme on polyacrylamide gels. Next, we also tested the cleavage of $mRNA_{acpP}$ in bacterial cells.



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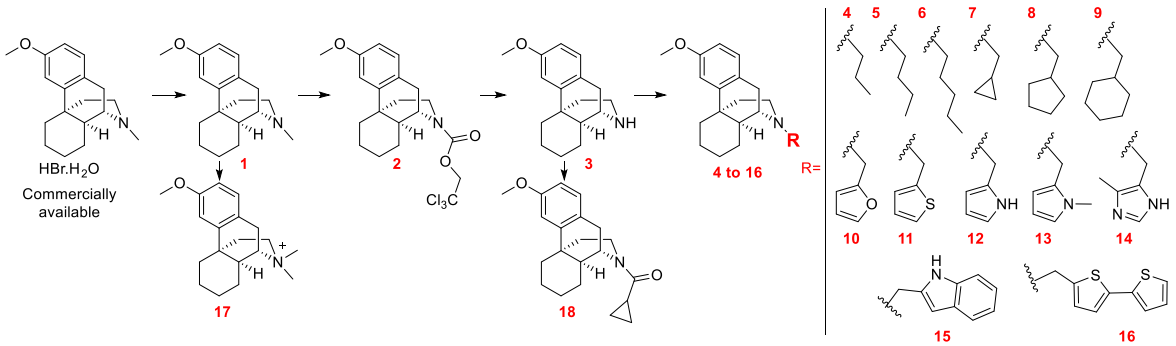
*Correspondence:
j.miszkiewicz@cent.uw.edu.pl

Simplified alkaloid mimics in the fight against malaria hepatic stage	***OC 06***

<u>Antoinette KEITA</u> [(1)], François-Hugues POREE [(2)], Romain DUVAL [(1)]

(1) UMR IRD 261, MERIT, Faculté de Pharmacie, Université de Paris, 4 Avenue de l'Observatoire, 75006 Paris
(2) UMR CNRS 6226 COrInt, Faculté de Pharmacie, Institut des Sciences Chimiques de Rennes, 2 Avenue du Pr. Léon Bernard, F-35043 Rennes CEDEX

Despite significant progresses in the prevention and treatment of malaria, the disease remains a major cause of death worldwide, the parasite *Plasmodium falciparum* (*Pf*) accounting for the majority of malaria mortality[1]. Our investigation is oriented towards the study of structurally-related Tazopsine alkaloids, which was described for the first time in 2006. Tazopsine, an alkaloid isolated from the endemic Malagasy plant *Strychnopsis thouarsii* and traditionally used as a decoction to combat malaria was shown to be active on *Pf in vitro* both on the blood stage and the liver stage with IC_{50} of 5 µM and 4 µM, respectively. Unfortunately, toxic effects were identified *in vivo* on mice infected by *Plasmodium yoelii* (*Py*)[2]. Besides, easily accessible and well-established medicinally antitussive drug dextromethorphan (DXM) can be seen as a "naked Tazopsine" which also demonstrates activity on *Pf in vitro* at IC_{50} of 52 µM for the blood stage and a selectivity for the hepatic stage with IC_{50} of 5 µM[3]. Unfortunately, it was also toxic *in vivo* on mouse infected by *Py*. In the search for prophylactic drugs, we carried out a structural diversification by a semisynthesis approach from DXM base (compound **1**) leading to the preparation of a small library of compounds (figure1). I*n vitro* prescreen on *Plasmodium berghei* expressing the Green Fluorescent Protein allowed us to identify four hits, in which one is active on *Plasmodium falciparum* in the low micromolar range.



<u>Figure</u>1: Route of molecules synthesis

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*** Correspondence**: antoinette.keita@parisdescartes.fr

Predicting potential EDCs binding to ERα using a pipeline combining SB and LB in silico methods

Asma SELLAMI*, Nathalie LAGARDE, Matthieu MONTES

Laboratoire GBCM, EA 7528, Conservatoire National des Arts et Métiers, Hésam Université, F-75003 Paris, France

OC 07

Estrogen receptors α (ERα) are transcription factors involved in several physiological processes belonging to the nuclear receptors (NRs) protein family. Beside the endogenous ligand (estrogen), several other chemicals are able to bind to these receptors **[1]**. Among them are endocrine disrupting chemicals (EDCs) that can trigger toxicological pathways **[2]**. Many studies have focused on predicting EDCs based on their ability to bind NRs, mainly ER, TR, AR, GR and PPARγ.

In this work we suggest a pipeline designed for the prediction of ERα binding activity for potential EDCs prediction. It is a combination of structure based (docking and pharmacophore models) and ligand based (pharmacophore models) methods.

The models have been constructed using the EPA data **[3]** encompassing a large number of structurally diverse compounds. All docking calculations were performed using three different softwares with free academic license accounting for 6 scoring functions i.e smina **[4]** (smina-ad4, smina-dkoes, smina-vina, smina-vinardo), Surflex-dock **[5]** and PLANTS **[6]**. Single and ensemble structures docking approaches were investigated using the predictiveness curve **[7]** to define scoring thresholds able to efficiently discriminate binding from non-binding compounds. Structure based (SB) and ligand based (LB) pharmacophores were constructed from 31 pdb structures of Erα and the EPA dataset, respectively. Both SB and LB models were combined into a unique SBLB collection of pharmacophores after removal of redundancies. Different combination approaches were investigated, i.e. cumulative method and the hierarchical method. A validation step was finally achieved using two external databases: the NR-DBIND **[8]** and the EADB **[9]**.

Results showed that docking and pharmacophores taken individually yielded good overall results. Single structure docking performed better than ensemble structure approach and the best software was Smina with the scoring function Dkoes **[4]**. Pharmacophore methods yielded high specificity values comparing to the associated sensitivities. Hopefully, combination of methods led to alleviate performances. The hierarchical approach was shown to be better suited for our toxicological study with high specificities. The cumulative method was described as better suited for drug design projects use as it yielded high sensitivity values.

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* Correspondence: asma.sellami@lecnam.net ; nathalie.lagarde@lecnam.net

The influence of 4-oxoquinoline tautomerism in the MAOs inhibition

Francesco Mesiti*[1, 2], Annalisa Maruca[1], Vera Silva[2, 4], Roberta Rocca[1], Carlos Fernandes[2], Fernando Remião[4], Eugenio Uriarte[6], Stefano Alcaro[1], Alexandra Gaspar[2], and Fernanda Borges[2]

[1]Net4Science Srl, spin-off accademico, Viale Europa, Loc. Germaneto, 88100, Catanzaro, Italy

[2]CIQUP, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, 4169-007, Portugal

[4]UCIBIO-REQUIMTE, Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, 4050-313, Porto, Portugal

[6]Departamento Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, España

OC 08

The 4-oxoquinoline compounds underwent in several drug discovery programs due to their promising pharmacological properties. (1) In recent years, our research showed that chromones (1, 4 benzopyrone) are potent and selective MAO-B inhibitors. (2) Therefore, based on the chromone and 4-oxoquinoline structure similarity and adopting a bioisosterism changing, a small library of quinoline compounds was designed, synthetized, and screened towards MAOs isoforms. In fact, the bioisosteric approach is a validated method often used in medicinal chemistry to change structural fragment in order to diversify the scaffold space and to improve activity, reduce toxicity, and change pharmacokinetic profile. (3) The data showed that *N*-(3,4-dichlorophenyl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **10** was the most potent and selective MAO-B inhibitor (IC_{50}=5.30±0.74 nM and SI: ≥1887). Furthermore, the data analysis demonstrated that prototropic tautomerism markedly influences the biological activity, then a rational physical/chemical characterization of the quinoline derivatives was performed to understand the attained data. The tautomeric form of the quinoline derivatives have been assessed by 2D NMR techniques, namely ^{1}H-^{15}N-HSQC and ^{1}H-^{15}N HMBC, which are proposed as expedite tools for the unequivocal characterization of quinoline tautomers. The cytotoxicity studies of the active compounds performed in differentiated human neuroblastoma cell line (SH-SY5Y) did not evidenced cytotoxicity effects. Finally, computational studies on enzyme-ligand complexes, obtained after MM-GBSA calculation and molecular dynamics simulations, supported the experimental data.

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Correspondence: E-mail francesco.mesiti@studenti.unicz.it

Crystallography and Molecular Dynamics of hIDO1 to assist structure-based drug design

Manon Mirgaux*, Laurence Leherte, Johan Wouters.

(1) Laboratoire de Chimie Biologique Structurale (CBS), Namur Institute of Structural Matter (NISM), Namur Research Institute for Life Sciences (NARILIS), University of Namur (UNamur), Belgium, Rue de Bruxelles 61 5000 Namur

OC 09

Due to its degradation into numerous bioactive metabolites, L-Tryptophan (L-Trp) is the less abundant but essential amino acid in mammals (a). The main way to transform L-Trp is the kynurenine pathway (KP), allowing the transformation of 95% of the L-Tryptophan available from food (b). Through KP, L-Trp is converted into L-kynurenine to produce essential metabolites such as redox cofactors, neuroprotectors and neurotoxics. (c) As a result, the KP pathway confers to L-Trp a central role in many diseases. (b, d) Therefore, enzymes of the kynurenine pathway can be considered as a set of therapeutic targets. Particularly, the first step of this road, catalyzed by hIDO1, hIDO2 or hTDO, raised an interest in the cancer research in cancer immune escape and in the resistance to immunotherapy. (e) Over the years, hIDO1 inhibitors have been developed (Indoximod, Epacadostat, PF-06844003, Navoximod and Linrodostat) but, recently, the most advanced of them (Epacadostat) failed in the clinical trial (f).

Since 2006, several structures of hIDO1 are deposited on the PDB. However, a loop of the enzyme, the JK-loop, has never been resolved. As the JK-loop is very likely involved in the closure of the active site, this loop plays a key role in the mechanism and the inhibition (g). Recent fails in clinical trials of hIDO1 inhibitors trigger a revision of the enzyme functioning involving the JK-loop (f). It is recognized that there is a lack of structural information on hIDO1 to understand the key role of the JK-loop.

In the present work, the refinement of the JK-loop is obtained for the first time by X-ray diffraction experiment, thanks to its crystal packing mode. To support the X-ray observation, Molecular Dynamics trajectories are also carried out to provide a dynamical information about the loop in the presence of the cofactor (i). Such new structural and dynamical information highlights the importance of the JK-loop in confining the labile heme cofactor into the active site. A better structural characterization of this important therapeutic target will assist medicinal chemistry programs.

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* Correspondence: manon.mirgaux@unamur.be

Design and synthesis of Red-SMU1 disruptors as new antiviral agents against Influenza A virus	***OC 10***

A. Corio[(1)*], U. Ashraf[(2)], P.-O. Vidalain[(3)], N. Pietrancosta[(4)], L. Le Corre[(1)], T. Crepin[(5)], N. Naffakh[(2)], C. Gravier-Pelletier[(1)], P. Busca[(1)].

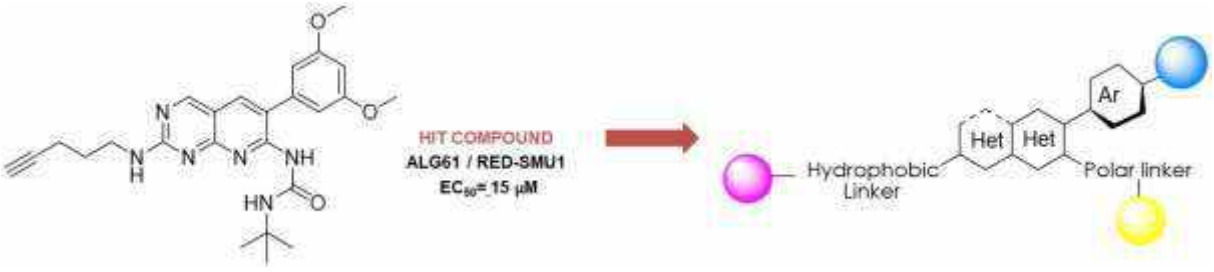
(1) Université de Paris, LCBPT, UMR 8601, Paris.
(2) Institut Pasteur, Paris.
(3) ENS, Centre International de Recherche en Infectiologie, Lyon.
(4) Institut de Biologie Paris Seine, UMR8246, Paris.
(5) Institut de Biologie Structurale, Grenoble.

Influenza A viruses represent a major global health treat. Despite the existence of a seasonal vaccine and the availability of antivirals, each year they cause between 3 and 5 million of severe cases and the death of more than 300 thousand people in the world. The available drugs are rapidly becoming inefficient because of the drug resistance. Consequently, there is an urging need for the development of an innovative antiviral agent.[a]

During the last years, Naffakh's team and collaborators demonstrated that the complex of Red and SMU1, which are human spliceosomal factors, has a fundamental role in the replication cycle of the Influenza A virus. Red-SMU1 complex can be considered as a promising target in the optic of host directed therapy, which has the advantage to virtually avoid the risk of the development of drug-resistance.[b] The *in silico* screening of more than 3000 compounds from the chemiolibrary of the University of Paris, followed by the *in cellulo* biological evaluation of the best candidates, allowed the identification of ALG61 which is able to both inhibit the RED-SMU1 interaction and decrease the viral replication *in cellulo*.[c]

With the objective of improving its biological activity and selectivity toward the Red-SMU1 interaction, we designed series of analogues, with different scaffolds or substitution patterns.

The recent results in their synthesis and activity evaluation will be presented in this oral communication.



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* Correspondence: corio.alessandra@gmail.com

Co-encapsulation of pioglitazone and curcumin into chitosan nanoparticles and in vivo antidiabetic potential	***OC 11***

Lupaşcu Florentina*, Apotrosoaei Maria, Constantin Sandra, Herciu Lorena, Alexandru Sava, Ababei Andra, Vasincu Ioana, Drăgan Oana, Iacob Andreea, Confederat Luminița, Profire Lenuța

[1]University of Medicine and Pharmacy "Grigore. T. Popa" Iași, Universității Street, no. 16, 700115, Iași, Romania

Diabetes mellitus (DM) is a one of the most serious chronic diseases in the world. DM associated with oxidative stress causes destruction of cellular structures leading to severe tissue damage. Curcumin has been shown to have significant antioxidant activity and a potential hypoglycemic effect in experimental diabetic animal models. Unfortunately, its therapeutic efficacy is limited because it has low oral bioavailability, reduced digestive absorption and fast metabolism. Pioglitazone is an efficient hypoglycemic therapeutic agent but with a low oral absorption, low solubility and short half –life. Developing of new multi-target polymer systems for improving the antidiabetic agents' bioavailability is a major concern of researchers. The aims of this study were to develop chitosan nanoparticles (CsNp) loaded with pioglitazone and curcumin and to investigate its hypoglycemic effects on DM induced with streptozotocin to rats. CsNp and drugs loaded into CsNp were obtained by ionic gelation method. In order to optimize the method for obtaining CsNp was varied the concentration of chitosan (Cs), TPP and of the active drug. Following the optimization process, it has been established the optimal formula for loading the two active substances in the polymer matrix. Antioxidant activity of pure substances and of its Cs nanoformulations was analyzed on DPPH radical scavenging activity assay. Moreover, compared to the pure curcumin, curcumin loaded into CsNp showed a dose-dependent manner with a higher radical scavenging activity. To determine the in vivo hypoglycemic effect, Pioglitazone, Curcumin, and Cs nanoformulations (CS-P, CS-C, CS-PC) were orally administered on diabetic rats, once per day, for a period of 21 days. During the experiment it was investigated glycaemia and at the final of experiment was analyzed glycosylated hemoglobin (HbA1c). Oral administration of these new polymeric systems significantly reduced the blood glucose levels and HbA1c in reference with the diabetic rats. The best hypoglycemic effect was recorded for CS-PC formulation.

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*Correspondence: lupascu.geanina@yahoo.com

Thiazolidine-2,4-dione derivatives: Synthesis, α-glucosidase and α-amylase inhibition potential and acute toxicity evaluation

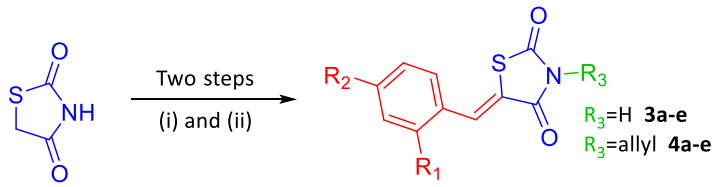
Saad Fettach [(1)], Fatima Zahra Thari [(2)], Khalid Karrouchi [(3)], Yahia Cherrah [(1)], Hamza Tachallait [(2,4)], Khalid Bougrin [(2,4)*], My El Abbes Faouzi [(1)].

(1) Laboratory of Pharmacology and Toxicology, Biopharmaceutical and toxicological analysis research team, Faculty of Medicine and Pharmacy, University Mohammed V in Rabat, Morocco. (2) Equipe de Chimie des Plantes et de Synthèse Organique et Bioorganique, URAC23, Faculty of Science, B.P. 1014, Geophysics, Natural Patrimony and Green Chemistry (GEOPAC) Research Center, Mohammed V University in Rabat, Morocco. (3) Laboratory of Analytical Chemistry and Bromatology, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco. (4) Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Benguerir, Morocco.

OC 12

Diabetes is a rapidly spreading life threatening disease with a global prevalence of 171 million in the year 2000 and is expected to increase to 366 million by 2030.[(a)] It is a chronic metabolic disorder characterized by persistent hyperglycemia, related to failure of insulin function (insulin resistance) over extended periods in peripheral tissues. It can be managed by enhancing the functionality of pancreatic α-amylase and intestinal α-glucosidase.[(b)] Thiazolidine-2,4-dione (TZD) also known as Glitazone is an eminent class of oral antidiabetic agents that directly target insulin resistance[(c)], it is an important scaffold associated with several other biological activities.[(d)]

We herein report the green synthesis of thiazolidine-2,4-dione derivatives *via* solvent-free microwave irradiation knoevenagel condensation, followed by NaOH catalyzed *N*-alkylation in aqueous media. All the synthesized products were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS. Aiming to develop new enzyme inhibitors, we screened our newly prepared products for their *in vitro* α-glucosidase and α-amylase inhibition potential, most of them were active against the latter enzymes, some of them were more potent than the clinical drug Acarbose. Thereafter we evaluated the acute toxicity of the lead products. The enzymatic screening revealed that three compounds were highly active against the two enzymes. The most potent compounds were also found to be non-toxic at concentration of 2000mg/kg.



Experimental conditions : (i) RCHO, solid support, solvent-free, MW, 100-110°C; (ii) Allyl bromide, NaOH, EtOH/H_2O (v/v 2:1), 75°C, 5-6h.

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* Correspondence: kbougrin@yahoo.fr (K. Bougrin)

Analyzing Cellular Effects and Pharmacokinetic Properties of Human Protein Kinase CK2 Inhibitors with an Indeno[1,2-*b*]indole Scaffold

Robin Birus [1]*, Laurens Ballentin [1], Faten Alchab [2], Dagmar Aichele [1], Laurent Ettouati [2], Christelle Maminon [2], Marc Le Borgne [2], Joachim Jose [1]

(1) Institute of Pharmaceutical and Medicinal Chemistry, PharmaCampus, Westfälische WilhelmsUniverstität Münster, 48149 Münster, Germany. (2) EA 4446 Bioactive Molecules and Medicinal Chemistry, Faculté de Pharmacie-ISPB, SFR Santé Lyon-Est CNRS UMS3453-INSERM US7, Université Claude Bernard Lyon 1, Université de Lyon, F-69373 Lyon, France

OC 13

CK2 is a ubiquitous and highly pleiotropic Ser/Thr kinase. Thus, it is involved in a multitude of cellular processes. However, its exact function is not yet fully understood. Previous studies have shown that the CK2 concentration and activity is increased in tumor cells compared to normal cells. Since the protein kinase causes anti-apoptotic and proliferation-enhancing effects in neoplastic tissues, CK2 appears an interesting target for tumor therapy nowadays [1,2].

Indeno[1,2-b]indoles represent a class of potent ATP-competitive CK2 inhibitors. These substances have already exhibited promising results in in vitro experiments. Some indeno[1,2-b]indoles inhibit the protein kinase with IC50 values in the range of low nanomolar concentrations [3,4]. To further evaluate their suitability as anti-cancer drugs, indeno[1,2-b]indoles were investigated in cell culture experiments in this study. CX-4945, the first CK2 inhibitor in clinical trials (phase II) [5], was used as a reference.

We could show that three indeno[1,2-b]indoles had comparable effects to CX-4945 on the proliferation of different tumor cell lines. One of these indeno[1,2-b]indoles had a considerable lower effect on the proliferation of non-cancer cells than the other CK2 inhibitors. Furthermore, we could proof the ability of these substances to induce apoptosis in A431 squamous carcinoma cells using live cell imaging. In addition, indeno[1,2-b]indoles could be shown to reduce the migration of A549 lung cancer cells. To analyze whether the cellular effects were due to CK2 inhibition, the intracellular activity of the kinase in tumor cells after treatment with the inhibitors was determined. One indeno[1,2-b]indole reduced intracellular CK2 activity in the same dimension as CX-4945, while the other inhibitors were less effective. To ascertain reasons for differences and similarities of the substances with regard to their cellular effects, their pharmacokinetic properties were analyzed. Focusing on the cellular uptake, the intracellular inhibitor concentrations were determined via HPLC-MS/MS. CX-4945 exhibited lower intracellular concentrations than the other CK2 inhibitors tested. On the other hand, CX-4945 proved to be metabolically more stable compared to the indeno[1,2-b]indoles. The results of this study indicate that indeno[1,2-b]indoles, although still to be investigated in further experiments, are promising candidates for possible application in tumor therapy.

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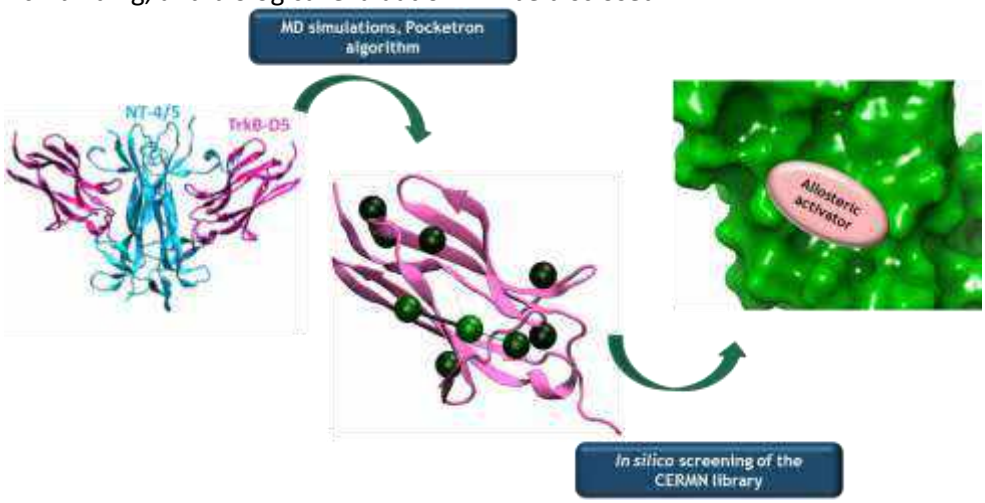
* Correspondence: robin.birus@uni-muenster.de

Discovery of an allosteric pocket network of the TrkB neurotrophin receptor, a promising target in the development of new ligands	*OC 14*
M. Antonijevic *, M. Benabderrahmane , R. Bureau, P. Dallemagne, C. Rochais *Normandie Univ., UNICAEN, CERMN, 14000 Caen, France*	

Numerous studies have been published about the implication of the neurotrophin tyrosine kinase receptor - TrkB in the pathogenesis of several neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis and motor neuron disease[a,b,c]. Brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) activate the TrkB receptor with high potency and specificity, promoting neuronal survival, differentiation and synaptic function[d]. Since natural ligands for TrkB receptor are large proteins, it is a challenge to discover small molecule capable to mimic their effects. Even though, the surface of receptor that is interacting with BDNF or NT-4/5 is known, there was always a question which pocket and interaction is responsible for activation of it. In order to answer this challenging question, we have used molecular dynamic (MD) simulations and Pocketron algorithm[e] which allowed us to detect pocket network existing in the interacting domain (D5) of the receptor. During this communication we will describe for the first time how pockets are communicating with each other as well as the exploitation of the allosteric pockets to identify potential modulator. *In silico* screening of CERMN library has recently generated a potential allosteric activator of the TrkB receptor which binding, and biological evaluation will be disclosed.



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* Correspondence: mirjana.antonijevic@unicaen.fr

This project has received funding from the European Union's Horizon 2020 framework programme for research and innovation under grant agreement No 765704.

In vivo photopharmacology of adrenergic receptors.

Davia Prischich [1,2], Alexandre M. J. Gomila [1,2], Santiago Milla-Navarro [3], Gemma Sangüesa [4,5], Rebeca Diez-Alarcia [6,7], Beatrice Preda [1], Carlo Matera [1,2], Montserrat Batlle [4,5], Laura Ramírez [3], Ernest Giralt [8,9], Jordi Hernando [10], Eduard Guasch [4,5], J. Javier Meana [6,7], Pedro de la Villa [3,11], Pau Gorostiza [1,2,12]*

(1) Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology, Barcelona (BIST), Spain. (2) Centro de Investigación Biomédica en Red – Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain. (3) Department of Systems Biology, University of Alcalá (UAH), Madrid, Spain. (4) Institut Clínic Cardiovascular, Hospital Clinic, University of Barcelona (UB), IDIBAPS, Barcelona, Spain. (5) Centro de Investigación Biomédica en Red – Enfermedades Cardiovasculares (CIBER-CV), Spain. (6) Department of Pharmacology, University of the Basque Country, Leioa, Bizkaia, Spain. (7) Centro de Investigación Biomédica en Red - Salud Mental (CIBER-SAM), Spain. (8) Department of Inorganic and Organic Chemistry, University of Barcelona, Barcelona, Spain. (9) Institute for Research in Biomedicine (IRB), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain. (10) Departament de Química, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. (11) Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain. (12) Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.

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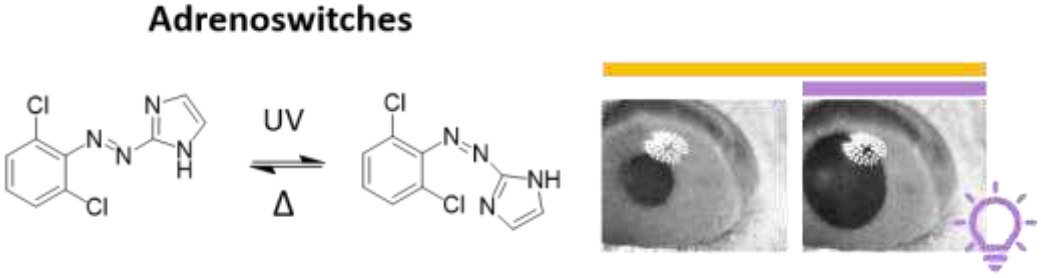
Adrenoceptors are ubiquitous and regulate most vital functions in the human body, including heart and respiratory rate, digestion, smooth-muscle contraction, gland secretion, and pupil diameter among others. In addition, adrenergic neurons firing from the locus coeruleus towards different areas of the central nervous system mediate alertness, responses to acute stress and danger, pain modulation, arousal, sleep-wake cycles, as well as neuroplasticity and cognitive behaviour. Despite the physiological relevance of adrenergic neurotransmission, molecular methods to precisely modulate the activity of endogenous adrenoceptor and to functionally dissect their pathways *in vivo* are not available.

Here we present a set of photochromic ligands, that we call adrenoswitches, to switch on and off adrenoceptor activity with high spatio-temporal resolution. Using a non-canonical azologization approach, we have designed novel arylazoheteroarene units that we have characterized *in vitro* and in two animal models (zebrafish locomotion and pupillary reflex in mice). The drug-like properties of these molecules, their efficacy and absence of acute toxicity in zebrafish larvae, and most remarkably the fact that specific adrenergic photomodulation was readily and reversibly achieved in the mammalian eye by topical application without formulation, all indicate that adrenoswitches could be a disruptive tool to dissect physiological adrenergic signalling and to develop safe and effective therapies. For example, photocontrol of adrenoceptors at specific locations might allow to single out individual adrenergic projections from the locus coeruleus, or to selectively decouple pupil tone from environmental illumination.



* Correspondance: pau@icrea.cat

An ionizable supramolecular dendrimer nanosystem for effective siRNA delivery with a favorable safety profile

Dinesh Dhumal[1]*, Wenjun Lan[1,2], Ling Ding [1,3], Yifan Jiang[1], Zhenbin Lyu[1], Laurini Erik[4], Marson Domenico[4], Tintaru Aura[5], Dusetti Nelson[2], Suzanne Giorgio[1], Juan Lucio Iovanna[2], Pricl Sabrina[4], Ling Peng[1]*.

[1] Aix-Marseille Univ, CNRS CINaM UMR 7325, Marseille, France.
[2] Aix-Marseille Univ, Inserm, CNRS, Institut Paoli-Calmettes, CRCM, Marseille, France
[3] Aix-Marseille Univ, CNRS, CRMBM, Marseille, France.
[4]Department of Engineering and Architectures, University of Trieste, Trieste, 34127, Italy
[5] Aix-Marseille Univ, CNRS, ICR, UMR7273, Marseille, France.

OC 16

Gene therapy involving small interfering RNA (siRNA) is emerging as a novel therapeutic approach for treating diseases.[a] However, safe and efficient delivery of siRNA therapeutics constitutes the major obstacles for its clinic implementation.[b] We report here an ionizable supramolecular dendrimer nanosystem, formed with a small amphiphilic dendrimer, was able to act as an effective vector for siRNA delivery with a favorable safety profile.[c] By virtue of the ionizable tertiary amine terminals, the supramolecular dendrimer had a low positively charged surface potential and was devoid of cytotoxicity at physiological pH. The ionizable feature imparted sufficient surface charge to the supramolecular dendrimer, allowing the formation of stable complex with siRNA via electrostatic interaction. The resulting siRNA/dendrimer delivery system had a surface charge that was neither neutral, thus avoiding aggregation, nor too high, thus avoiding cytotoxicity, but was sufficient for favorable cell uptake and endosomal release of siRNA. When tested in different cancer cell lines and patient-derived cancer organoids, this dendrimer-mediated siRNA delivery resulted in effective gene silencing of oncogenes with a potent ant proliferative effect, outperforming the gold standard vector, Lipofectamine 2000. This ionizable supramolecular dendrimer therefore represents a promising vector for siRNA delivery. The concept of supramolecular dendrimer nanovectors via self-assembly is new, yet easy to implement in practice,[d] offering a new perspective for supramolecular chemistry in biomedical applications.

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* Correspondence: E-mail, ling.peng@univ-amu.fr and dhumaldinesh212@gmail.com

Effect of naringenin and naringenin complexed with hydroxypropyl-β-cyclodextrin on neonatal sepsis. Role of COX-2, NFκB, MAPK pathways.	***OC 16***

Luana Heimfarth#, Jullyana de Souza Siqueira Quintans, Lucindo José Quintans- Junior

Health Sciences Graduate Program, Federal University of Sergipe, 49.060-100, Aracaju-SE, Brazil

Sepsis is a severe and dangerous clinical syndrome that results in elevate morbidity especially in intensive care units. It is caused by a dysregulation in host response to bacterial endotoxin. This uncontrolled activation of immune systems leads to systemic inflammatory response syndromes and multiple organ failure. The neonatal immune response could generate an uncompensated inflammatory response to LPS that affects the development of different systems, such as lung, heart and kidney. The available treatment for endotoxemia, mainly neonatal sepsis, are often few effective. One group with potential skills for treat neonatal inflammatory diseases are the flavonoids. Naringenin (NGN) is a flavonoid with anti-inflammatory, antioxidant, anti-nociceptive and anti-cancer effects. However, NGN has low water solubility, which affects its bioavailability. Thus, the present study investigates the effect of NGN pure and complexed with hydroxypropyl-β-cyclodextrin (HPβCD) on neonatal sepsis in a rodent model and describes the probable molecular mechanisms involved in NGN activities. We used a bacterial lipopolysaccharide (LPS) exposure to induce neonatal sepsis. Mice were divided in control, LPS, NGN (100 mg/kg, i.p.) and NGN/ HPβCD (100 mg/kg, i.p.) group and the viability was determined, and the inflammatory and oxidative parameters was assessed 4, 6 or 12 hours after LPS administration in lung, heart, kidney and cerebral cortex. It was found that NGN/ HPβCD increases the animals survival compared to LPS and NGN group. Moreover, NGN exposure during the neonatal period reduces leukocytes migration and decrease pro-inflammatory cytokines levels in lung, heart, kidney and cerebral cortex. In addition, NGN upregulated IL-10 production in lung and kidney of neonate's mice. The administration of NGN also enhanced antioxidants enzymes catalase and SOD activity and reduced the lipoperoxidation and protein carbonylation and increased the reduced sulfhydryl groups in an organ-dependent manner, attenuating the oxidative damage caused by LPS exposure. Finally, NGN and/or NGN/ HPβCD downregulated COX-2, ERK1/2 and NFκB activation. Therefore, NGN attenuated inflammatory and oxidative damage caused by neonatal sepsis. The complexation of NGN in HPβCD improve the bioavailable of the drug and consequently decrease the animal death. These results support the benefic role of NGN against neonatal sepsis and could be useful for achieving optimum effect of this flavonoid in neonatal inflammatory injuries. However, further studies are necessary due to the lack of information on potential toxicity.

*Corresponding Authors: luahei@yahoo.com.br).

Development of 5'-Nucleotidase inhibitors for cancer immunotherapy

Félix Grosjean [(1)] *, Pr. Christophe Mathé [(1)], Dr. Jean-Pierre Uttaro [(1)], Dr. Laurent Chaloin [(2)], Dr. Lars P. Jordheim [(3)] and Dr. Suzanne Peyrottes [(1)].

(1) Institut des Biomolecules Max Mousseron (IBMM), UMR 5247 CNRS, Université de Montpellier, ENSCM, Campus Triolet, cc1705, Place Eugene Bataillon, 34095 Montpellier (France)

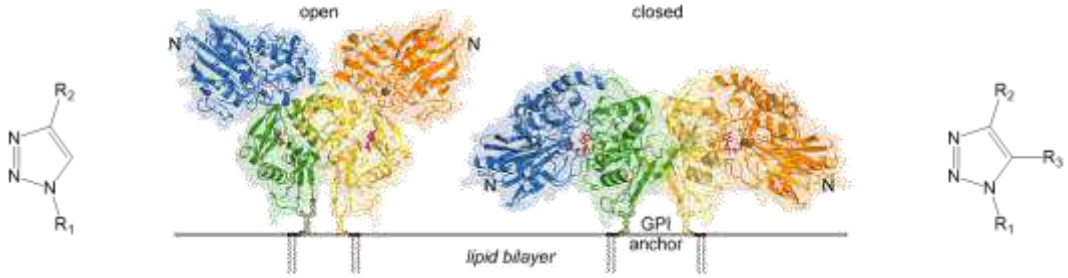
(2) Institut de Recherche en Infectiologie de Montpellier (IRIM), Université de Montpellier, CNRS, 34293 Montpellier, France

(3) Univ. Lyon, Université Claude Bernard Lyon 1, INSERM U-1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, 69008 Lyon, France.

OC 18

Ecto-5'-nucleotidase CD73 is a glycophosphatidylinositol-anchored di-Zn^{2+} metallophosphatase. This enzyme is involved in the extracellular catabolism of adenosine monophosphate (AMP) into adenosine in human blood and tissues.[1] Overexpression of this protein leads to an immunosuppressed and pro-angiogenic microenvironment inducing low efficiency of T-lymphocytes against tumor growth.[2] Thus, CD73 became a valuable target for cancer and immunotherapy and as already been tested with monoclonal antibodies, siRNA or adenosine analogues such as AMPCP[3].

By crystallizing this enzyme in the presence of Zn^{2+} and AMPCP, Knapp *et al*. found a dimerization cavity that distinguished two conformers of the protein, as shown in *scheme 1*.[4] *In silico* studies displayed the potential efficiency of small molecules in this dimerization interface to inhibit its activity by freezing the enzyme into one conformation.[5] In this communication will be presented the synthesis of potential allosteric inhibitors bearing a triazole scaffold, their biological evaluation on the recombined enzyme and MDA-MDB-231 cancer cells.



Scheme 1: Structure of CD73 as open and closed form

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* Correspondence: felix.grosjean@etu.umontpellier.fr

FLASH POSTER ABSTRACT

Drugs and mucus: an innovative mucus model to investigate the diffusion of drugs	FP 01

Cosmin Butnarasu (1)*, Daniela Pacheco (2), Paola Petrini (2), Livia Visai (3) Sonja Visentin(1).

(1) Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università degli Studi di Torino, 10125 Italia
(2) Dipartimento di Chimica, Materiali e Ingegneria Chimica, Politecnico di Milano, 20133 Italia
(2) Dipartimento di Medicina Molecolare, Università degli Studi di Pavia, 27100 Italia

A thin layer of mucus covers all the wet epithelia of the human body ensuring protection against exogenous compounds. Orally or pulmonary administered drugs have to overcome the mucosal layer in order to be absorbed and then to be effective. However, mucus can represent a strong barrier to overcome even for drugs (Figure 1). Despite the crucial role played by mucus on drug absorption, there are no standard mucus models to be employed in the early drug discovery process for the screening of potential drug candidates.

We have developed a biosimilar mucus model that mimics a pathological mucus[(a)]. The viscoelastic behavior of mucus was recreated by using a natural polysaccharide while the composition was mimicked by adding mucin, the main glycoprotein forming mucus. By coupling the biosimilar mucus model to the state-of-art permeability platforms (i.e. parallel artificial membrane permeability assay), the diffusion of some drugs through both mucus and cellular membrane, was investigated. The amount of diffused drug was quantified and reported as percent of total.

The herein presented mucus model represents not only a physical barrier but it really is an interactive filter. Respect to PAMPA test, different molecular structures have been differently retained by mucus reflecting the interaction with mucin[(b)].

Since the drug development is characterized by a high rate of failure, the mucus platform could help to reduce the number of non-effective drugs that reach the preclinical trials. Moreover, the model is completely tunable as the production method allows to easily include other molecules present within mucus (lipids, DNA, proteins).

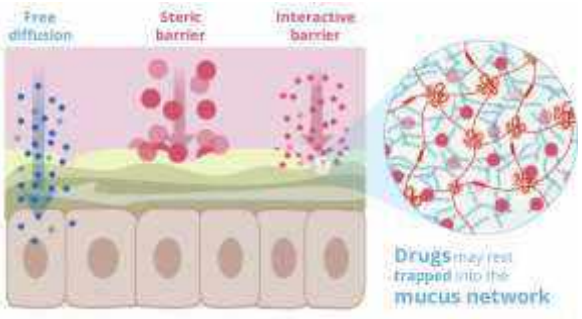

Figure 1. Mucus barriers reduces the absorption of drugs

Bibliographic references:

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* Correspondence: cosminstefan.butnarasu@unito.it

DRUGS AND MUCUS

AN INNOVATIVE MUCUS MODEL TO INVESTIGATE THE DIFFUSION OF DRUGS

Cosmin Butnarasu[a], Daniela Pacheco[b], Paola Petrini[b], Livia Visai[c], Sonja Visentin[a]

[a] Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università degli Studi di Torino, 10125 Italia
[b] Dipartimento di Chimica, Materiali e Ingegneria Chimica, Politecnico di Milano, 20133 Italia
[c] Dipartimento di Medicina Molecolare, Università degli studi di Pavia, 27100 Italia

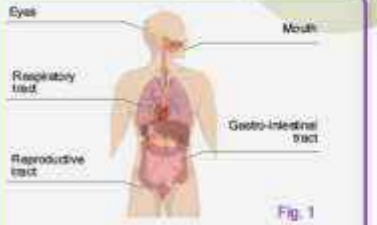


Fig. 1

Mucus distribution

Mucus covers all the wet tissues of the human body. Mucus is helping us staying healthy. It is a natural barrier (Fig 1).

Mucus barriers

Mucus is a selective barrier against pathogens however, it is an obstacle even for drugs orally administered.
Drugs may rest trapped into the mucus network (Fig 2).

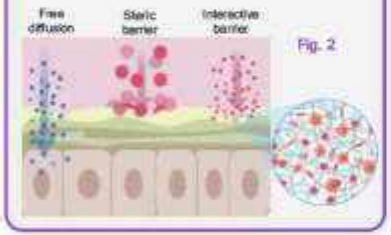


Fig. 2

The need

Up until now there are no standard protocols that model the passage of molecules through mucus.

Pharmaceutical companies need an *in vitro* screening mucus model in order to reduce the number of non effective drugs reaching preclinical trials.

Physico-chemical characteristics

The mucus model Mu^{3}Gel, produced by Bac^{3}Gel (www.bac3gel.com) reproduces the physico-chemical properties of cystic fibrosis mucus.

Rheological parameters such as the elastic (G') and the viscous (G") modulus of the biosimilar mucus are as similar as possible to the pathological mucus (Fig. 3).

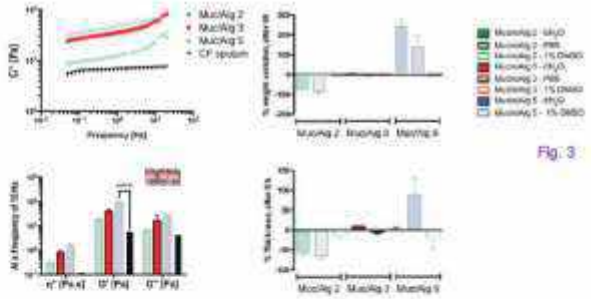


Fig. 3

Diffusion of drugs

The mucus model can be coupled to classic diffusion platforms (e.g. PAMPA, PermeaPad) for high throughput analysis.

The diffusion across the mucus model of different drugs was studied by means of PAMPA and compared with the diffusion rates in absence of mucus (Fig. 4).

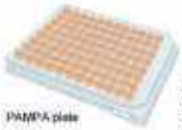
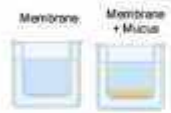
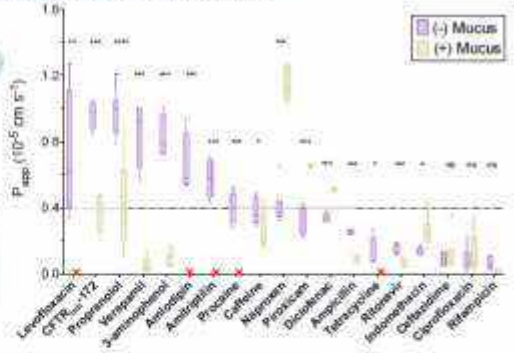


Fig. 4





Take home messages

- Mu^{3}Gel is fully tuneable. The production method allow to incorporate other mucus components
- Mu^{3}Gel reproduces the interactive and steric barrier of human mucus
- Mu^{3}Gel is cytocompatible
- Mu^{3}Gel can host different and competitive microorganisms
- Mu^{3}Gel is easy to use and easy to produce

Scan me

Design, pharmacomodulation, synthesis and biological evaluation of bioactive diarylmethanes

Ameni Hadj Mohamed [1] [2]*, Aline Pinon [3], Bertrand Liagre[3], Moncef Msaddek[2], Maité Sylla-Iyarreta Veitía[1].

(1) Equipe de Chimie Moléculaire, Laboratoire GBCM (EA 7528), Conservatoire national des arts et métiers, 2 rue Conté, 75003 Paris, HESAM Université, France.

(2) Laboratoire chimie hétérocyclique, produits naturels et réactivité, (LR11ES39), Faculté des Sciences de Monastir, avenue de l'environnement, 5000, Monastir, Tunisie.

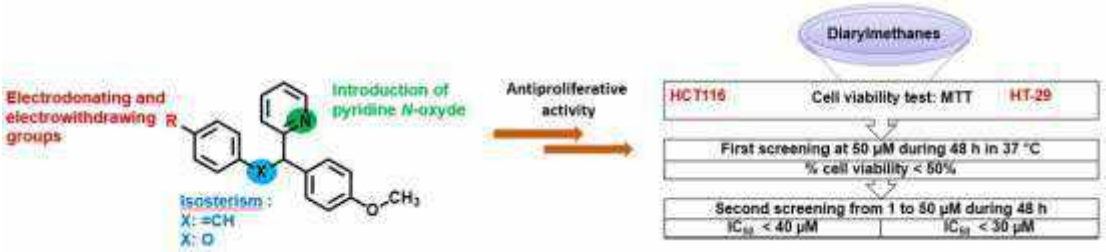
(3) Laboratoire PEIRENE (EA 7500), Faculté de Pharmacie de l'Université de Limoges, 2 rue de docteur Marcland, 87025, Limoges, France

FP 02

The process of drug discovery or lead optimization involves an efficient synthesis of bioactive molecules and the creation of chemical libraries. For this reason, rapid and efficient strategies for generation of new bioactive molecules are essential due to the emergence for the creation of new drugs. Thereof, many strategies are currently used in medicinal chemistry to generate new interesting lead compounds such as selective optimization of side activities or the development of analogues of existing drugs (SOSA approach).[a-c]

Diarylmethane molecules are described in the literature as privileged structures in medicinal chemistry. They have attracted much attention due to their biological and therapeutic relevancy in several areas such as antimicrobials, anti-inflammatory and anticancer. [d-e]

We presented herein the design, pharmacomodulation and synthesis of two heterocyclic series of diarylmethanes: olefinic and aryloxy diarylmethanes. The anticancer activity as well as cytotoxicity were studied *in vitro*.The best compounds showed an interesting antiproliferative activity against colorectal cell lines HT-29 and HCT116 with IC_{50} in the range of 20-40 µM. The prediction of the ADME profile of the best molecules was also performed. In conclusion, we demonstrated in this study the interesting antiproliferative potential of these compounds for the treatment of colorectal cancer and the absence of cytotoxicity on normal cells.



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*Correspondence: amenihajmohamed@gmail.com, maite.sylla@lecnam.net

Design, pharmacomodulation, synthesis and biological evaluation of bioactive diarylmethanes

Ameni Hadj Mohamed,[1,2] Aline Pinon,[3] Bertrand Liagre,[3] Moncef Msaddek,[2] Maité Sylla-Iyarreta Veitia[1]

[1] *Equipe de Chimie Moléculaire du Laboratoire de Génomique Bioinformatique et Chimie Moléculaire, GBCM, EA7528, Conservatoire national des arts et métiers, 2 rue Conté, 75003 Paris, France, HESAM Université.*

[2] *Laboratoire chimie hétérocyclique, produits naturels et réactivité, (LR11ES39), Faculté des Sciences de Monastir, avenue de l'environnement, 5000, Monastir, Tunisie.*

[3] *Laboratoire PEIRENE (EA 7500), Faculté de Pharmacie de l'Université de Limoges, 2 rue du docteur Marcland, 87025, Limoges, France.*

Background [1-4]

Anticancer

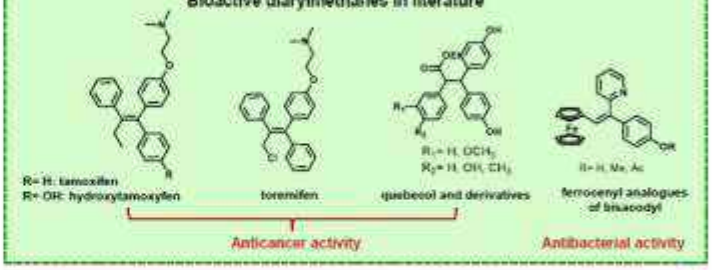
Antibacterial

Anti-inflammatory

Diarylmethanes

Privileged structures

Bioactive diarylmethanes in literature

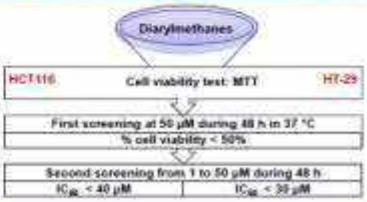


Objectives

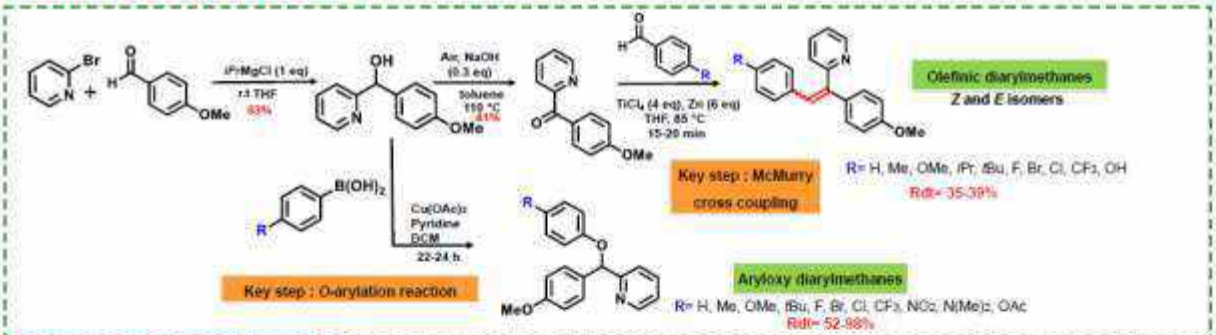
Design, synthesis and pharmacomodulation

Biological evaluation : Antiproliferative activity on human colorectal cancer cell lines

The antiproliferative activity of two series of diarylmethanes was evaluated on human colorectal cancer cell lines : HT-29 and HCT116 using MTT test.



Chemical strategy and results [5]

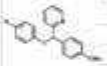


Structure activity relationships [5]

Structure	R	Isomer	IC_{50} ± SEM (µM) HT-29	IC_{50} ± SEM (µM) HCT116
	H	Z	30.34 ± 1.78	35.56 ± 2.06
		E	> 50	> 50
	Me	Z	35.00 ± 1.99	> 50
		E	n.d.	> 50
	OMe	E	n.d.	> 50
	Cl	Z	28.48 ± 1.82	42.69 ± 2.24
		E	> 50	> 50
	Br	Z	24.03 ± 2.03	33.67 ± 0.21
		E	> 50	26.13 ± 2.06
	F	Z	43.43 ± 0.31	> 50
		E	> 50	> 50
	CF_3	Z	23.02 ± 1.27	31.44 ± 1.74
		E	> 50	> 50
	OH	E	> 50	> 50
	$C(CH_3)_3$	Z	25.70 ± 1.21	> 50
		E	25.15 ± 1.29	> 50
	$CH(CH_3)_2$	Z	> 50	> 50
		E	34.98 ± 0.78	> 50

Compounds containing halogens and bulky groups are the most active

No influence of introduction *N*-oxyde pyridine on antiproliferative activity

Structure	R	IC_{50} ± SEM (µM) HT-29	IC_{50} ± SEM (µM) HCT116
	H	> 50	> 50
	Me	> 50	> 50
	OMe	> 50	> 50
	$C(CH_3)_3$	> 50	> 50
	Cl	> 50	> 50
	Br	37.96 ± 1.87	41.51 ± 1.48
	F	> 50	> 50
	CF_3	> 50	> 50
	NO_2	> 50	> 50
	$N(CH_3)_2$	> 50	> 50
	Ac	> 50	> 50

X: =CH

Olefinic diarylmethanes

Generally *Z* isomers are more active than *E* isomers.

CF_3 > Br > tBu > Cl

IC_{50} < 25 µM (HT-29, HCT116)

X: O

Aryloxy diarylmethanes

Brominated compound is active on HT-29 and HCT 116 cell lines.

R=Br

HT-29 : IC_{50}= 37 µM

HCT116 : IC_{50}= 41 µM

Olefinic diarylmethanes are more active than aryloxy diarylmethanes. All compounds are more active on HT-29 than HCT116 cell lines.

Conclusions

- **2** isosteric series of diarylmethanes was synthetized as a potential anticancer compounds.
- **19** olefinic diarylmethanes were straightforwardly synthesized using McMurry cross coupling reaction.
- **11** aryloxy diarylmethanes was synthesized with good yields using *O*-arylation reaction.
- Olefinic diarylmethanes and especially *Z* isomers showed an interesting antiproliferative activity on human colorectal cancer cell lines with IC_{50}<25 µM.
- *In vitro* experiments are in progress in order to determine the mechanism of action of these compounds.

(1) Guerineau, Sylla-Iyarreta Veitia M., Trigo F., El Aroi, Ferroud, C. J. Organomet. Chem. 794, 2015, 274-281.

(2) Gauthier, S.; Vostrikl, J.; Labrie, F. J. Org. Chem. 61 (11), 1996, 3646–3653.

(3) Vakkilainen, R., Pyrkilinen, S., Heikkinen, M., Rissanen, P. Eur. J. Cancer Clin. Oncol. 24 (4), 1988, 785–790.

(4) Pedireddy, K., Shinde, A. N., Kanneganti Rao, V., Thati, R. K., Cappoli, N. Bioorg. Med. Chem. Lett. 23 (19), 2013, 5329–5331.

(5) Hadj Mohamed, A., Pinon, A., Mouhsine, H., Liagre, B., Msaddek, M., Ferroud, C., Sylla-Iyarreta Veitia, M. Publication in progress.

SCT's Thiverny Research Fellows Meeting 28th 2021

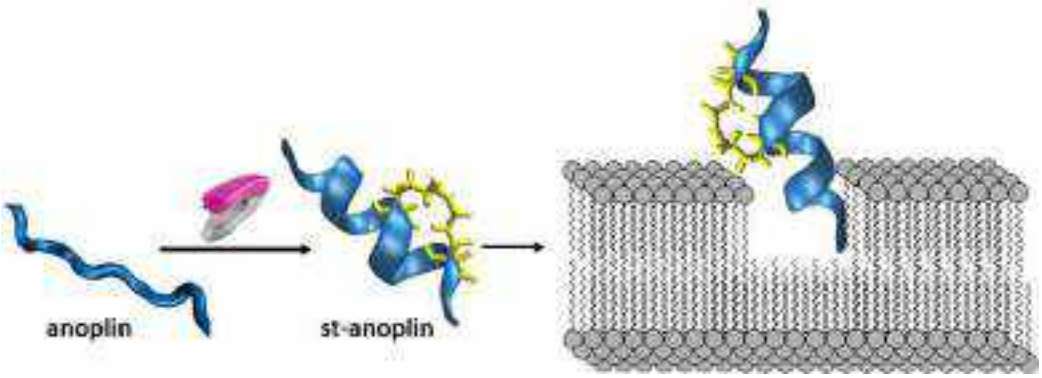
Modifications of anoplin that stabilize its α-helical structure and enhance antibacterial activity

Julia Macyszyn *, Joanna Trylska, Monika Wojciechowska

Center of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

FP 03

Significant increase in drug resistance of microorganisms contributes to the intense searching for new antibiotics. Antimicrobial peptides (AMP) are promising candidates for combating multi-drug resistance bacteria. Anoplin is a linear AMP of natural origin, isolated from the venom of the spider wasp *Anoplius samariensis* [(a)]. Anoplin (GLLKRIKTLL-NH_2) in water shows a disordered structure while after binding to the bacterial cell membrane it adopts an α-helix [(b)]**.** We assume that this helical structure is the active structure of this peptide. The aim of the project is to modify anoplin to stabilize its α-helical structure to possibly increase its antibacterial activity (Scheme 1).



Scheme1. *Representation of a linear and helical structure of anoplin. The structure of the α-helix is stabilized by hydrocarbon stapling link (in yellow), which probably increases anoplin penetration through the bacterial membrane.*

Increasing the stability of the helical structure of anoplin was achieved by metathesis reaction with the use of 1st generation Grubbs catalyst [(c)]. The reaction of hydrocarbon stapling of the peptide was performed between two olefinic side chains of non-natural amino acids (*S*-1-(4'-pentene)alanine) incorporated at position 2 instead of Leu and at position 6 instead of Ile of the anoplin sequence. The stability of the secondary structure of the unmodified and modified anoplin was determined in the presence of various lipid environments (micelles, liposomes, lipopolysaccharides) using the circular dichroism (CD) spectroscopy. Additionally, in order to investigate the correlation between the stability of the secondary structure of anoplin and its antibacterial activity, liposome degradation and activity against the *Escherichia coli* were tested confirming our hypothesis.

Acknowledgements: This work was supported by National Science Cent***re*** *(Sonata 15, 2019/35/D/NZ1/01957)*

Bibliographic references:

(a) Konno, K. *et al.. Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol.* **1550**, 70–80 (2001).
(b) Uggerhøj, L. E. *et al. ChemBioChem* **16**, 242–253 (2015).
(c) Mourtada, R. *et al. Nat. Biotechnol.* **37**, 1186–1197 (2019).

* j.macyszyn@cent.uw.edu.pl

Modifications of anoplin that stabilize its α-helical structure and enhance antibacterial activity

Julia Macyszyn, Joanna Trylska, Monika Wojciechowska

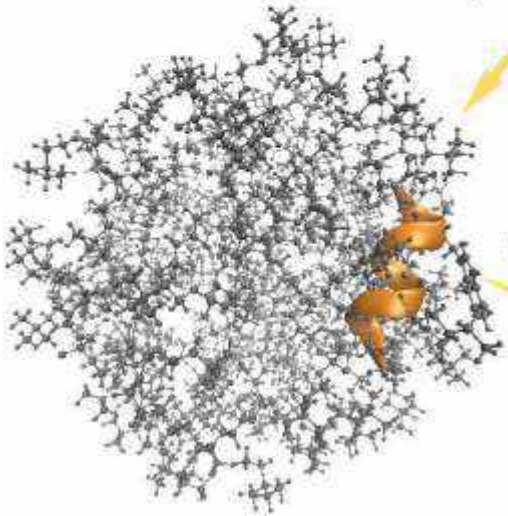
Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

j.macyszyn@cent.uw.edu.pl

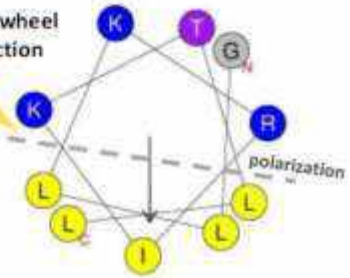
Anoplius samariensis

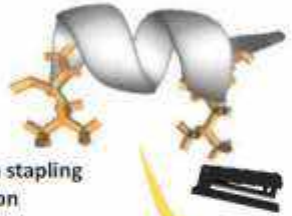
Anoplin

Interaction with micelle



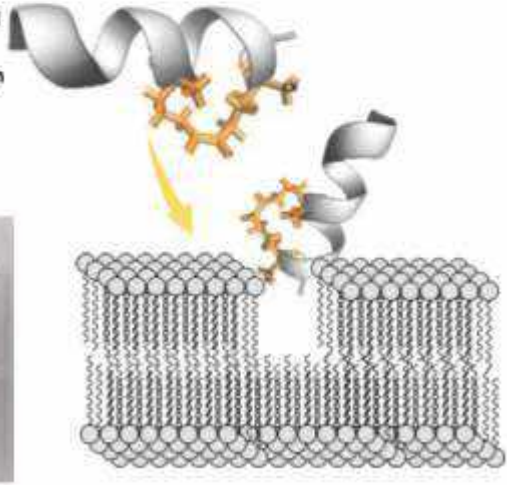
Helical wheel projection



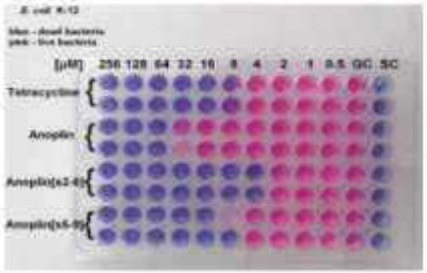


Hydrocarbon stapling reaction

Representation of helical structure of anoplin stabilized by hydrocarbon stapling link



Results of the Minimum Inhibitor Concentration

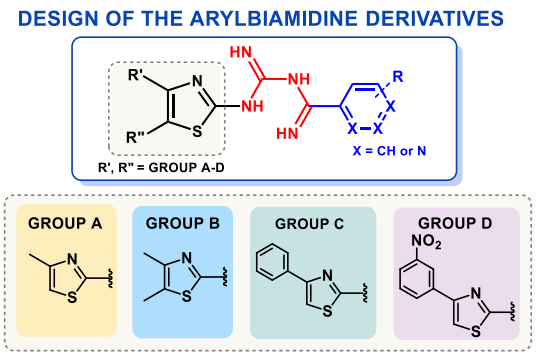


Insights into the characterization of arylbiamidines function with therapeutic potential	***FP 04***

Rostyslav Bardovskyi [(1)] *, Oleksandr Grytsai [(1)], Leticia Christina Pires Gonçalves[(1)], Nedra Hamouda-Tekaya [(2)], Stéphane Rocchi [(2)], Cyril Ronco [(1)] * and Rachid Benhida [(1)] *

(1) Institut de Chimie de Nice CRNS UMR7272, Université Côte d'Azur, Nice, France.
(2) Centre Méditerranéen de Médecine Moléculaire (C3M), INSERM, U1065, Université Côte d'Azur, Nice, France.

An uncommon chemical group - arylbiamidine – was studied through the synthesis of four novel series of heteroaryl derivatives. Thiazolebiamidine was chosen as scaffold due to a number of H-bond donating and accepting possibilities - a key point for the interaction with biomolecules and the apparent hydrosolubility properties of the biamidine function. We synthesized a new family of 37 thiazole-based diarylbiamidine derivatives and characterized it by X-ray crystallography, spectroscopic methods, pK_a and stability determinations. The analysis revealed particular tautomeric and H-bonding structure. Their antimelanoma activity highlights the promising druglike features of this unfamous scaffold.



Bibliographic references:

* Correspondence: Cyril.RONCO@univ-cotedazur.fr
Rachid.BENHIDA@univ-cotedazur.fr
Rostyslav.BARDOVSKYI@univ-cotedazur.fr

Insights into the characterization of arylbiamidine function with therapeutic potential

Rostyslav Bardovskyi,[a] Oleksandr Grytsai,[a] Leticia Christina Pires Gonçalves,[a] Nedra Hamouda-Tekaya,[b] Stéphane Rocchi,[b] Cyril Ronco *[a] and Rachid Benhida *[a]

[a] Institut de Chimie de Nice CRNS UMR7272, Université Côte d'Azur, Nice, France.

[b] Centre Méditerranéen de Médecine Moléculaire (C3M), INSERM, U1065, Université Côte d'Azur, Nice, France.

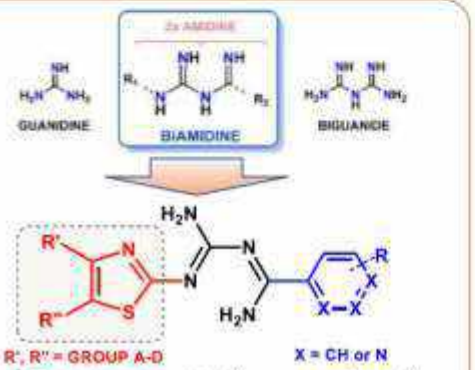


Figure 1. Chemical structure of biamidine, compared to guanidine, and biguanide.

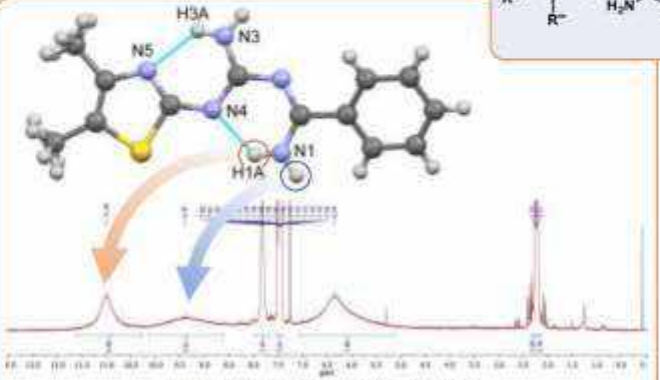
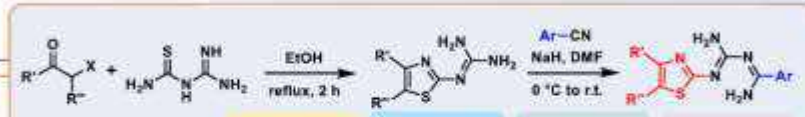


Figure 2. X-Ray structure of **B1** and ^{1}H NMR.



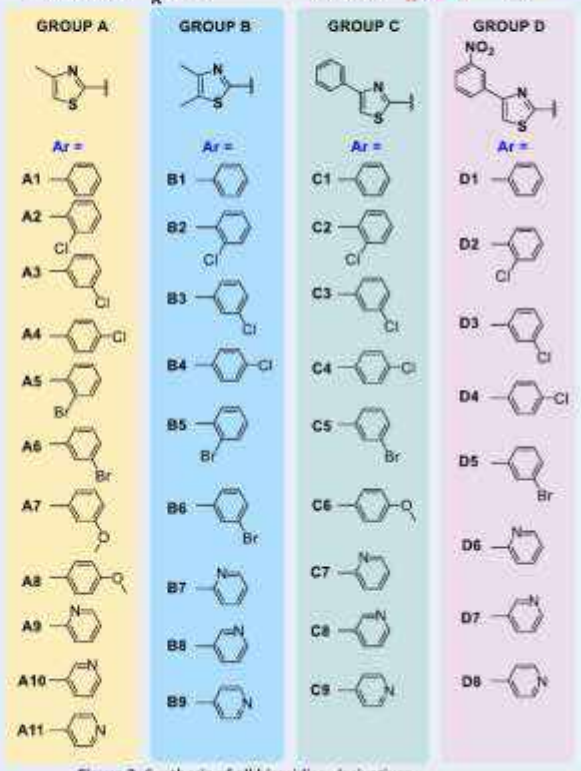


Figure 3. Synthesis of all biamidine derivatives.

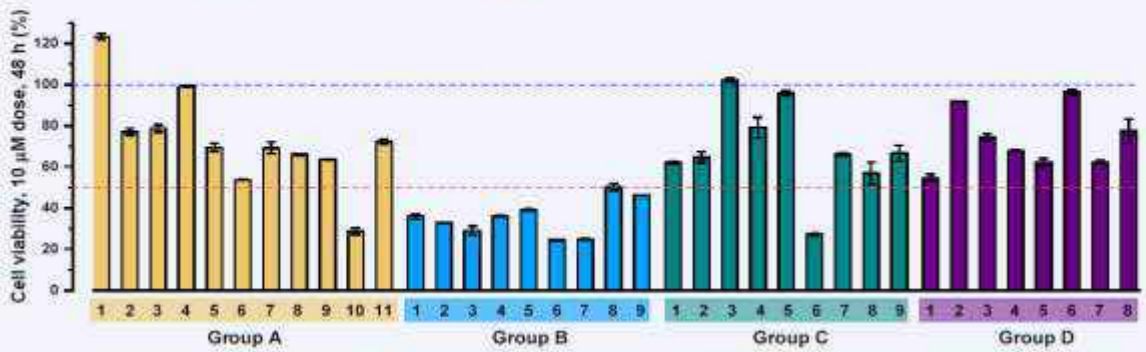


Figure 4. Cell viability of A375 melanoma cells relative to DMSO upon incubation at 10 µM after 48 h incubation. Red trace indicates EC50. N=3 ± SD.

1,10-Phenanthroline Derivative as Competent Nondoped Blue Fluorescent OLEDs with enhanced Quantum Efficiency of 28.4% at 1000 cd m^{-2}	***FP 05***

E.H. Edinsha Gladis[1] and K. Nagashri[2*]

[1]Research Scholar, Dept. of Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627012, Tamil Nadu, India.
[2]Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627012, Tamil Nadu, India.

Efficiency is crucial for organic light emitting diodes (OLEDs) to be energy-saving and to have a long lifetime for display and solid state lighting applications. Numerous approaches have been proposed to attain high efficiency OLEDs through the synthesis of novel organic materials, the design of light extraction structures and the design of efficiency-effective device architectures. Therefore, developing competent blue-emitting materials suitable for nondoped device is of pronounced significance in terms of practical utilities. In the present investigations was focused on the 1,10-Phenanthroline derivative may attain high efficiency at greater luminescence in nondoped OLEDs. The maximum external quantum efficiency (EQE) of nondoped device is 15.20% which is acquired at the luminescence of 1000 cd m^{-2}, maximum external quantum efficiency (EQE) of 28.4%. Our research outcomes offers a new perception into the design and architecture of high-efficiency blue-light OLEDs with modified device structure for practical utilities.

Reference:

1. Vilas Venunath Patil, Kyung Hyung Lee and Jun Yeob Lee, Dibenzo[c,g]indolo[3,2,1-jk]carbazole as a new chromophore for blue organic light-emitting diodes, J. Mater. Chem. C, 2019, 7, 14301. DOI: 10.1039/C9TC04783B.

E-mail: shrik1810@gmail.com

Discovery of new inhibitors of the SARS-CoV-2 3CL protease by High Throughput Screening.	***FP 06***

Lucile Brier [(1)]*, Valérie Landry [(1)], Julie Dumont [(1)], Sandrine Warenghem [(1)], Adrien Herledan [(1)], Xavier Hanoulle [(2)], Sandrine Belouzard [(3)], Jean Dubuisson [(3)], Florence Leroux [(1)], Benoit Deprez [(1)], Julie Charton [(1)].

(1) Université de Lille, INSERM, Institut Pasteur de Lille, U1177 - Drugs and Molecules for Living Systems, F-59000 Lille, France.
(2) ERL 9002 CNRS, U1167 INSERM, Institut Pasteur de Lille, Université de Lille, F-59000 Lille, France.
(3) Université de Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019-UMR 8204-CIIL-Center for Infection and Immunity of Lille, F-59000 Lille, France.

Coronaviruses are single-stranded RNA viruses that cause respiratory, enteric, hepatic and neurological diseases of varying severity in different species including humans. Seven coronaviruses can infect humans. Strains HCoV-OC43, HCoV-NL63, HCoV-229E and HCoV-HKU1 lead to mild respiratory tract infections. However SARS-CoV, MERS-CoV and SARS-CoV-2 are potentially lethal and resulted in major pandemics. As a matter of fact, these viruses cause also respiratory tract infections but some patients develop serious symptoms like severe acute respiratory syndrome which is life-threatening. The lack of specific antiviral treatment against coronaviruses and the risk of emergence of new viruses of this family require research for new effective therapeutics.

To discover new antiviral compounds against coronaviruses we decided to target an essential component of these viruses: the 3CL protease [(a,b)]. We will present here the High Throughput Screening of a 100000 compounds library on the 3CL protease of SARS-CoV-2 which led to the identification of new inhibitors that entered a hit-to-lead optimization phase.

Bibliographic references:

[(a)] Ullrich, S. & Nitsche, C. The SARS-CoV-2 main protease as drug target. *Bioorg. Med. Chem. Lett.* 30, 127377 (2020).

[(b)] Liu, Y. *et al.* The development of Coronavirus 3C-Like protease (3CLpro) inhibitors from 2010 to 2020. *Eur. J. Med. Chem.* 206, 112711 (2020).

* Correspondence: lucile.brier@univ-lille.fr

Discovery of new inhibitors of the SARS-CoV-2 3CL protease by High Throughput Screening

28th Young Research Fellows Meeting of SCT
February 11-12, 2021 - Paris

Lucile Bidet (1), Valérie Landry (1), Fanny Bourgeois (1), Sarah Benhabiles (1), Julie Dumont (1), Sandrine Warenghem (1), Adrien Herledan (1), Xavier Hanoulle (2), Sandrine Belouzard (3), Jean Dubuisson (3), Florence Leroux (1), Benoit Deprez (1), Julie Charton (1)*

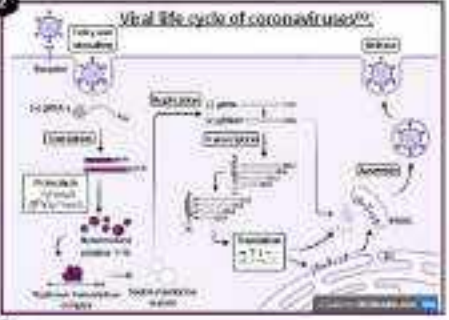
(1) Université de Lille, INSERM, Institut Pasteur de Lille, U1177 - Drugs and Molecules for Living Systems, F-59000 Lille, France. (2) EA 7002 ONFR, U1167 INSERM, Institut Pasteur de Lille, Université de Lille, F-59800 Lille, France. (3) Université de Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019-UMR 9004-CIIL-Center for Infection and Immunity of Lille, F-59000 Lille, France.

1

Coronaviruses are single-stranded RNA viruses that cause respiratory, enteric, hepatic and neurological diseases of varying severity in different species including humans. Seven coronaviruses can infect humans. Strains HCoV-OC43, HCoV-NL63, HCoV-229E and HCoV-HKU1 lead to mild respiratory tract infections. However SARS-CoV, MERS-CoV and SARS-CoV-2 are potentially lethal and resulted in major pandemics. As a matter of fact, these viruses cause also respiratory tract infections but some patients develop serious symptoms like severe acute respiratory syndrome which is life-threatening. The lack of specific antiviral treatment against coronaviruses and the risk of emergence of new viruses of this family require research for new effective therapeutics. To discover new antiviral compounds against coronaviruses we decided to target an essential component of these viruses conserved among this virus family: the 3CL protease[1,2]. A High Throughput Screening of a 90 000 compounds library on the 3CL protease of SARS-CoV-2 led to the identification of new inhibitors that entered a hit-to-lead optimization phase.

2

Viral life cycle of coronaviruses[1,2]:

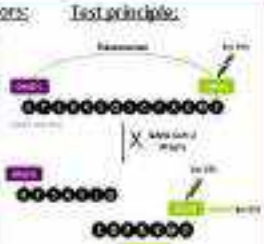


3

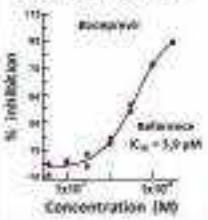
HTS for the identification of SARS-CoV-2 3CL^{pro} inhibitors:

Test principle:

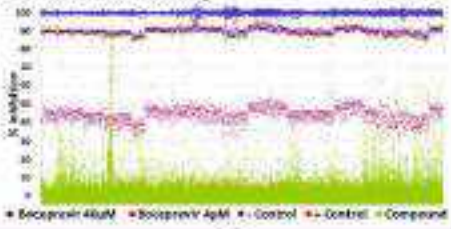
A semi-automated enzymatic FRET-based assay was optimized and miniaturized in 384-well plates for HTS on the ARIADNE-screening platform (UMS2014-US41). The assay was conducted at room temperature using a reaction volume of 20 µL and a buffer containing 50 mM HEPES, 0.1 mg/mL BSA, 0.01% TRITON and 2 mM GSH at pH 7.4. A library of 90 000 compounds stored at 10 mM stock solutions in DMSO was dispensed with an Echo Liquid Handler (Labcyte). The native enzyme, produced in high yield and purity, was prepared in assay buffer (25 nM final concentration), added to the tested compounds (10 µM) and preincubated for 30 min. Enzyme reaction was initiated with 10 µL of FRET-based substrate dissolved in assay buffer (20 µM final concentration). After 30 min, the fluorescence intensity was monitored with a Victor V2 multilabel (PerkinElmer) using excitation and emission wavelengths of 320(20) nm and 405(10) nm.



Dose-response curve:



Overview of the screening:



4

Screening cascade:

Dose-response curves were done in duplicate with 7 concentrations using the same assay conditions as the primary screen, except for the preincubation period fixed at 60 min. At the same time, an enzyme emission assay enabled the identification of compounds interfering with the fluorescence. These compounds were then tested in a kinetic assay, with a continuous fluorescence reading during the incubation period.

A library of 2 560 fragments was also tested at 200 µM. Among them, 33 showed an enzyme inhibition greater than 50%. 6 hits were confirmed with K_{ia} between 27 and 154 µM.

5

Perspectives:

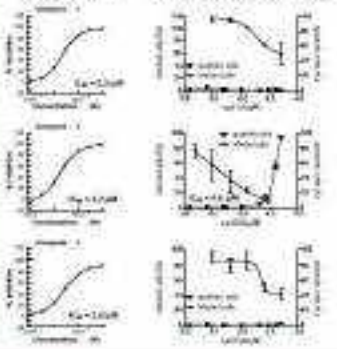
After the screening phase, a selection among the 41 hits has been made based on the structure and potency to enter an optimization phase. The objective of this medicinal chemistry program is the development of broad-spectrum 3CL protease inhibitors with physicochemical, pharmacokinetic and pharmacodynamic characteristics suitable for proof-of-concept in animal models and a future use in humans.

6

In vitro preliminary results:

SARS-CoV-2 3CL^{pro} inhibition assay:

Cytotoxicity assay and antiviral activity on HCoV-229E:



SARS-CoV-2 3CL^{pro} enzymatic assay:
Dose-response curves were obtained as described above using the kinetic assay.

High-Content screening apoptosis assay:
The High-Content screening (HCS) performed on the platform ARIADNE, Lille), allows the rapid detection and quantification of apoptotic Huh 7 cells by detection of Caspase-3/7 activity in real-time imaging using Nucview™ 488 substrate containing peptide sequence DEVD attached to a nucleic acid dye.

hCoV-229E antiviral assay:
In cellulo (Huh-7 cells) antiviral activity on the human coronavirus 229E was assessed using recombinant hCoV-229E expressing the *Renilla luciferase* (*Rluc*) reporter gene. *Rluc* activity was measured using the Renilla-Glo luciferase assay system (Promega).

6

Coronavirus 3CL protease is a promising therapeutic target as it is an essential component for the viral life cycle that is conserved among coronaviruses. Here, we report the development and the High Throughput Screening of a library of more than 90 000 compounds that allowed the identification of several chemical series of reversible and irreversible 3CLpro inhibitors. That represent promising starting points for further development of therapeutics against SARS-CoV-2 and new emerging coronaviruses.

[1] Anand, K. & Ziebuhr, J. The SARS-CoV-2 main protease as drug target. Bioorg. Med. Chem. Lett. 30, 127377 (2020). [2] Liu, Y. et al. The development of Coronavirus 3C-like protease (3CLpro) inhibitors from 2010 to 2020. Eur. J. Med. Chem. 206, 112703 (2020).

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Synthesis and anticandidosic activities of some imidazo[1,2-*a*]pyridines with arylacrylonitrile pharmacophore

Déto U. Jean-Paul N'guessan #1(1)*, Songuigama Coulibaly #2(1), Fulgence Kassi K. #3(2), Pierre-Olivier Delaye #4 (3), Enguehard-Gueiffier Cécile #5 (3), Mahama Ouattara #6 (1)

(1) Département de Chimie Thérapeutique et Chimie Organique, UFR Sciences Pharmaceutiques et Biologiques, Université FHB, 01 BP V34 Abidjan, Côte d'Ivoire. (2) Département de Parasitologie-Mycologie-Zoologie, UFR Sciences Pharmaceutiques et Biologiques, Université FHB, 01 BP V34 Abidjan, Côte d'Ivoire. (3) Laboratoire de chimie thérapeutique, UFR Sciences pharmaceutiques, Université de Tours, 31 avenue Monge, 37200 Tours, France

FP 07

Candidiasis cause superficial and visceral infections which can become a significant concern to clinicians (a). With the increased use of fluconazole, azole-resistant fungi as well as non-*albicans* Candida species rapidly emerged as common pathogens in immunocompromised patients(b). It is therefore necessary to search for new molecules that will be able to bypass resistance mechanisms. Thus, we are interested in imidazo[1,2-*a*]pyridine derivatives containing an acrylonitrile group and their acrylamide analogues. The objective of this work was to synthesize and evaluate the antifungal activities of some imidazo [1,2-*a*] pyridine hybrids against three Candida species in order to identify an anticandidosis leader.

The acrylonitrile derivatives were obtained by chemical synthesis following a Knoevenagel condensation reaction between imidazo[1,2-*a*]pyridin-2-carboxaldehyde and various phenylacetonitriles or 2-cyano-*N*-alkylacetamides. An antifungal screening of these derivatives on clinical strains of the three species of Candida (*C. albicans*, *C. tropicalis* and *C. glabrata*) was carried out by the bioautography method then the Minimum Inhibitory Concentrations (MIC) were determined according to the technique microplate dilution compared to fluconazole.

Six imidazo[1,2-*a*]pyridinyl phenylacrylonitrile or acrylamide derivatives were synthesized and characterized by spectroscopic methods. The antifungal results show that these are all endowed with anticandidosis activities with MICs ranging from 408 to 0.52 µM. On *C. albicans*, with a MIC of 2.79 µM, the 3-chlorinated derivative was found to be the most effective. On this species, this compound was 100 times more effective than fluconazole (MIC = 326.5 µM). Likewise, the best performance on *C tropicalis* was obtained with the piperazine derivative with a MIC of 0.52 µM. This is comparable to the activity of fluconazole against *C. tropicalis* (MIC = 0.64 µM). On *Candida glabrata*, with MICs of between 264.6 and 408 µM, the antifungal activities of the six imidazo[1,2-*a*]pyridine derivatives remain low compared to that of fluconazole (MIC = 20.41 µM). Ultimately, the best anticandidosis profile on the three species was obtained with the 3-chlorinated compound, the MICs of which varied between 357.5 and 1.4 µM.

Our pharmacochemical approach validated the acrylonitrile chain carried by the heterocycle imidazo [1,2-*a*] pyridine as a new antifungal pharmacophore against *C. albicans* resistant to Fluconazole..

Keywords: Antifungal. Imidazo[1,2-*a*]pyridine. acrylonitriles. *Candida*

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* Correspondence: E-mail : *jacque.nguessan@univ-fhb.edu.ci*

Synthesis and anticandidosis activities some imidazo[1,2-*a*]pyridines with arylacrylonitrile pharmacophore

Déto U. Jean-Paul N'guessan[1], Songrigama Coulibaly[1], Pulgence Kassi K.[2], Pierre-Olivier Delaye[3], Enguehard-Gueiffier Cécile[3], Mahama Ouattara[1*]

1 Department of Therapeutic Chemistry and Organic Chemistry, UFR Pharmaceutical and Biological Sciences, FHB University, 01 BP V34 Abidjan, Côte d'Ivoire
2 Department of Parasitology-Mycology-Zoology, UFR Pharmaceutical and Biological Sciences, FHB University, 01 BP V34 Abidjan, Côte d'Ivoire
3 Laboratory of therapeutic chemistry, UFR Pharmaceutical Sciences, University of Tours, 31 Avenue Monge, 37200 Tours, France
*Contact person: Ouattara, Mahama * 01 BP V34 Abidjan, UFR SPB, University FHB, Ivory Coast, Tel 00 74 10 23; Email: mahama.ouattara@univ-fhb.edu.ci*

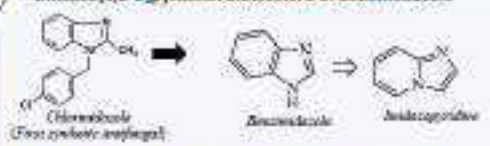
INTRODUCTION AND OBJECTIFS

The increase of immunodeficiency situations such as HIV and Cancer, is at the origin of the expansion of fungal infections due to the genus *Candida* . Although *Candida albicans* remains the most widespread species in pathogenic isolates, its epidemiological impact in human infectiology has declined in favor of new emerging species of *Candida* refractory to conventional treatment.
In addition, drug treatment of these candidiasis has become a public health problem since the misuse of antifungals has contributed to the proliferation and emergence of drug- resistant strains of *Candida* .
Faced with this situation, we decided to contribute to the development of some Imidazo[1,2-*a*]pyridinyl-arylacrylonitriles, as potential new anticandidosics.
Therefore, the objective assigned to this work is to identify a molecular hit likely to be developed as a drug candidate.
Specifically, for us it will be a question of determining the minimum inhibitory concentrations of Imidazo[1,2-*a*]pyridinyl-arylacrylonitriles with respect to *Candida albicans*, *Candida glabrata* and *Candida tropicalis*;
Then to establish the structural elements favorable to the induction, maintenance or even improvement of said anticandidosis activities.

Material and methods

Conceptual origin

✓ **Imidazo[1,2-*a*]pyridine: Bioisostere of benzimidazole**



✓ **Phenylacrylonitrile: isostere of chalcones phenylpropenone**

Anti-infectious properties: antiparasitic, antibacterial, antifungal

1. CHEMISTRY

The imidazo[1,2- *a*]pyridinyl phenylacrylonitrile derivatives and analogs were synthesized following a Knoevenagel condensation reaction between imidazo[1,2- *a*] pyridin-2-carboxaldehyde and various phenylacetonitriles or 2-cyano- *N* - alkylacetamides. They were characterized by the usual spectroscopic methods

2. BIOLOGIE

Material

- Products tested: 6-Imidazo[1,2-*a*]pyridinyl-arylacrylonitriles derivatives
- Reference substance: FLUconazole
- Fungal species: : *Candida (C. albicans, C. glabrata, C. tropicalis)*

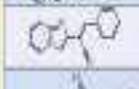
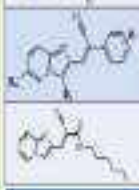
Methods

- Bioautography: Antifungal screening method
- Microplate dilution: In vitro method for determining MICs

1. Preparation of stock solutions of the products to be tested (1 mg in 1 ml of DMSO)
2. Production of a range of product concentration by successive dilution in RPS containing the fungal inoculum in a 96-well microplate
3. Incubation of the titration microplates at 30 ° C for 48h
4. Reveal by color appreciation after addition of MTT

Determination with the naked eye of the smallest concentration which inhibits any growth of *Candida*

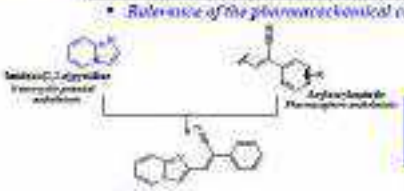
Results -Discussion

Structure	Compound	R	R_1	R_2	Candida albicans (µM)	Candida Tropicalis (µM)	Candida glabrata (µM)
	1				6,4	3,2	408,8
	2				408,8	3,2	408,8
	3	4-Cl	H	H	178,7	1,6	357,5
	4	4-Cl	H		66,2	1,6	264,8
	5	H	Cl	H	2,8	0,5	357,5
	6				5,3	1,3	-
Fluconazole					328,5	0,64	20,43

The six derivatives are endowed with anticandidosis activities with MICs varying between 408 - 0,52 µM

Study of structure-activity relationships

- *Relevance of the pharmacochemical concept*



Anti - Candida activities
***C. albicans* : MIC = 6,4 µM**
***C. tropicalis*: MIC = 3,2 µM**
***C. glabrata* : MIC = 408 µM**

The attachment of the phenylacrylonitrile linkage to the Imidazo[1,2- *a*]pyridine ring leads to a new hybrid molecule endowed with anticandidosis properties

Validation of the juxtaposition of bioactive entities

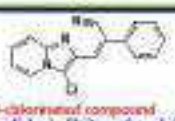
- *Optimization trials of anticandidosis activities*

These tests show that:

1. Displacement of the nitrile a to the β position did not improve anticandidosis activities
2. Para-chlorination of the benzene homocycle improved the activities against *C. tropicalis* in detriment of activities with respect to *C. albicans*
3. The replacement of the benzene homocycle by a hexylamine group, made it possible to obtain excellent activity against *C. albicans* and *C. tropicalis*. However, this derivative has lost all activity against *C. glabrata*
4. On *C. albicans* and *C. tropicalis* , the 3-chlorinated derivative was found to be the most effective.
5. The best performance on *C. glabrata* is obtained with the piperazine derivative.

Conclusion

✓ The pharmacochemical concepts of bioisosteria and molecular hybridization have enabled us to develop new Imidazo [1,2-*a*]pyridinyl-arylacrylonitriles endowed with anti-candidal properties.
✓ Furthermore, the best anticandidosis profile on the three *Candida* species tested was obtained with the 3-chlorinated compound, the MICs of which vary between 357,5 - 0,52 µM.



3-chlorinated compound
Anticandidosis "hit molecule"

Development of new inhibitors of SK3 channel to prevent metastasis occurrence

K.Brugemann[1], A. Chantôme[2], M. Potier-Cartereau[2], C. Vandier[2] et F. Buron[1], S. Routier[1]

[1]*Institut de Chimie Organique et Analytique, Université d'Orléans, UMR CNRS 7311*

[2]*Inserm UMR 1069 « Nutrition, Croissance et Cancer », Université de Tours*

FP 08

Currently, there is no treatment able to prevent bone metastasis. We discovered that while the abnormal expression of the SK3 channel by cancer cells promotes cancer cell migration and bone metastasis development, its suppression reduces them. Here, we propose to develop SK3 channel inhibitors as a new class of anti-metastatic drugs in targeted and personalized cancer therapy (targeted to SK3 channel and dedicated to patients with cancer cells expressing the SK3 channel).

Lead compound **NS8593** is currently one of the SK negative modulator inhibitor. However, the selectivity is current limitation in order to use this compound as anti-metastatic agent.

Our team is developing new synthetic strategies in order to provide novel polyfunctionalized pyridopyrimidines to explore structure-activity relationships and to improve the selectivity of final compounds. To achieve these objectives, we have developed efficient and modular strategies using S_NAr and palladium-catalyzed coupling reactions to modulate the main scaffold.

Among all the 25 compounds tested using patch clamp technique we identified **GF495**, a chiral compound, as strong inhibitor of SK3 channel with an IC_{50} = 18.4 nM (n=12). This compound was also found to inhibit the SK2 channel with an IC50 of around 1nM (n=7). In vitro experiments showed that this compound was not toxic until 10 µM. Our results show that **GF495** (100nM) significantly reduces the migration of five cancer cell lines, expressing SK3 channel including the MDA-MB435s. Furthermore, **GF495** has on effect on the migration of breast cancer cells that do not expressed SK3 (MDA-MB231) or in which SK3 channel were knockdown (MDA-MB435-shSK3). In vivo experiments showed that GF495 was not toxic until 20 mg/Kg (i.p. 5 times a week for 2 weeks). Finally, **GF495** was tested on a murine model of metastatic breast cancer (i.p.1 mg/kg, 3 times a week for 15 weeks). **GF495** treatment reduces dramatically bone metastasis (88.8%) and suppresses uterine and ovarian metastases,

To conclude, **GF495** is a new and potent inhibitor of SK3 channel, that show a capacity to reduce the development of metastasis. These promising results encourage us to develop analogues of **GF495** molecule, with at least a better selectivity (no effect on SK2 channel). In addition, it seems necessary to characterize the role of SK2 channel in cancer cell biology.

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* Correspondence: kevin.brugemann@univ-orleans.fr

Development of new inhibitors of SK3 channel to prevent metastasis occurrence

K.Brugemann[1], S. Hammoud[1], A. Chartier[1], A. Chantôme[2], M. Potier-Cartereau[2], F. Verrecchia[3], C. Vandier[2], F. Buron[1] and S. Routier[1]

[1]Institut de Chimie Organique et Analytique, Université d'Orléans, UMR CNRS 7311
[2]Inserm UMR 1069 « Nutrition, Croissance et Cancer », Université de Tours
[3]Inserm U1238, Faculté de Médecine, Université de Nantes

Introduction

In 2020, 20 million new cases of cancer worldwide are diagnosed. 80% of these cancers, such as breast cancer, showed the development of bone metastasis and the vital prognostic is engaged. [1,2] Metastases are considered as secondary tumors; they are formed *via* a cancer cells from a primary tumor that migrates through lymphatic or blood vessels. Currently, there is no treatment able to prevent bone metastasis. We discovered that while the abnormal expression of the SK3 channel by cancer cells promotes cancer cell migration and bone metastasis development, its suppression reduces them.[3] We propose to develop SK3 channel inhibitors as a new class of anti-metastatic drugs in targeted and personalized cancer therapy (targeted to SK3 channel and dedicated to patients with cancer cells expressing the SK3 channel).

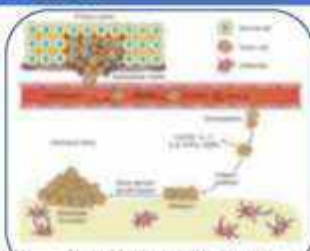

Figure 1 : Metastasis formation. [3]

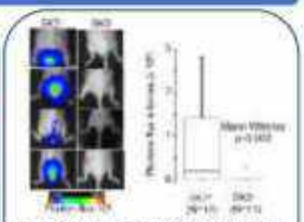

Figure 2 : Cells injected into the tail vein have the SK3 channel forms bone metastases, which reduces in the case of SK3- cells. [3]

Synthesis and biological results

As part of preliminary studies to the project, we identified from a series of several molecules derived from NS8593 (a commercial SK3 channel inhibitor), GF495, as an effective inhibitor of SK3 channel activity and with an IC_{50} estimated at 7 nM. [4]

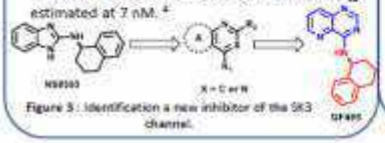

Figure 3 : Identification a new inhibitor of the SK3 channel.

Figure 4 : Synthesis of GF495. [4]

Synthesis in 4 steps starting from the commercially available 3-aminopicolinic acid with an overall yield of 14%

The IC_{50} of GF495 was determined by the patch clamp technique (Figure 5). It is around 7 nM for the SK3 channel.

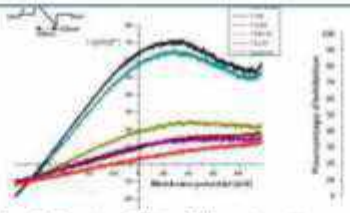

Figure 5 : Percentage inhibition of SK3 current sensitive to apamine, measured by the patch-clamp technique.

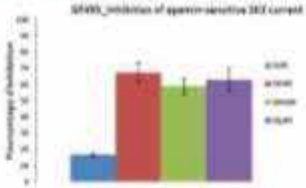

Figure 6 : Activity of GF495 on the SK3 channel in a dose-dependent manner.

GF495 is a chiral molecule for which we have not observed toxicity *in vitro* up to 10 µM on various cancer lines (HuH7, CaCo-2, MDA-MB231, MDA-MB 435s, HCT116, PC3, HaCaT) and on fibroblasts. *In vivo* toxicity experiments on healthy mice in acute doses and in repeated doses (ip, 5 times / week for 2 weeks) revealed an absence of toxicity up to 25 mg/kg. In addition, hepatic toxicity was not observed (1 mg / kg, ip, 3 times / week for 15 weeks).

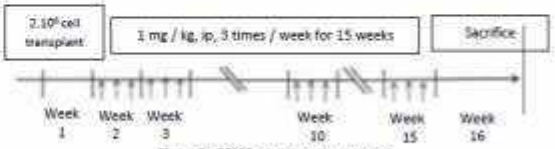

Figure 7 : GF495 administration protocol.

GF495 was tested on a mouse model of metastatic breast cancer (Figure 2). We have shown that GF495 administered in ip, at 1 mg/kg for 15 weeks (3 times per week) had no effect on the primary tumor volume or on the development of pulmonary metastases but interestingly led to a strong reduced development of bone metastases and suppression of ovarian and uterine metastases (Figure 2).

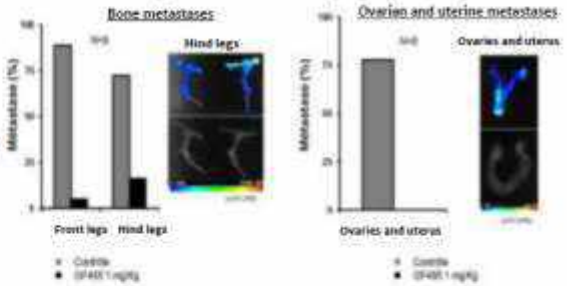

Figure 8 : Effect of GF495 on the development of bone and ovarian / uterine metastases.

Conclusion

GF495 was developed and identified as a strong SK3 ion channel inhibitor with anti-metastasis activity with an IC_{50} of 7.0 ± 0.3 nM on the SK3 channel. *In vitro* experiments showed that this compound was not toxic until 10 µM. *In vivo*, compound GF495 at 1 mg/kg reduced the number of bone metastases and completely abolished the development of ovarian and uterine metastases. These promising results encourage us to develop analogues of GF495 molecule, with at least a better selectivity (no effect on SK2 channel). In addition, it seems necessary to characterize the role of SK2 channel in cancer cell biology.

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[4] S. Routier, F. Buron, N. Rodrigues et al. *Ion channel inhibitor compounds for cancer treatment*. WO **2018**-215557 A1.

kevin.brugemann@univ-orleans.fr

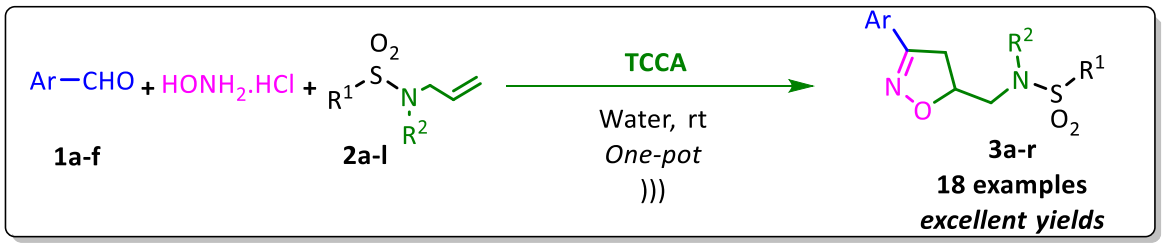
Ultrasound-assisted one-pot, three-component synthesis of new isoxazolines bearing sulfonamides as anti-leukemia agents.

Ayoub Elmahmoudi,[(1)] Aicha Talha,[(1)] Guillaume Robert,[(2)] Patrick Auberger,[(2)]Rachid Benhida,[(3,4)] Anthony R. Martin,[(3)] Khalid Bougrin [(1,4)]*

*(1). Equipe de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculty of Science, Geophysics, Natural Patrimony and Green Chemistry (GEOPAC) Research Center, Mohammed V University in Rabat, Rabat, Morocco. (2). Université Côte d'Azur, INSERM U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Bâtiment ARCHIMED, 151 Route de Saint-Antoine de Ginestière, BP 2 3194, 06204 Nice Cedex 3, France. (3). Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR 7272 - 06108 Nice, France. (4). Chemical & Biochemical Sciences Green-Process Engineering (CBS-GPE) Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Benguerir, Morocco. **

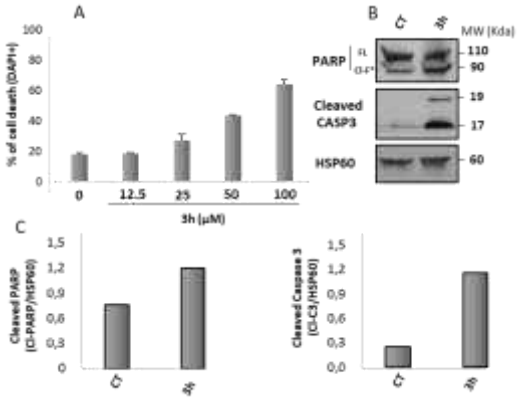
FP 09

Sulfonamides have received significant relevance in modern organic chemistry and medicinal chemistry.[a] A major goal of current medicinal research is to identify new, safer and more effective treatments for a wider range of neoplastic conditions, with the hope of reducing morbidity and mortality. In line with this consideration, and in continuation of our recent research in identifying new and original routes to bioactive anticancer agents,[b] we synthesized a series of new isoxazoline-linked sulfonamide following a one-pot two-steps method *via* a highly regioselective 1,3-dipolar cycloaddition catalyzed with the trichloroisocyanuric acid (TCCA) as a safe and ecological oxidant under sonication (Scheme), and evaluated their biological activity against CML and APL.



Scheme

Among this series of isoxazoline analogues one compound, **3h**, displayed promising cytotoxic activities against K562 and HL-60 cells. In addition, **3h** behaved as an apoptotic cell death inducer with an EC_{50} of 94 µM (HL-60 cell line) as shown in the figure on the right. Altogether, these results clearly demonstrated the potential of this class of molecules for the optimization of apoptosis inducer owing to their ease of synthesis the herein report two-step one-pot strategy.



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* Correspondence E-mail: kbougrin@yahoo.fr.

Ultrasound-assisted one-pot, three-component synthesis of new isoxazolines bearing sulfonamides as anti-leukemia agents

Ayoub Elmahmoudi,[1] Aicha Talha,[1] , Cécile Favreau,[2] Maxence Bourgoin, [2] Guillaume Robert,[2] Patrick Auberger,[2] Rachid Benhida,[3,4] Anthony R. Martin,[3] Khalid Bougrin [1,4]*

(1) Equipe de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculty of Sciences, Geophysics, Natural Patrimony and Green Chemistry (GEOPAC) Research Center, Mohammed V University in Rabat, Rabat, Morocco.

(2) Université Côte d'Azur, INSERM U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Bâtiment ARCHIMED, 151 Route de Saint-Antoine de Ginestière, BP 2 5194, 06204 Nice Cedex 3, France.

(3) Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR 7272 - 06108 Nice, France.

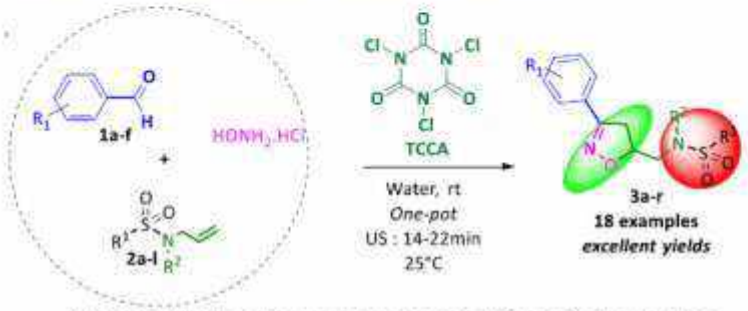
(4)). Chemical & Biochemical Sciences Green-Process Engineering (CBS-GPE) Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Benguerir, Morocco.

* Correspondence: E-mail : kbougrin@yahoo.fr

Introduction

Sulfonamide and Isoxazoline moieties are among the most important pharmacophore in modern organic and medicinal chemistry .There are currently a large number of compounds that contain these pharmacophores, which exhibit diverse and powerful biological activities [1]. In This work, following a *one-pot* two-steps protocol, we have synthesized a series of novel sulfonamides-isoxazolines hybrids **3a-r** *via* a highly regioselective 1,3-dipolar cycloaddition under mild and eco-friendly conditions , we have developed a new methodology using trichloroisocyanuric acid (TCCA) as a safe and ecological oxidant for the *in-situ* conversion of aldehydes **1a-f** to nitrile oxides in the presence of hydroxylammonium chloride under sonication (Scheme.1). All the sulfonamides-isoxazolines hybrids **3a-r** are screened for anti-leukemia activity against ***K562*** cell line (Chronic Myeloid Leukemia) and ***HL-60*** cell line (Promyelocytic Leukemia)

Synthesis of new sulfonamides-isoxazolines hybrids 3a-r



Scheme 1. Synthesis in *one-pot* two-steps of sulfonamides-isoxazolines under sonication.

Anti-leukemia activity

All these newly synthesized isoxazoline **3a-r**, were evaluated for their anti-cancer activity against ***K562*** cell line at 1 µM 10 µM and 50 µM (48 h) using XTT and DAPI assays. This first screening against ***K562*** cells revealed a couple of compounds (**3h** and **3l**) displaying a moderate cytotoxic activity between 10 and 50 µM. Thus, we decided to investigate further the biological effect of **3h**. we have evaluated the dose-response effect of **3h** (12.5-100 µM) on ***HL-60*** cell line (Figure 2).The determined value of EC_{50}= 62 ± 2 µM on ***HL-60*** cell of compound **3h**

In conclusion these results clearly demonstrated the potential of this class of molecules for the optimization of apoptosis inducer owing to their ease of synthesis the herein report two-steps one-pot strategy.

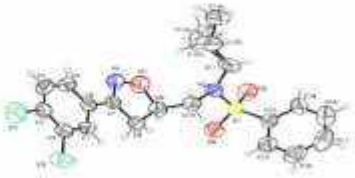
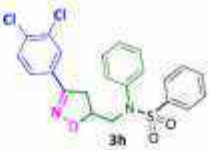


Figure 1. The ORTEP of novel single crystal of product (**3k**).



EC50= 62 ± 2 µM (*HL60*)

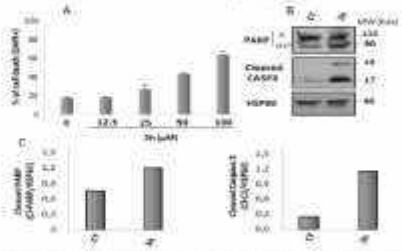


Figure 2. The evalution of the dose reponse of **3h** on ***HL60*** cell line.

[1] (a) Liu, J., & Cao, C. (2020). 145(14), 4901-4905.(b) Alaoui, S., Dufies, M., Driowya, M., Demange, L., Bougrin, K., Robert, G., ... & Benhida, R. (2017). 27(9), 1989-1992.

(c) Bernal, C. C., Vesga, L. C., Mendez-Sánchez, S. C., & Bohórquez, A. R. R. (2020). 29(4), 675-689.

Exploring β-turn stabilization strategies in cyclic peptide ligands of the VEGF (Vascular Endothelial Growth Factor)

Xiaoqing Ye *, **Jean-François Gaucher, Michel Vidal, Sylvain Broussy**

(1) UMR CiTCoM CNRS 8038 and INSERM U1268, Faculty of Pharmacy of Paris, University of Paris 4 avenue de l'Observatoire, 75006 Paris, France

FP 10

Angiogenesis regulation is involved in numerous physiological processes and diseases like cancer and age-related macular degeneration. Cytokines of the Vascular Endothelial Growth Factor (VEGFs) family and their transmembrane receptors have been validated as therapeutic targets: VEGF-A, VEGF-B, VEGF-C and PlGF in particular. The current available molecules in the clinic are derived from recombinant proteins, like antibodies. A next generation of drugs must be developed, with better pharmacological properties, reduced production costs, and the capacity to be engineered for specific applications. The objective of our research program is to develop antagonist small peptidomimetic molecules, able to bind the VEGFs with high affinity and selectivity.

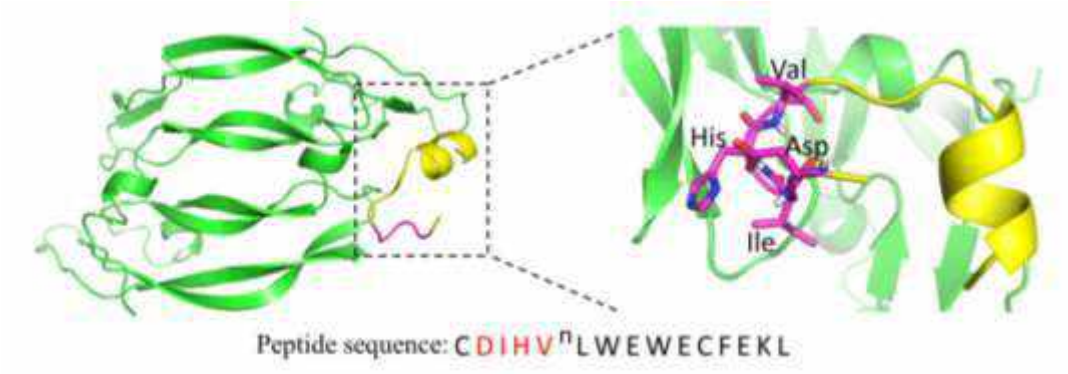


Figure. Co-crystal structure of a peptide in complex with VEGF-A. The VEGF dimer is represented in green and the peptide in yellow. The β-turn of the peptide is shown in magenta, and relevant residues are labelled three-letter abbreviation. The peptide is cyclized by a disulfide.

Starting from a known peptide ligand of VEGF-A, our objective is to stabilize the β-turn conformation present at the C-terminal position in its bioactive conformation. This strategy could improve the binding affinity by reducing the entropic penalty of binding. We will present the design (including non-natural amino acids), the synthesis and the biochemical evaluation of the new series of peptides. Their evaluation was achieved by ELISA-type assays with VEGF-A and VEGF-B, and by isothermal titration calorimetry (ITC) with VEGF-A.
Further structural studies by NMR and biological evaluation of VEGF inhibition activity in HUVEC (Human Vein Endothelial Cells) will be achieved with the most affine peptidomimetics.

Bibliographic references:
P. Carmeliet and R. K. Jain, *Nature*, **2011**, *473*, 298; M. Reille-Seroussi et al. *Biochemistry*, **2015**, *54*, 5147; L. Trapiella-Alfonso et al. *Anal. Biochem*. **2018**, *544*, 114; R. S. Apte et al. *Cell*, **2019**, 176, 1248.

* Correspondence: xiaoqing.ye@etu.u-paris.fr

Exploring β-turn stabilization strategies in cyclic peptide ligands of the VEGF (Vascular Endothelial Growth Factor)

Xiaoqing Ye (1)*, Jean-François Gaucher (1), Michel Vidal (1), Sylvain Broussy (1)

(1) UMR CiTCoM CNRS 8038 and INSERM U1268, Faculty of Pharmacy of Paris, University of Paris 4 avenue de l'Observatoire, 75006 Paris, France

Introduction

Angiogenesis regulation is involved in numerous physiological processes and diseases like cancer and age-related macular degeneration [1]. Cytokines of the Vascular Endothelial Growth Factor (VEGFs) family and their transmembrane receptors have been validated as therapeutic targets: VEGF-A, VEGF-B, VEGF-C and PlGF in particular [2]. The current available molecules in the clinic are derived from recombinant proteins, like antibodies [3]. A next generation of drugs must be developed, with better pharmacological properties, reduced production costs, and the capacity to be engineered for specific applications. The objective of our research program is to develop antagonist small peptidomimetic molecules, able to bind the VEGFs with high affinity and selectivity.

Starting from a known peptide ligand of VEGF-A, our objective is to stabilize the β-turn conformation present at the C-terminal position in its bioactive conformation. This strategy could improve the binding affinity by reducing the entropic penalty of binding. We present the design (including non-natural amino acids), the synthesis and the biochemical evaluation of the new series of peptides. Their evaluation was achieved by ELISA-type assays with VEGF-A and VEGF-B, and by isothermal titration calorimetry (ITC) with VEGF-A.

The design of β-turn stabilization strategies in cyclic peptide ligands

Peptide sequence: CDIHV^{a}LWEWECFEKL

Co-crystal structure of a known peptide in complex with VEGF-A. The VEGF dimer is represented in green and the peptide in yellow. The β-turn of the peptide is shown in magenta, and relevant residues are labelled three-letter abbreviation. The peptide is cyclized by a disulfide bond between two cysteines and lactam bridge between glutamate 10 and lysine 14.

peptide	β-turn transformation	amino acid sequence
prototype	-DIHV^{a}L-	phenylacetyl-CDIHV^{a}LWEWECFERL-NH$_2$
XY-1	-DIHV^{b}L-	phenylacetyl-CDIHV^{b}LWEWECFERL-NH$_2$
XY-2	-D^{c}P HV^{a}L-	phenylacetyl-CD^{c}PHV^{a}LWEWECFERL-NH$_2$
XY-3	-DAHV^{a}L-	phenylacetyl-CDAHV^{a}LWEWECFERL-NH$_2$
XY-4	-DPHV^{a}L-	phenylacetyl-CDPHV^{a}LWEWECFERL-NH$_2$
XY-5	-DAib^{d}V^{a}L-	phenylacetyl-CDAibV^{a}LWEWECFERL-NH$_2$
XY-6	-DAib^{e}A V^{a}L-	phenylacetyl-CDAib^{e}AV^{a}LWEWECFERL-NH$_2$
XY-7	-D^{f}A V^{a}L-	phenylacetyl-CD^{f}AV^{a}LWEWECFERL-NH$_2$
XY-8	-DFreidinger's lactam$^{[4]}$V^{a}L-	phenylacetyl-CDFreidinger's lactamV^{a}LWEWECFERL-NH$_2$

This table shows the design of β-turn stabilization strategies and the whole sequences of synthesized peptides by solid phase peptide synthesis. [a] L: Norleucine, [b] DL: D-Norleucine, [c] DP: D-proline, [d] Aib: 2-Aminoisobutyric acid, [e] DA: D-Alanine, [f] NMeA: N-Methylalanine

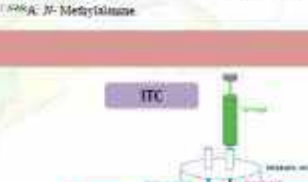
Experimental Section

Peptide synthesis

ELISA Displacement Assay

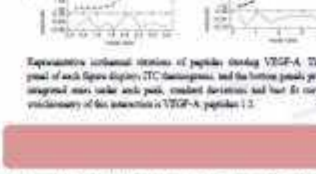
ELISA illustration is adapted from Analytical Biochemistry [illegible]

ITC



ITC illustration is from [illegible]

Results



Representative isothermal titrations of peptides binding VEGF-A. The upper panel of each figure displays ITC thermograms, and the bottom panels present the integrated areas under each peak, standard deviations and best fit curves. The stoichiometry of this interaction is VEGF-A: peptides 1:2.

ELISA displacement assays of XY-8 binding to VEGF-A (red) and VEGF-B (green), respectively. The analysis was performed with the software GraphPad Prism using a "log[inhibitor] vs. response" non-linear regression. The bottom and top values were fixed at 0 % and 100 %, respectively.

Peptide	IC_{50} VEGF-A (µM)[a]	IC_{50} VEGF-B (µM)[b]	K_d VEGF-A (µM)[c]
prototype	6.0 [4.6, 7.7]	7.5 [5.2, 10.2]	0.048 [0.041, 0.056]
XY-1	7.4 [5.5, 9.8]	18.6 [11.6, 30.0]	ND[d]
XY-2	15.1 [8.4, 27.0]	39.6 [16.9, 93.1]	ND
XY-3	21.0 [16.4, 26.8]	7.5 [4.5, 11.9]	0.234 [0.202, 0.269]
XY-4	>100	>100	0.110 [0.097, 0.125]
XY-5	28.5 [14.0, 58.1]	34.8 [9.4, 128.6]	ND
XY-6	17.4 [9.4, 32.2]	37.1 [13.5, 101.5]	1.811 [1.601, 2.002]
XY-7	17.4 [8.0, 38.0]	17.5 [5.6, 55.0]	ND
XY-8	41.7 [19.3, 90.0]	46.6 [20.0, 118.8]	ND

[a] IC_{50} VEGF-A refers to IC_{50} values determined by ELISA displacement assay for VEGF-A. [b] IC_{50} VEGF-B refers to IC_{50} values determined in ELISA displacement assay for VEGF-B. [c] K_d values were determined by ITC for peptides binding to VEGF-A. [d] ND refers to not determined because of the insufficient affinity. 95% confidence intervals are given in the brackets.

Conclusion and perspectives

Compared to prototype peptide, the 8 new peptides show a decrease in binding affinities, indicating that our attempts to stabilize the β-turn conformation and therefore improve the bioactivity was not successful. However, these results reversely demonstrate the vital importance of certain fine structural features, which were disturbed by stabilization attempts: the β-turn types and the steric constraints of side chains obviously disrupted the binding surface and the network of water molecules at the interface. What's more, these results imply that it may be possible to stabilize the bioactive structure to improve binding affinity. Different results were obtained for some peptides between the two assay methods (Elisa and ITC). Previous studies showed that the ITC assay was more reliable, therefore our next step is testing these peptides by ITC for VEGF-B binding. Our future research will focus on structure modifications targeting other amino acids to stabilize the bioactive conformation in solution. Then, further structural studies by NMR and biological evaluation of the inhibitory activity of VEGF in HUVECs will be carried out with the most potent peptidomimetics.

References: [1] Carmeliet, P. and R.K. Jain, Molecular mechanisms and clinical applications of angiogenesis. Nature, 2011. 473(7347): p. 298-307. [2] Park, S.A., et al., Structure and function of vascular endothelial growth factor and its receptor system. BMB Rep, 2018. 51(2): p. 73-78. [3] Ferrara, N. and A.P. Adamis, Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov, 2016. 15(6): p. 385-403. [4] Freidinger, R.M., et al., Bioactive conformation of luteinizing hormone-releasing hormone: evidence from a conformationally constrained analog. Science, 1980. 210(4470): p. 656-8. [5] Reille-Seroussi, M., et al., Vascular Endothelial Growth Factor Peptide Ligands Explored by Competition Assay and Isothermal Titration Calorimetry. Biochemistry, 2015. 54(33): p. 5147-56.

* Correspondence: [illegible]

New 2-heteroaryl-4-quinolones as potential multi-drug resistant ESKAPEE antivirulence agents

Marine Duplantier*, **Elodie Lohou, Pascal Sonnet**

AGIR, UR4294, UFR of Pharmacy, Jules Verne University of Picardie, 80037 Amiens, France.

FP 11

Over the last decades, massive misuse of antibiotics prompted the apparition of resistances in many microorganisms such as ESKAPEE pathogens responsible for various nosocomial infections.[(a)] Indeed, the selective pressure put on sensitive bacteria by conventional antimicrobial molecules that cause their death promotes resistant strain survival. The development of antivirulence agents that could attenuate bacteria pathogenicity without affecting their growth, seems to be a new promising therapeutic strategy. This could facilitate the host's defence by immune system and restore the associated efficiency of conventional treatments.[(b)]

The quorum sensing (QS), that makes reference to communication systems of bacteria, constitute a pool of promising pharmacological targets for the development of antivirulence agents. The inhibition of QS could disrupt, especially in *P. aeruginosa*, intra/inter-species protective interactions that stimulate virulence pathways (biofilm formation, pyocyanin or rhamnolipid production). The interest of *Pseudomonas* Quinolone Signal receptor (PqsR) that regulates virulence gene expression in response to environmental factors and population density once activated by its natural ligand (PQS), has recently emerged for the development of inhibitors.[(c)] Among PqsR antagonists described in the literature, the 6-nitro-2-heptyl-4(*1H*)-quinolone-3-carboxamide revealed efficient to inhibit pyocyanin production with an IC_{50} of 2 µM in *P. aeruginosa* PA14 strains.[(d-f)]

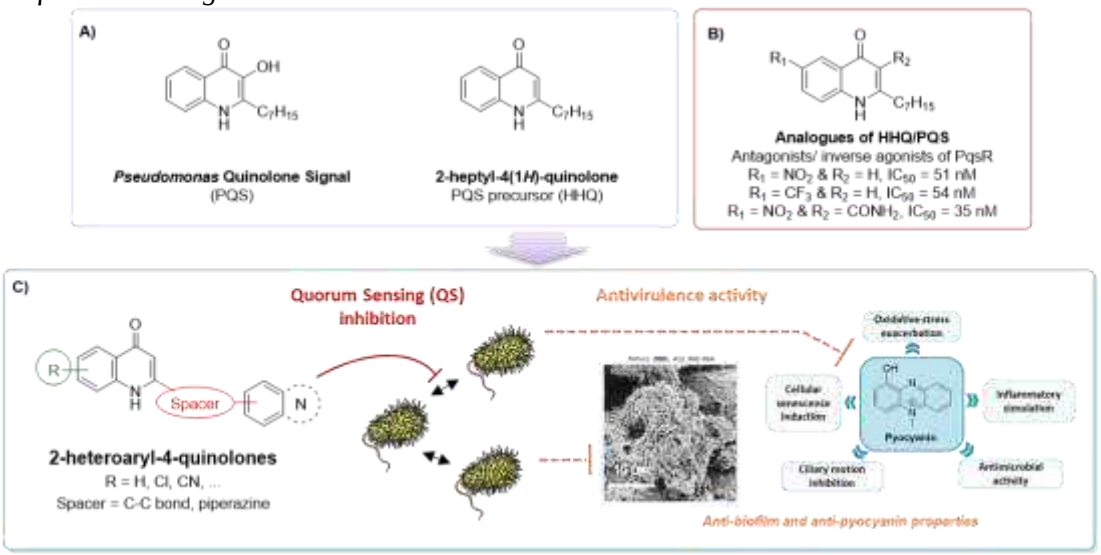


Figure 1: A) Natural ligands of PqsR produced by *P. aeruginosa*; B) Quorum sensing inhibitors described in literature and C) Development of 2-heteroaryl-4-quinolones as potential QS inhibitors.

Taking these studies into account, we aim to develop a new 2-heteroaryl-4-quinolone family potentially active against ESKAPEE pathogens as QS inhibitors. The synthesis pathways based on pallado-catalyzed coupling reactions, but also the physicochemical and biological evaluations of these compounds (MIC in various ESKAPEE strains and anti-biofilm activity) will be described in the presentation.

Bibliographic references:

(a) *Pharm. Ther*., **2015**, 40 (4), 277-283; (b) *RSC Med. Chem*., **2020**, 10.103; (c) *Chem. Sci*., **2017**, 8, 7403-7411; (d) *Org. Biomol. Chem*., **2017**, 15 (21), 4620–4630; (e) *Chem. Biol.*, **2012**, 19 (3), 381–390 ; (f) *Angew. Chem. Int. Ed*., **2014**, 53 (4), 1109–1112.

* Correspondence: marine.duplantier@etud.u-picardie.fr

New 2-heteroaryl-4-quinolones as potential multi-drug resistant ESKAPEE anti-virulence agents

Marine Duplantier [1]*, Elodie Lohou [1], Pascal Sonnet [1]

[1] AGIR, UR4294, UFR of Pharmacy, Jules Verne University of Picardie, 80037 Amiens, France.

* Correspondence: marine.duplantier@etud.u-picardie.fr

INTRODUCTION

Over the last decades, massive misuse of antibiotics (ATB) prompted the emergence of resistances in many microorganisms such as ESKAPEE pathogens responsible for various nosocomial infections.[1] Indeed, the selective pressure put on sensitive bacteria by conventional antimicrobial molecules that cause their death promotes resistant strain survival. The development of anti-virulence agents (AVA) that could attenuate bacteria pathogenicity without affecting their growth, seems to be a new promising therapeutic strategy. Such AVA could **potentiate the host's immune system response** in monotherapy and **restore efficiency of conventional ATB** in combination therapy (Fig. 1).[2]

The quorum sensing (QS), that makes reference to communication systems of bacteria, constitutes a pool of promising pharmacological targets for the development of AVA. The inhibition of QS could disrupt, especially in *P. aeruginosa*, intra/inter-species protective interactions that stimulate virulence pathways (biofilm formation or pyocyanin production). The interest of ***Pseudomonas* Quinolone Signal receptor (PqsR)** that regulates virulence gene expression in response to environmental factors and population density once activated by its **natural ligand (PQS)**, has recently emerged for the development of inhibitors.[3]

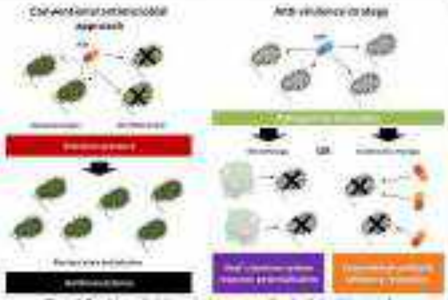


Figure 1. Promising anti-virulence strategy vs conventional antimicrobial approach.

Figure 2. A) Natural ligands of PqsR produced by *P. aeruginosa*; B) Quorum sensing inhibitors described in the literature and C) Development of 2-heteroaryl-4-quinolones as potential QS inhibitors.

AIMS AND STRATEGY

Different 2-heptyl-4-quinolone analogues of PQS revealed efficient as **PqsR inhibitors** (Fig. 2A-B).[4,5] Taking these studies into account, we aim to develop a new **2-heteroaryl-4-quinolone family** potentially active against ESKAPEE pathogens as quorum quenchers. The building block coupled in position 2 of the quinolone pharmacophore via a C-C bond or different amine spacers was chosen for its bio-isosterism with several heteroaryl cores of various PqsR antagonists described in the literature.

Herein, we reported the synthesis of two series of 2-heteroaryl-4-quinolones: i) derivatives possessing a C-C bond between the two aromatic fragments and ii) analogues bearing a **piperazine spacer** (Fig. 2C). With the great impermeability of Gram-negative bacteria cell envelop in mind, a prediction of their physicochemical properties was performed using QikProp, a Schrödinger software, to analyse their **drugability**. The biological evaluation of these compounds on different ESKAPEE strains was carried out in terms of their bactericidal and anti-virulence properties, as well as their cytotoxicity in a human hepatoma cell line.

SYNTHESIS

The design of new 2-heteroaryl-4-quinolones relies on pallado-catalyzed cross-coupling reactions between different 2-bromo-4-chloroquinoline precursors 1 and various second heteroaryl derivatives (Fig. 3) such as:

- 4, 5 or 6-heteroarylboronic esters 2a-c in the series I (Suzuki C-C couplings, Table 1),
- or *N*-heteroarylpiperazine derivatives 5 in the series II (Buchwald-Hartwig C-N couplings).

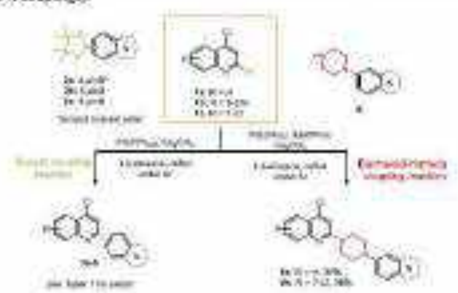


Figure 3. Synthesis of 2-heteroaryl-4-quinolones.

Quinolinone (1)	Second building block	3a-k Yields (%)		4a-k Yields (%)	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 1. Coupling and final products in the series I.

PHYSICOCHEMICAL AND *IN VITRO* BIOLOGICAL EVALUATIONS

The QikProp-predicted octanol/water partition coefficients (cLog $P_{o/w}$) of derivatives 4c, 4e and 7b revealed their strong lipophilicity. However, the compound 4d bearing a cyano polar group presented an increased hydrophilicity. This could be in favour of a mediated transport inside the bacterial cell by porins.

The minimal inhibition concentrations (MIC) of synthetized 2-heteroaryl-4-quinolones have been measured on various ESKAPEE strains (Table 2). The molecules have no effect on bacterial growth that is a favorable result in the case of anti-virulence strategy.

Compounds		cLog $P_{o/w}$	*S. aureus* CIP 103.429 MIC (µg/mL)	*A. baumannii* 15-[illegible] MIC (µg/mL)	*P. aeruginosa* DSM 1117 MIC (µg/mL)	*E. coli* DSM 1103 MIC (µg/mL)
Ciprofloxacin		0.280	0.06	Resistance by efflux	0.06	0.05
4c-e	4c, R = H	2.570	>128	>128	>128	>128
	4d, R = 6-CN	1.647	>128	X	>128	>128
	4e, R = 7-Cl	2.818	>128	X	>128	>128
7b		3.517	>128	X	>128	>128

Table 2. Log $P_{o/w}$ values calculated for 2-heteroaryl-4-quinolones and their MIC on ESKAPEE bacterial strains.

Their inhibitory properties on biofilm formation have been evaluated on *P. aeruginosa* PAO1 strain using purple crystal dyeing (Fig. 4A). Compounds 4d and 4e **inhibit biofilm** production with respective ratios of 32% and 32% at 50 µM (Fig. 4B-C), whereas the derivative 4c was inactive. The presence of an electron-withdrawing substituent in position 6 or 7 of the quinolone core seems to be promising for the development of anti-virulence agents. In contrast, compound 7b appeared able to stimulate biofilm formation with an overproduction ratio of 21% at 50 µM (Fig. 4D). The nature of the coupling between the two fragments could thus orientate the activity towards a biofilm overproduction or inhibition. No cytotoxicity of the compounds 4c-e was observed in a human hepatoma cell line (HepG2 from ECACC) after 48 hours of treatment at 100 µM.

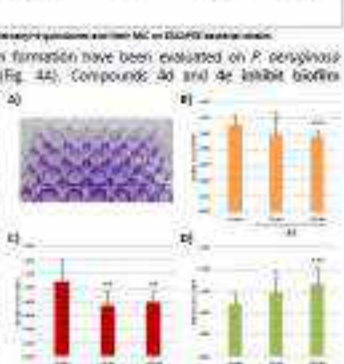


Figure 4. A) Biofilm dyeing with purple crystal and B) Effect of compound 4e, C) 4d and D) 7b on biofilm formation (***p < 0.001, **p < 0.01 and *p < 0.05 indicate statistically significant differences from the untreated control group).

CONCLUSION

Eleven 2-heteroaryl-4-quinolones have been synthesized in 4-5 steps with global yields of 8 to 50% for the series I and of 1 to 3% for the series II. The 6-cyano and 7-chloro derivatives 4d and 4e showed a promising anti-biofilm activity without affecting the bacterial growth. Taking this into account, extended pharmacomodulations in positions 4 to 5 of the quinolone core are currently under progress to develop anti-virulence agents, as well as the evaluation of their anti-pyocyanin properties.

References: [1] Pharm. Ther. 2015, 40 (4), 277-283; [2] RSC Med. Chem. 2020, 11, 556-568; [3] Chem. Sci. 2017, 8, 7403-7411; [4] Org. Biomol. Chem. 2017, 15 (21), 4620-4630; [5] J. Med. Chem. 2020, 63, 10921-10945.

[illegible] Young Research Fellows Meeting, Paris, France
February 11-12, 2021

Synthesis and characterization of ^{13}C-labelled carnosine derivatives and their application as internal standards for the measurements in biological matrices	***FP 12***

Marco Maspero(1)*, Ettore Gilardoni (1), Graziella Messina (2), Luca Regazzoni (1), Giancarlo Aldini (1), Clelia Dallanoce (1).

(1) Department of Pharmaceutical Sciences, University of Milan, via L. Mangiagalli 25, 20133 Milan, Italy
(2) Department of BioSciences, University of Milan, Via Celoria 26, 20133 Milan, Italy

Carnosine is an endogenous dipeptide, composed of β-alanine and L-histidine, highly concentrated in skeletal muscle and other excitable tissues (Figure 1a). Its physiological roles, based on its biochemical properties, include pH-buffering, metal-ion chelation, antioxidant capacity, as well as the ability to protect against the formation of advanced glycation and lipoxidation end-products.[a] For these reasons, besides its nutritional ergogenic application in the sport community,[b] carnosine supplementation presents a therapeutic potential for the treatment of numerous diseases in which ischemic or oxidative stress are involved.[a] Besides carnosine, most animals also possess the methylated analogues anserine and/or balenine (Figure 1a). Quantitation of these histidine dipeptides in biological matrices results to be crucial for their potential applications, and LC-MS procedures with isotope-labeled internal standards are the state-of-the-art approach for this analytical need.[c] The use of these standards allows to account for variations during the complex sample preparation process, different matrix effects between patient samples, and variations in instrument performance.

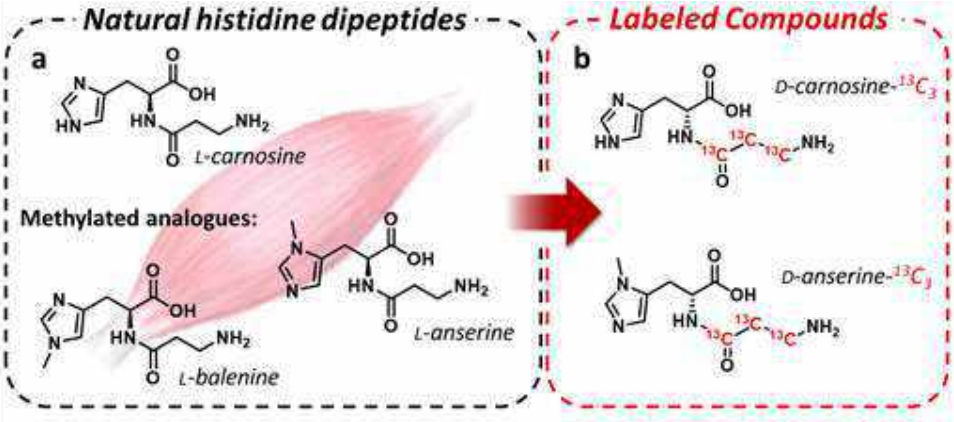


Figure 1: a) Structures of histidine dipeptides, b) 13C-labelled carnosine analogues.

In this study we present a fast, flexible and convenient strategy for the synthesis of 13C-labelled carnosine analogues (Figure 1b), and their application as internal standards for the quantitation of carnosine and anserine in a biological matrix.

Bibliographic references:

(a) Boldyrev, A.A.; Aldini, G.; Derave, W. Physiol Rev 2013, 93, 1803–1845

(b) Brisola, G.; Zagatto, A. J. Strength Cond. Res. 2019, 33(1), 253-282.

(c) Stokvis, E.; Rosing, H.; López-Lázaro, L.; et al. Biomed. Chromatogr. 2004, 18, 400-402

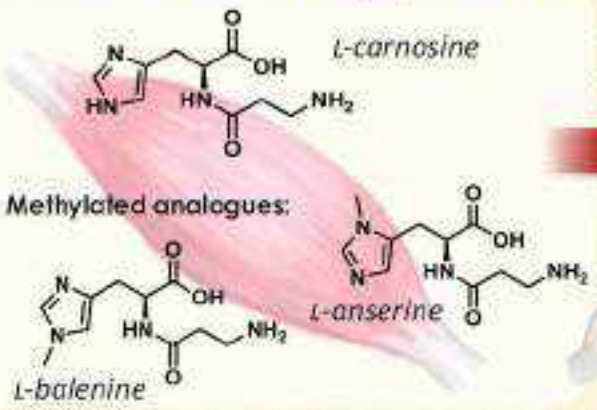
* marco.maspero@unimi.it

SYNTHESIS AND CHARACTERIZATION OF ^{13}C-LABELLED CARNOSINE DERIVATIVES AND THEIR APPLICATION AS INTERNAL STANDARDS FOR THE MEASUREMENTS IN BIOLOGICAL MATRICES

Marco Maspero,* Ettore Gilardoni,* Graziella Messina,** Luca Regazzoni,* Giancarlo Aldini,* Clelia Dallanoce,*

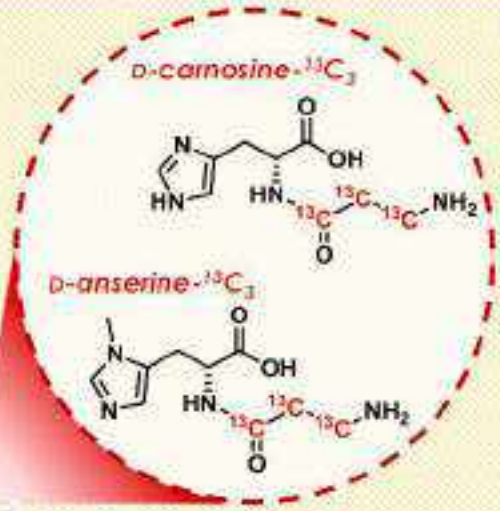
*Department of Pharmaceutical Sciences DISFARM, University of Milan, Via L. Mangiagalli 25, 20133 Milan, Italy.
**Department of BioSciences, University of Milan, Via Celoria 26, 20133 Milan, Italy

Natural histidine dipeptides

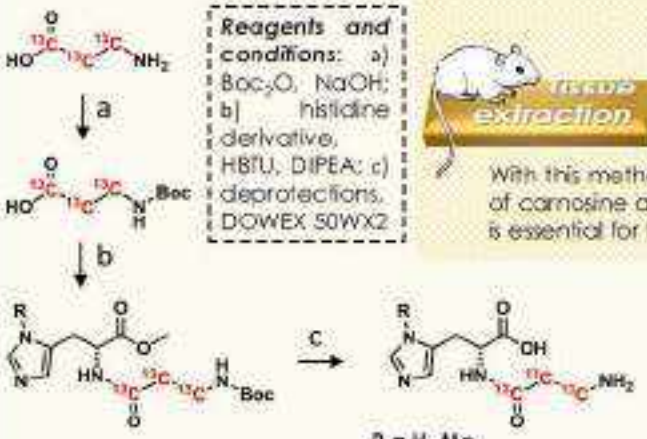


Isotope labeled internal standard

- State-of-the-art approach in LC-MS procedures
- Able to correct either random or systematic error
- Essential tool for quantitation of carnosine and derivatives in complex matrices



Synthesis



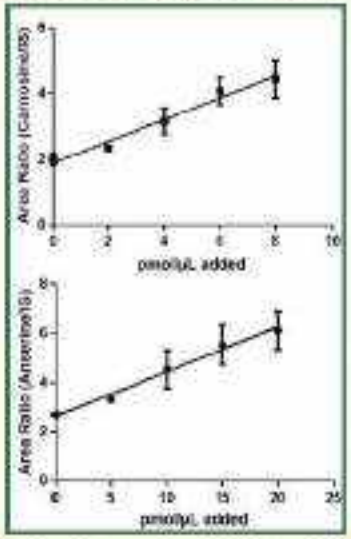
Reagents and conditions: a) Boc_2O, NaOH; b) histidine derivative, HBTU, DIPEA; c) deprotections, DOWEX 50WX2

With this method it is possible to have a correct quantitation of carnosine and its derivatives in biological matrices, which is essential for their ergogenic and therapeutic applications

Advantages:

- High isotopic purity
- No chromatographic interference
- Flexible synthetic pathway
- High metabolic stability

Accurate quantitation



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One-pot synthesis of bioactive dihydropyrimidines *via* eco-friendly phosphorus derivatives catalysis.	***FP 13***

Zakaria MOUTAOUKIL[(1,2)], Cyril RONCO [(2)], Rachid BENHIDA [(1,2)]

[(1)] Department of Chemical and Biochemical Sciences – Green Proces Engeneering (CBS-GPE), Mohammed VI Polytechnic University, UM6P, 43150, Ben Guerir, Morocco

[(2)] Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR 7272, 06108 Nice, France.

The Biginelli reaction is one of the most useful and efficient protocol for the preparation of biologically active *(dihydro)pyridine(thi)ones and pyrimidin(thi)ones* (DHPMs)[1]. This reaction uses readily accessible starting materials, presents high atom economy and allows the access to a great diversity of products (Figure), with broad spectrum of pharmacological activities (*e.g.* Monastrol, a potent anticancer drug; SQ 32926 and SWO2, antihypertensive agents)[2].

Phosphate derivatives are versatile and efficient catalyst for many organic transformations[3]. Herein, we report the use of different phosphorus derivatives as acid catalysts for the straightforward synthesis of various dihydropyrimidines under mild conditions. A first series of 30 biologically relevant DHPM analogues has already been prepared. Other related series have been designed and their synthesis is currently ongoing. These compounds will be evaluated for their anticancer cytotoxic activity and on antibacterial assays.

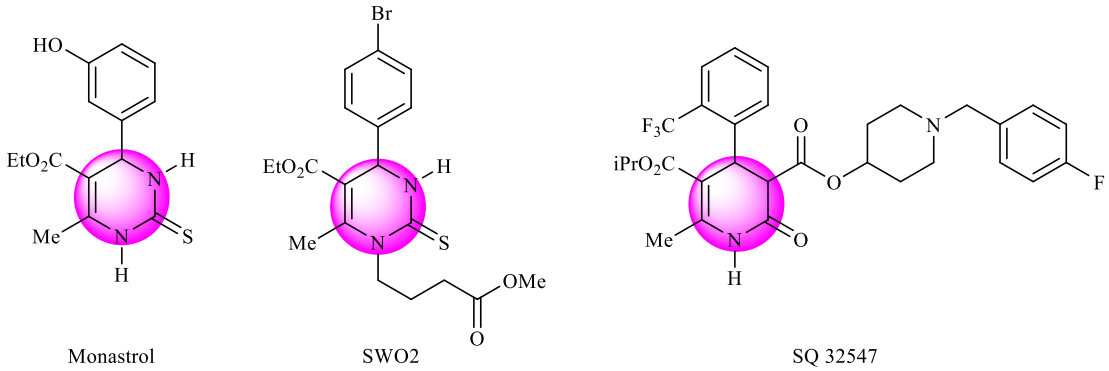

Figure: Pharmacologically active DHPMs

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*Correspondence E-mail: zakaria.moutaoukil@um6p.ma

XXVIIIth Young Research Fellows Meeting – 2021
Société de Chimie Thérapeutique
Faculté de Pharmacie
Paris, France

One-pot synthesis of bioactive dihydropyrimidines *via* eco-friendly phosphorus derivatives catalysis

Z. Mostaoukil [1,2], C. Ronco [2], R. Benhida [1,2*]
[1] Department of Chemical and Biochemical Sciences - Green Process Engineering (CBS-GPE), Mohammed VI Polytechnic University (UM6P), Ben-Guerir, Morocco
[2] Institut de Chimie de Nice-UMR7272, Université Côte d'Azur, CNRS, Nice, France.
Correspondence : rachid.benhida@unice.fr

Introduction

"GREEN CHEMISTRY": several approaches have emerged with significant potential for **minimizing byproducts, waste reduction, the lowering of energy costs.** The development of new methodologies towards previously inaccessible materials using existing technologies has been also very appealing

3,4-dihydropyrimidin-2(1H)-ones (DHPMs): important class of heterocyclic compounds, which have attracted much interest due to their pharmacological and biological properties. Nevertheless, the common accesses to these structures rely on strong acidic conditions, or display low yields, or requires the use of expensive catalyst and/or toxic solvents. Therefore, the development of new eco-friendly syntheses of this scaffold is currently the subject of active research.

Green-Phosphorus Chemistry

Phosphate derivatives have recently gained much interest as reagents and catalysts, because of their **large availability, low cost and efficiency in numerous organic processes.**

The need for alternatives for non-"rare metals" catalysts is now widely acknowledged and has become a major research focus both in industry and academia.

In this context, we report the use of **hypophosphorus acid** as inexpensive and ecofriendly catalyst for the **Biginelli tricomponent reaction**, which allowed the production of a **broad panel of DHPM derivatives** diversely substituted.[1] Other related more complex heterocyclic systems have also been afforded following this methodology. The biological evaluation of these series of compounds on anti-cancer, inflammation and antibacterial models is currently ongoing and will be reported in due time.

Experimental Process

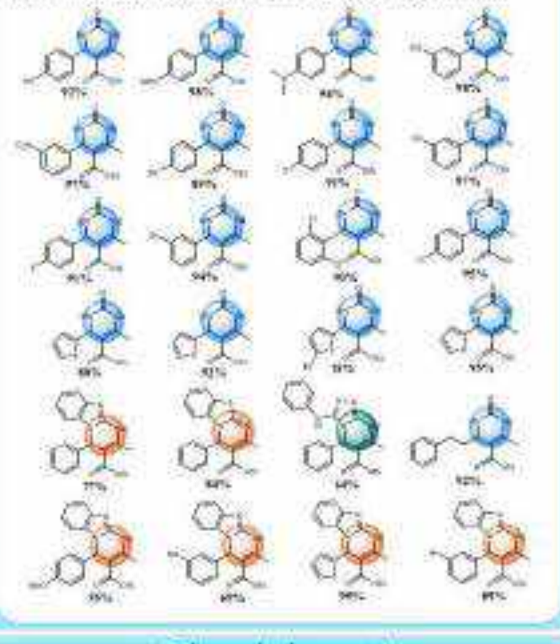
Benzaldehyde, ethyl acetoacetate and urea derivatives react in a tricomponent reaction in the presence of **20 mol%** of catalyst to convert quantitatively into DHMP scaffold.

Condition optimization

Entry	Catalyst (%)	Amount of catalyst	Solvent	Time	Temp. [°C]	Yield [%]
1	—	—	Ethanol	8h	80	13%
2	phosphoric Acid	20%	Ethanol	6h	80	51%
3	N-(Phosphonomethyl)iminodiacetic acid hydrate	20%	Ethanol	6h	80	80%
4	2-ethylhexyl phosphate	20%	Ethanol	6h	80	98%
5	**hypophosphorus acid**	**20%**	**Ethanol**	**4h,6h**	**80**	**97%,99%**
6	phosphogypsum	20%	Ethanol	6h	80	12%
7	apatite	20%	Ethanol	6h	80	3%
Ref.[2]	$H_3[PW_{12}O_{40}]$	0.1g	Ethanol	5h	80	95%
Ref.[3]	A-zirconium sulfonylphosphonate	12%	—	10h	80	89%

Synthesis of different scaffolds

Large numbers of substrates were screened and found to give excellent yield of the desired products using **20%** of the **hypophosphorus acid**.



Conclusions

The described protocol proposes the **hypophosphorus acid** as **green alternative catalyst to access functionalized DHPMs in excellent yields.** The DHPMs derivatives produced represent interesting drug-like scaffolds with great functional diversity **to be tested on anti-cancer, inflammation and antibacterial models.**

Acknowledgements

This work was supported by OCP (Maroc), Mohammed VI Polytechnique University (Maroc), Institut de Chimie de Nice (France), Université Côte d'Azur, CNRS and Région PACA.

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Improvement of the activity of topical antibiotics against Staphylococcus aureus isolated from diabetic foot wounds

Diana Oliveira[1,2,3], Anabela Borges [1,2], Fernanda Borges [3], Manuel Simões [1,2]*.

(1) LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal (2) DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal (3) CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal

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Diabetes mellitus is a severe endocrinal disorder particularly prevalent in the past decades. Its incidence is increasing globally and, as a result, secondary complications are also mounting. The most devastating complication of diabetes mellitus is diabetic foot ulcer (DFU). It is estimated that 15% of diabetic patients will suffer from DFU at least once in their lifetime and, if not proper care is taken, it can cause severe harm to the patient as amputation being a possible outcome in extreme cases[(a)]. Usually these wounds are colonized by pathogenic bacteria and infection is facilitated by patients' immunological deficits[(b)]. Among the bacteria present at the wound site, *Staphylococcus aureus* is the most prevalent bacterial species[(c)]. Typically, the conventional treatment comprises the use of systemic antibiotics, however, due to the limited lack of blood supply at the wound surface, this will produce limited results and it may ultimately increase antibiotic resistance and the risk of drug-related toxic effects. As such, the use of topical antibiotics appears as an alternative towards systemic ones, especially when combined with adjuvants that enhance their activity and minimize or even block resistance. Plants are an important source of such adjuvant compounds that not only potentiate antibiotics but also possess numerous therapeutic properties[(d)]. Therefore, in this study three different phytochemicals were selected - trigonelline, chalcone and juglone and their potentiation effect on fusidic acid and mupirocin antibiotics against seven clinical isolates of *S. aureus* (MSSA and MRSA isolates) was evaluated through modified disc diffusion method. Firstly, the minimal inhibitory and bactericidal concentrations were determined for phytochemicals by a microtiter plate microdilution method. For disc diffusion experiment, phytochemicals were tested at MIC/sub-MIC and antibiotics according to the CLSI guidelines (200 µg/disc for mupirocin and 10 µg/disc for fusidic acid). The results show that the phytochemicals only potentiated the effect of mupirocin. Chalcone, with a concentration 10× and 20× less than MIC, potentiated against one MSSA and one MRSA isolates, while trigonelline (at 1 mg/mL as the MIC was not determined) potentiated against one MSSA and two MRSA isolates. Juglone, at MIC, potentiated against only one MRSA isolate. Overall, an improvement of the activity of mupirocin against some of the *S. aureus* strains was verified, revealing the potential for the topical application of phytochemicals as adjuvants to the antibiotics to combat multi-drug resistant wound infections.

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[(c)] Kadam, S., et al. Biomedicines 7.2 (2019): 35.
[(d)] Borges, A.; et al. Molecules 21.7 (2016): 877.

*Correspondence: mvs@fe.up.pt

Improvement of the activity of topical antibiotics against *Staphylococcus aureus* isolated from diabetic foot wounds

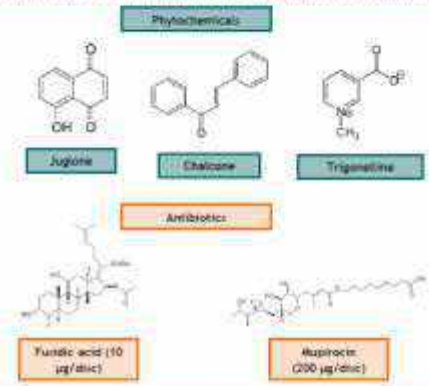
Diana Oliveira[1,2,3], Anabela Borges[1,2], Maria J. Saavedra[4], Fernanda Borges[3], Manuel Simões[1,2]

[1] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[2] DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[3] CIQUP - Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal
[4] CECAV-Veterinary and Animal Science Research Center, Veterinary Science Department, University of Tras-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal

Introduction

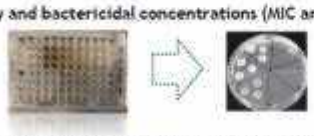
Diabetic foot ulcers (DFUs) are one of the most devastating complications of diabetes mellitus. It is estimated that 15% of the diabetic patients will suffer from a DFU at least once in their lifetime[1]. This condition, if not proper care is provided, may lead to infection that, in turn, may cause amputation or even death[2]. Typically, the conventional treatment comprises the use of systemic antibiotics, however, and due to the limited lack of blood supply at the wound surface, this will produce limited results and increased antibiotic resistance. Among the bacteria present at the wound site, *Staphylococcus aureus* is the most prevalent bacterial species[3]. Due to the increasingly frequent appearance of resistant microorganisms, it becomes urgent the search for new alternatives. So, it is proposed the use of topical antibiotics as an alternative towards systemic ones, specially in combination with natural adjuvants to enhance the antibiotics' activity and minimize or even block resistance[4]. For that, the effects of three different phytochemicals, trigonelline, chalcone and juglone, and their potentiation effect on fusidic acid and mupirocin antibiotics were evaluated against seven clinical isolates of *S. aureus* (MSSA and MRSA isolates).

Materials & Methods

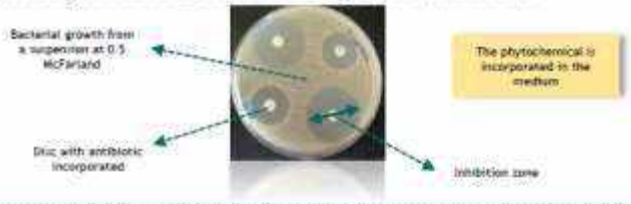


Bacterial strains: MSSA and MRSA clinical isolates (SA102, SA109, SA110, SA111, Xu212, 11998, RH4220)

Minimum inhibitory and bactericidal concentrations (MIC and MBC)



Antibiotic/Phytochemical dual combination assay - disc diffusion method



Results

Antibacterial activity (MIC and MBC)

For all the tested strains, juglone presented the lowest MIC value (12.5 µg/mL). Chalcone had MIC of 100 µg/mL, while for trigonelline the MIC value was not found. Both chalcone and trigonelline did not exhibit bactericidal effects for the range of concentrations tested (6.25-1000 µg/mL). The MBC value of juglone varied between 25 µg/mL and 50 µg/mL for the different strains.

Effect of the phytochemicals on the antibiotic activity

Chalcone at sub-MIC concentrations potentiate the effect of mupirocin against SA109 and SA111 strains. Trigonelline also potentiate the effect of mupirocin, however, with exception of SA109, the strains differ (SA110 and 11998). With respect to RH4220 strain, which was the one with the most marked effect, the potentiation of mupirocin occur with almost all the phytochemicals except trigonelline. All these results are shown in **Table 1**.

Table 1- Classification of the results for the combinatorial experiments between the different tested phytochemicals and antibiotics.

Antibiotic	Phytochemical	Concentration Antibiotic (µg/disc)/Phytochemical (µg/mL)	SA102			SA109			SA110			SA111			Xu212			11998			RH4220		
			ØDa (mm)	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C	ØDa	ØDa+p (mm)	C
Fusidic acid	Chalcone	10/10	21.8	21.8	-	22.0	24.5	-	20.0	22.5	-	23.0	27.8	+	0	0	-	19.0	20.3	-	0	0	-
		10/5		25.5	+		24.8	-		21.3	-		24.8	-		0	-		22.0	-		0	-
	Juglone	10/1.25		26.0	+		21.5	-		20.3	-		23.8	-		0	-		19.0	-		0	-
	Trigonelline	10/1000		24.0	-		24.0	-		22.8	-		24.3	-		0	-		21.5	+		0	-
Mupirocin	Chalcone	10/10	21.8	25.5	+	32.2	50.0	++	31.4	34.9	-	30.3	35.5	++	30.0	35.0	+	30.4	35.0	+	2.6	17.5	++
		10/5		24.3	-		37.8	++		35.2	+		33.8	-		35.0	+		35.0	+		16.3	++
	Juglone	10/1.25		24.0	+		35.0	+		34.3	-		32.0	-		32.5	-		33.0	-		18.0	++
	Trigonelline	10/1000		22.8	-		37.8	++		37.8	++		22.8	-		26.3	+		36.8	++		7.3	+

Classification (C): **Potentiation (++)**: (ØDa+p -ØDa) ≥ 6 mm; **Additive (+)**: 4< (ØDa+p -ØDa) < 6 mm; **Negative (-)**: (ØDa -ØDa+p) ≥ 6 mm; where ØD- inhibition zone diameter, a- antibiotic and p- phytochemical.

Conclusions

The phytochemicals appear as relevant mupirocin adjuvants, demonstrating great potentiation effects. These are promising results as topical formulations of mupirocin with natural compounds might be used to combat multi-drug resistant diabetic foot wound infections.

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Acknowledgments

This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy - LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); - PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219; POCI-01-0247-FEDER-035234; POCI-01-145-FEDER-006939, funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through CEECIND/01261/2017 (Anabela Borges), SFRH/BD/138217/2018 (Diana Oliveira) and SFRH/BSAB/150379/2019 (Manuel Simões).

Conception of new NDM-1 inhibitors for antibiotic treatment by combination of FBDD, docking, synthesis and NMR screening evaluation

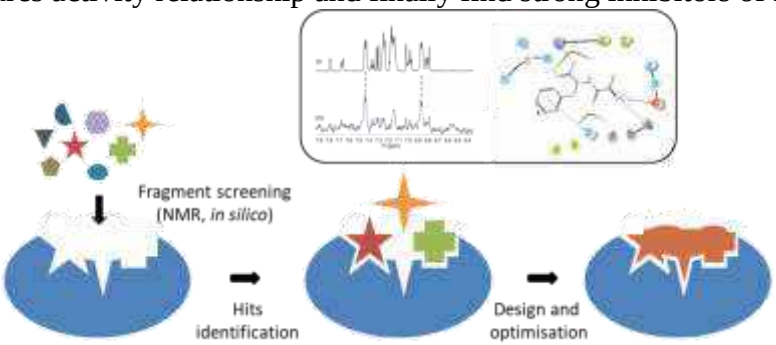
Jérémy CABURET[(1, 2)*], Serge CROUZY[† (2)], Benjamin BOUCHERLE [(1)], Marine PEUCHMAUR [(1)].

(1) Univ. Grenoble Alpes, CNRS, DPM, Grenoble, France
(2) Univ. Grenoble Alpes, CEA, CNRS, IRIG, CBM, Grenoble, France

FP 15

Antibiotic resistance remains currently a major public health problem[(a)]. One of the most problematic mechanism of resistance is the enzymatic hydrolysis of antibiotics of the β-lactams family. The New-Delhi Metallo-betalactamase-1 (NDM-1) is a protein with two zinc atoms in its active site. It can cleave a large part of beta-lactams, even carbapenems which are the last line of antibiotics[(b)]. Since its discovery in 2009, no inhibitor was marketed thus making NDM-1 a major target to fight antibiotic resistance. Our strategy to find new inhibitors of this enzyme is based on Fragment Based Drug Design (FBDD)[(c)] in combination with docking, NMR screening, and synthesis and bio-assays.

Virtual screening of 800,000 molecules allowed us to identified 30 fragments as potential hits for the inhibition of NDM-1. These fragments were synthesized and analyzed by STD NMR experiments in presence of the protein. About 30 % have been confirmed as actual ligands of NDM-1. In parallel, a part of the library of the "Centre de RMN à Très Haut Champs" (150 fragments) were also screened by NMR, giving 30 more hits. A correlation between all results (virtual screening, NMR and bibliography) highlighted two major fragments. We are now setting up a FBDD strategy to design and synthetize molecules by linking active fragments together and/or using growing methods. The newly designed structures will then be tested by bio-assays to carry out structures activity relationship and finally find strong inhibitors of NDM-1.



Bibliographic references:

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[(b)] : Yong D. *et al, A.A.C.,* 2009, *53,* 12, 5046 – 5054.

[(c)] : Bancet A. *et al, J. Med. Chem.,* 2020, *63*, 11420 - 11435.

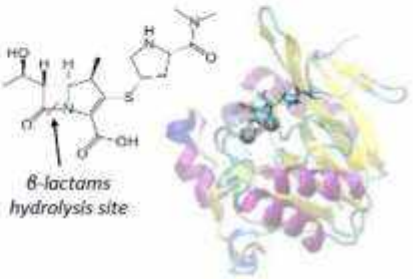
* Correspondence: jeremy.caburet@univ-grenoble-alpes.fr

Conception of new NDM-1 inhibitors for antibiotic treatment by combination of Fragment-Based Drug Design, docking, synthesis and NMR screening evaluation

CABURET Jeremy (1,2), CROUZY Serge (1), MOREAU Hélène (2), BOUCHERLE Benjamin (1), PELUCHARLIK Marine (1)
(1) Univ. Grenoble Alpes, DPM, Grenoble, France
(2) Univ. Grenoble Alpes, CEA, DRF(I), IRIG, CBM, Grenoble, France

GENERAL CONTEXT

Antibiotic resistance is a major public health problem. Bacteria developed several processes of defense to counteract antibiotics. One of the most problematic mechanism is the hydrolysis of β-lactams by New-Delhi Metallo-betalactamase-1 (NDM-1). This enzyme, identified in 2008, possesses two zinc atoms in its active site. NDM-1 can cleave all β-lactams even carbapenems which are the last line of antibiotics. This protein is considered as a very pertinent therapeutic target as, since its discovery, no effective inhibitor was marketed.



1. Introduction: FBDD strategy

Our strategy to find new inhibitors is based on Fragment Based Drug Design (FBDD) including docking, NMR screening, synthesis and bio-assays. Using known inhibitors of NDM-1 (**A**), a 3D model of the protein was elaborated to carry out virtual screening (**B**). Then, the best molecules were used to identified fragments, which were synthesized (**C**) and screened simultaneously with the CRMN fragment library (**E**) by NMR to find NDM-1 ligands (**D**). From these results, fragments modulations and/or combinations have been undertaken assisted by molecular modelling (**F**) to find NDM-1 potent inhibitors. Finally, structure-activity relationships obtained from bio-assays (**G**) will allow design and synthesis of fine tuned molecules.

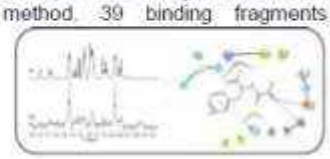
2. Molecular modelling fragments identification

To identify fragments which can potentially binds to NDM-1, a virtual screening of 800,000 molecules was realized using Glide and GOLD software. Different steps of selection allowed us to identify the 375 best molecules. Using two software, eMolFrag and DataWarrior, associated to a visual inspection, 30 fragments potentially ligands of NDM-1 were designed and synthesized.

800,000 Molecules → 375 Molecules → 30 Fragments

3. Fragments NMR screening

120 fragments of the CRMN library, combined to the 30 previously synthesized, were screened in presence of the protein by NMR using Saturation Transfer Difference method. 39 binding fragments were identified.

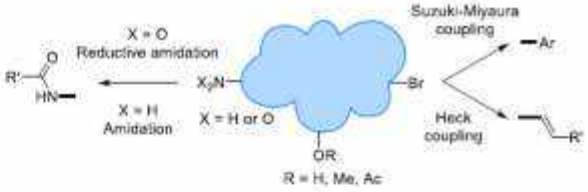


4. Pharmacophore determination

Starting from results of NMR and virtual screenings, a pharmacophore structure was defined using Phase software. This structure was checked through comparison with already known NDM-1 inhibitors. Two fragments binding to Zn ion presents in the active site were selected for further development.

5. From fragments to molecules

Fragments are currently modulated on various positions based on structural information deriving from molecular modelling. Types of link, flexibility and polarity of the incorporated groups were varied to explore the catalytic pocket volume.



CONCLUSION / PERSPECTIVES

Data compilation from NMR and virtual screenings provide a pharmacophoric structure to undertake further design of new potent NDM-1 ligands. The first fragment modulation is now being finalized. Biological evaluations will start early in the development process to fine tune pharmacological properties of our compounds targeting a protein involved in bacterial resistance. These inhibitors will be tested in association with various antibiotics on resistant bacteria strains to evaluate their ability to restore their sensitivity to treatment.

Département de pharmacochimie moléculaire - DPM
Contact: jeremy.caburet@univ-grenoble-alpes.fr

Acknowledgement: Labex Arcane for funding (ANR-17-EURE-0003)

Photodegradation of pharmaceutical contaminants using phthalocyanine–grafted titanium dioxide nanoparticles

Joanna Musiał [(1)*], Rafał Krakowiak [(2)], Dariusz T. Mlynarczyk [(2)], Robert Frankowski [(3)], Agnieszka Zgoła-Grześkowiak[(3)], Tomasz Gośliński [(2)], Beata Stanisz [(1)]

(1) Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
(2) Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
(3) Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznań, Poland

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Frequent use of pharmaceuticals and their improper disposal increasingly contribute to environmental pollution [(a)]. Ibuprofen and naproxen, commonly used nonsteroidal anti-inflammatory drugs, are usually not completely removed in the wastewater treatment process [(b,c)]. To address this problem, we attempted to use photocatalysis to develop a new effective method for water remediation [(d)].

For this purpose, copper(II) phthalocyanine (CuPc) and zinc(II) phthalocyanine (ZnPc) were deposited on titanium dioxide nanoparticles (40 nm), yielding two types of photocatalytic materials: CuPc@TiO_2 and ZnPc@TiO_2. Briefly, TiO_2 nanoparticles were suspended in a Pc solution in dichloromethane. The mixture was left overnight stirring, the solvent was evaporated and the resulting solid dried at room temperature.

The photocatalytic experiment was carried out in a reactor composed of three vessels containing aqueous ibuprofen or naproxen solution (both 10 mg/L) and the photocatalytic material. The mixtures were stirred and irradiated with three UV lamps (λ_{max} = 365 nm), positioned on a circular line. After the irradiation started, samples were taken at six and seven timepoints for naproxen and ibuprofen, respectively. The concentration of the drugs in the reaction medium was determined using HPLC-MS/MS.

Ibuprofen and naproxen were photocatalytically degraded according to the first-order kinetics. When CuPc@TiO_2 was used, 92% of ibuprofen was removed after six hours of the experiment, whereas in the case of NAP ZnPc@TiO_2 displayed the highest effectiveness, resulting in 94% degradation of naproxen within one hour of the experiment.

This study was supported by the National Science Centre, Poland under grant number 2016/21/B/NZ9/00783.

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*** Correspondence**: joanna.musial@student.ump.edu.pl

Photodegradation of pharmaceutical contaminants using phthalocyanine-grafted titanium dioxide nanoparticles

Joanna Musiał [1]*, Rafał Krakowiak [2], Dariusz Młynarczyk [2], Robert Frankowski [3], Agnieszka Zgoła-Grześkowiak [3], Tomasz Gośliński [2], Beata Stanisz [1]

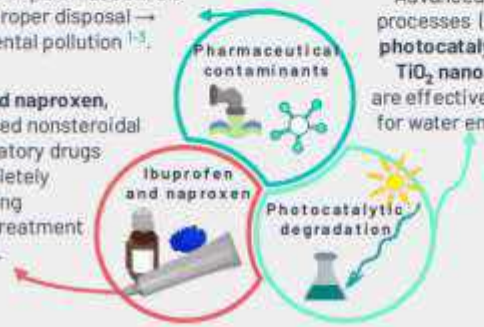
*e-mail: joanna.musial@student.ump.edu.pl

(1) Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
(2) Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
(3) Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznań, Poland

1. BACKGROUND

Frequent use of pharmaceuticals and their improper disposal → → environmental pollution [1-3].

Ibuprofen and naproxen, commonly used nonsteroidal anti-inflammatory drugs are not completely removed during wastewater treatment processes [4,5].



Advanced oxidation processes (AOP), e.g. **photocatalysis using TiO_2 nanoparticles**, are effective methods for water emediation.

2. MATERIALS AND METHODS

1. PREPARATION OF THE PHOTOCATALYTIC MATERIAL

nano- TiO_2 (40 nm) was suspended in a solution of
- copper(II) phtalocyanine (**CuPc**)
- zinc(II) phtalocyanine (**ZnPc**)

in dichloromethane (DCM)

→ the mixture was stirred overnight
→ DCM evaporated
→ resulting solid dried at room temperature

2. PHOTODEGRADATION

Ibuprofen or naproxen solution 10 mg/L

λ_{max} = 365 nm

3. LC-MS/MS ANALYSIS

4. TOXICITY ASSESSMENT

bacterial suspension used: *Aliivibrio fischeri*

Icons: Smart.servier.com, Flaticon.com

3. RESULTS

During the photodegradation of ibuprofen, CuPc@TiO_2 displayed the highest effectiveness, resulting in 92% removal of the drug within six hours. In the case of naproxen, 94% of the drug was removed using ZnPc@TiO_2. ZnPc@TiO_2 retained its activity after three consecutive experiments.

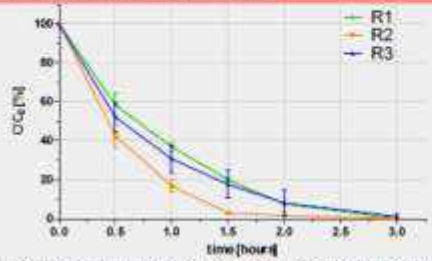


Fig. 2. Changes in naproxen concentration after three consecutive degradation cycles (UV irradiation) of the solution containing ZnPc@TiO_2.

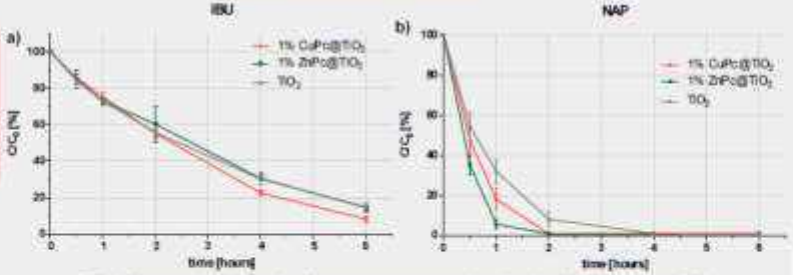
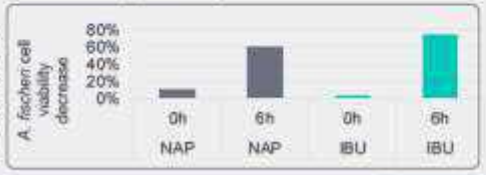


Fig. 1. Changes in a) ibuprofen, b) naproxen concentration after irradiation (365 nm light) of the solution containing a photocatalyst.

Fig. 3. Cell viability decrease of *A. fischeri* cells upon addition of naproxen and ibuprofen solutions before (0 h) and after (6 h) photodegradation experiment with ZnPc@TiO_2, when irradiated with UV light (λ_{max} = 385 nm).



4. CONCLUSIONS

Using UV radiation and photocatalytic materials, CuPc@TiO2 and ZnPc@TiO2, it is possible to completely degrade the ibuprofen and naproxen content.

The degradation of the drugs occurs according to the first-order kinetics.

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SCT Young Research Fellows Meeting
February 11-12, 2021

The experimental literature review was carried out using Reaxys

This study was supported by the National Science Centre, Poland, under grant number 2016/21/B/NZ9/00783

Elaboration of pharmaceutical interest nanosized crystals for co-administration and bioavailability improvement of drugs

Panpan Ma (1)*, René Laï-Kuen (2), Nathalie Mignet (1), Yohann Corvis (1).

(1) Université de Paris, CNRS, Inserm, UTCBS, Faculté de Pharmacie, 4 avenue de l'Observatoire, F-75006 Paris, France.
(2) Université de Paris, CNRS, Inserm, Cellular and Molecular Imaging Technology Platform, 4 avenue de l'Observatoire, F-75006 Paris, France.

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Solubility enhancement of poorly water-soluble drugs is a major challenge in the pharmaceutical field since 80% of the marketed drugs are formulated in the solid form, and since more than 70% of the drugs exhibit low solubility. A broad and comprehensive study of the various solid forms of the matter is needed to enhance its translation to clinic.[(a)] The different possibilities of a certain active pharmaceutical ingredient (API) can notably influence its properties, such as physical, chemical, and biopharmaceutical properties.[(b)] Therefore, the most suitable solid form has to be taken into consideration regarding *ex-* and *in-vivo* stability, targeting, solubility, dissolution rate, and bioavailability. With the development of nanotechnology in recent years, nanocrystals (NCs) have been used to modify the therapeutic impact of an API. They are recognized as carrier-free submicron colloidal drug delivery systems with an average particle size with the nanometer range. The emergence of drug nanocrystals formulation in the last 10 years proved that size reduction is a challenging, improving both drug loading capacity and bioavailability of active ingredients.[(a)] Moreover, nanocrystal formulations can be administrated through several routes and provide a better performance in term of safety, targeting efficiency, and pharmacokinetics.[(c)]

The UTCBS group has been recently developed the bottom-up approach to engineer etoposide (ETO) NCs, controlling the crystallization process.[(d)] The results obtained reinforce the legitimacy of drug NCs as potent forthcoming delivery systems for nanomedicines through intravenous routes and open the way to potential marketability of NCs using co-precipitation. Synthesized ETO NCs were first evaluated for *in vivo* studies on murine subcutaneous carcinoma model. It was shown that ETO NCs were more effective and safer than the conventional marketed product Toposar®. We propose here to embrace the advent of nanomedicine providing significant advancements to the development of new therapeutic treatments, since it offers the design of new properties and therapeutic strategies, such as co-administration, minimizing both administration protocol and potential side effects of drugs. For that purpose, prednisolone (PRD), a synthetic cortisol compound was chosen to extend the nanocrystallisation protocol to other APIs on the one hand and propose ETO-PRD nanocrystal based formulations on the other hand. By controlled variables technics and/or conditions, the proportions of API and stabilizing agent were adjusted to screen out the best formulation of nanocrystal with optimized yield and long-term stability.

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* Correspondence: panpan.ma@etu.parisdescartes.fr

Elaboration of pharmaceutical interest nanosized crystals for co-administration and bioavailability improvement of drugs

Panpan Ma[1], René Lai-Kuen [2], Nathalie Mignet[1], Yohann Corvis[1]

[1]Université de Paris, CNRS, Inserm, Chemical and Biological Techniques for Health (UTCBS), Faculté de Pharmacie, 4 avenue de l'Observatoire, F-75006 Paris, France

[2]Université de Paris, CNRS, Inserm, Cellular and Molecular Imaging Technology Platform, Faculté de Pharmacie, 4 avenue de l'Observatoire, F-75006 Paris, France

* Correspondence: panpan.ma@etu.u-paris.fr

Introduction and scientific objectives

95% of drugs currently in development present low solubility (*i.e.* 70% and 25% of Biopharmaceutics Classification System class II and IV, respectively) [1]. Therefore, their solubility enhancement represents a major challenge for the pharmaceutical industry. Consequently, physicochemical study of the drug matter is needed to promote its translation to clinic [2]. Differences in the organization of an active pharmaceutical ingredient (API) can notably influence its properties such as the biopharmaceutical properties [3]. Nanocrystals (NCs) have been engineered to enhance the therapeutic impact of an API by increasing the its loading and reducing the overall ingredients of a given formulation. Besides, nanocrystal formulations can be administrated to patients through various routes and provide improved efficiency regarding pharmaceutical benefit over risk ratio (*i.e.* bioavailability, targeting, and pharmacokinetics) [4]. The UTCBS pioneered the bottom-up approach to engineer etoposide (ETO) NCs, controlling the crystallization process, [4] which allows: *i/* reinforce the legitimacy of drug NCs as potent forthcoming delivery systems for nanomedicines through intravenous routes, *ii/* open the way to potential marketability of NCs using co-precipitation, and *iii/* more effective and safer than the conventional marketed product such as Toposar®, since synthesized ETO NCs have been evaluated via in vivo studies on murine carcinoma model. Our actual work is to prolong and enhance this effect by a concomitant activation with two APIs. Prednisolone (PRD), a synthetic cortisol compound was chosen to extend the nanocrystallisation protocol to other APIs on the one hand and propose ETO-PRD nanocrystal-based formulations on the other hand. By controlled variables technics and/or conditions, the proportions of API and stabilizing agent were adjusted to screen out the best formulation of nanocrystal with optimized yield and long-term stability.

Experimental protocol

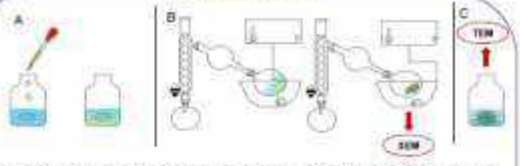


Figure 1. Bottom-up synthesis based on the drop-wise addition of a drug solubilized in an organic phase to a P407/P188 aqueous phase in which the drug is insoluble (A). Then, evaporation (B) followed by dispersion in aqueous solvent is needed to form NCs in suspension (C).

The drug/surfactant ratio has been screened to optimize the nanocrystal production.

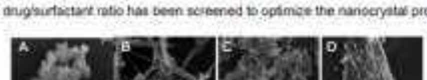
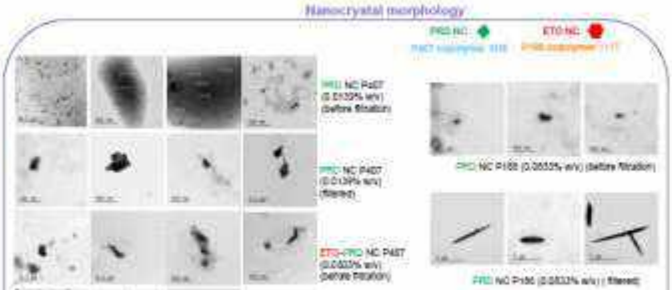


Figure 2. Scanning Electron Microscopy (SEM) images of aggregated raw PRD (A), PRD after organic solvent/water evaporation (B), PRD NC P407, 0.0139% w/v after water evaporation (C) and ETO-PRD NC P407, 0.0833% w/v after water evaporation (D).

Nanocrystal morphology



The TEM images deepen understanding the role of copolymer as stabilizing agent.

Figure 3. Transmission Electron Microscopy (TEM) images of PRD NC and ETO-PRD NC stabilized with P407 or P188 in water.

Stability evaluation of PRD nanocrystals stabilized with P407 or P188

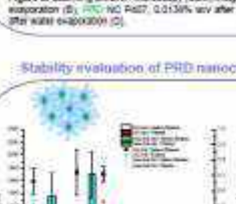


Figure 4. Size distribution and polydispersity (PDI) of PRD NC stabilized with P407 by Dynamic Light Scattering (DLS) and Nano-Kit.

Figure 5. Size distribution and PDI of PRD NC stabilized with P188 by DLS and Nano-Kit.

Table 1. Yield of obtention of PRD NC stabilized with P407 or P188 as a function of copolymer content

Formulation	PRD Yield (%)	
	Before filtration	Filtered
PRD NC P407 [illegible]	79.76 ± 1.82 %	77.18 ± 4.03 %
PRD NC P407 [illegible]	84.66 ± 2.58 %	55.78 ± 1.19 %
PRD NC P407 [illegible]	62.72 ± 1.16 %	70.90 ± 11.13 %
PRD NC P407 [illegible]	86.44 ± 1.09 %	73.99 ± 3.07 %
PRD NC P407 [illegible]	86.81 ± 1.26 %	74.25 ± 4.56 %
PRD NC P188 [illegible]	75.96 ± 4.77 %	64.18 ± 2.06 %
PRD NC P188 [illegible]	42.39 ± 1.94 %	74.22 ± 0.83 %
PRD NC P188 [illegible]	78.26 ± 1.96 %	69.02 ± 3.11 %

Stability of PRD NC formulations with P407 and P188: at least 120 days and 50 days, respectively.

Size distribution, stability and yield evaluation of ETO-PRD nanocrystals stabilized with P407

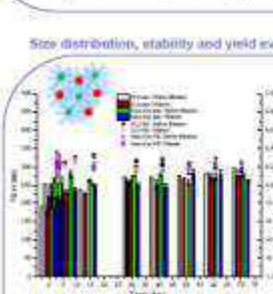


Figure 6. Size and PDI of ETO-PRD NC stabilized with P407(0.0833% w/v) over time.

Table 2. Yield of ETO-PRD NC P407(0.0833% w/v)

	ETO Yield (%)	PRD Yield (%)	Sample preparation prior UV-vis
Before filtration	46.41 ± 2.75%	59.03 ± 1.77%	Total evaporation + MeOH solubilization
Filtered	42.09 ± 2.48%	58.08 ± 4.03%	
Before filtration	49.58 ± 2.60%	64.30 ± 5.59%	Solubilization of the NC in MeOH (2mL H2O:MeOH volume ratio 1:9)
Filtered	48.27 ± 2.96%	61.46 ± 8.14%	

From the comprehensive comparison of the above results, the most suitable formulation is: 1:1 mass ratio of ETO-PRD, 5-10 drug-stabilizer mass ratio, and 0.0833% w/v of stabilizer in water.

Stability test performed with the ETO-PRD formulations did not show any significant changes in size, PDI and zeta potential data for at least 70 days.

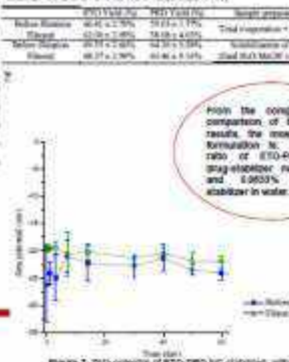


Figure 7. Zeta potential of ETO-PRD NC stabilized with P407(0.0833% w/v) over time.

Conclusions and perspectives

- Nanocrystal preparation for both prednisolone and etoposide-prednisolone formulations was optimized. Further, *in vitro and in vivo* evaluation of the nanocrystalline formulations will be tested as codelivery tool.
- Given nanocrystalline suspensions reaching FDA approval in all aspects of applications, these results obviously demonstrate that co-administration form has the potential to embrace the advent of nanomedicine providing significant advancements to the development of new cancer treatments.

[1] [illegible]
[2] [illegible]
[3] [illegible]
[4] [illegible]

(SIPr)Pd(allyl)Cl Complex for Microwave-Irradiation C-H Arylation and Suzuki-Miyaura Reactions of N-Heteroarenes

Chaimae Ouazzani Chahdi [(1), (2)]*, Abdelmoula El Abbouchi [(2)], Jamal Koubachi [(1)], Nabil El Brahmi [(2)], and Saïd El Kazzouli [(2)]

(1) Faculté Polydisciplinaire de Taroudant, B.P 271, 83000 Taroudant, Laboratoire de Chimie Appliquée et Environnement (LACAPE), Faculté Sciences, Université Ibn Zohr d'Agadir, Morocco

(2) Euromed Research Center, School of Biomedical and Biotechnology, Euromed University of Fes (UEMF), Route de Meknes, 30000 Fes, Morocco

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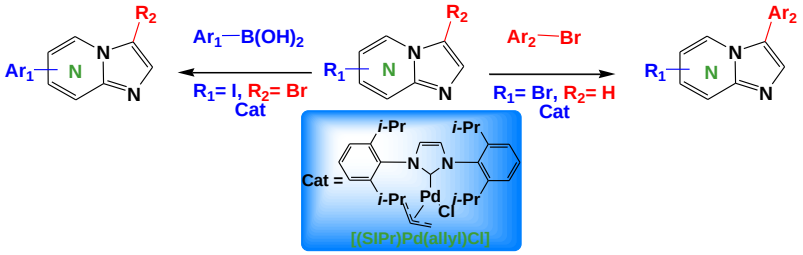
N-Heterocyclic carbenes (NHCs) have received a great deal of attention from a number of researchers over the past few decades [(1)]. The [Pd(NHC)] complexes have been employed as catalysts for Suzuki Miyaura coupling, Buchwald-Hartwig amination reaction, Kumada coupling, Sonogashira coupling, Heck reactions, Stille coupling, dehalogenation reaction, ketone arylation reactions as well as C-H arylation reactions[(2)]. In this area, our group has a long-standing interest in C-H activation of 6, 5-fused heterocyclic systems [(3)]. In this work we decided to focus on an efficient direct arylation and Suzuki-Miyaura coupling of *N*-Heteroarene derivatives with various aryl halides or aryl boronic acids as coupling partners[(4)]. Under the assistance of microwave. The use of phosphine-free (SIPr)Pd(allyl)Cl as the catalyst lead to substituted *N*-Heteroarenes in good to excellent yields



Keywords: Direct arylation; Suzuki-Miyaura coupling; *N*-Heteroarenes; Microwave-irradiation.

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* Correspondence: E-mail: chaimaeouazzani1996@gmail.com

(SIPr)Pd(allyl)Cl complex for microwave-irradiation C-H Arylation and Suzuki-Miyaura reactions of N-Heteroarenes

C. Ouazzani Chahdi [a,b], A. El Abbouchi [b], J. Koubachi [a], N. El Brahmi [b] and S. El Kazzouli [b]

[a] Faculté Polydisciplinaire de Taroudant, B.P. 271, 83000 Taroudant, Laboratoire de Chimie Appliquée et Environnement (LACAPE), Faculté Sciences, Université Ibn Zohr d'Agadir, Morocco
[b] Euromed Research Center, Euromed Faculty of Engineering, Euromed University of Fez (UEMF), Route de Meknes, 30000 Fes, Morocco

For correspondence: Email: chaimaeouazzani1996@gmail.com

1. Introduction

The *N*-containing heterocycles compound system such as imidazo[1,2-*a*]pyridines derivatives were reported as e.g., melatonin receptor ligands[1], ligand for detecting β-amyloid[2a], cyclin-dependent kinases (CDK) inhibitors[2b], as well as GABAA receptor modulator[2c],...

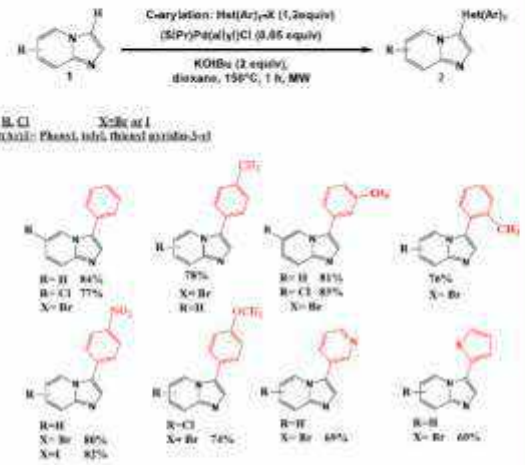
N-Heterocyclic carbenes (NHCs) have received a great deal of attention from a number of researchers over the past few decades [3]. The [Pd(NHC)] complexes have been employed as catalysts for Suzuki-Miyaura coupling, Buchwald-Hartwig amination reaction, Kumada coupling, Sonogashira coupling, Heck reaction, Stille coupling, dehalogenation reactions, ketone arylation reactions as well as *C*-H arylation reactions[4]. In this area, our group has a long-standing interest in *C*-H activation of 6,5-fused heterocyclic system[5].

In this work we decided to focus on an efficient direct arylation and Suzuki-Miyaura coupling of *N*-Heteroarenes derivatives with various aryl halides or aryl boronic acids as coupling partners[6]. The use of phosphine-free (SIPr)Pd(allyl)Cl as the catalyst lead to substituted *N*-Heteroarenes in good to excellent yields during 1 hour under the assistance of microwave (Scheme 1).

Scheme 1

2. Direct *C*-arylation of imidazo[1,2-*a*]pyridines under microwaves irradiation catalyzed by (SIPr)Pd(allyl)Cl complex

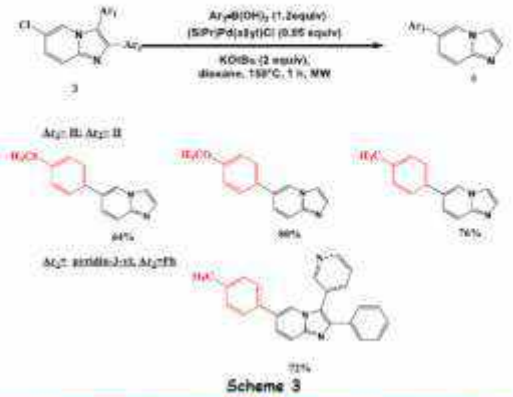
The palladium-catalyzed direct *C*3-(hetero)arylation of imidazo[1,2-*a*]pyridines **1** were achieved in the presence of aryl halides with KOtBu as base and $Pd(OAc)_2$/ PPh_3 as catalytic system. The reactions were carried out under microwave irradiation in dioxane in optimal reaction conditions (at 150 °C). The desired products **2** were isolated in yields ranging between 60% and 84% (Scheme 2).



Scheme 2

3. Suzuki-Miyaura coupling of 6-chloro imidazo[1,2-*a*]pyridines under microwaves irradiation catalyzed by (SIPr)Pd(allyl)Cl complex

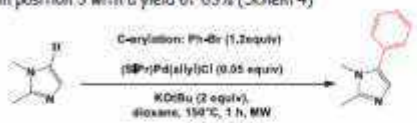
We imported an efficient procedure for synthesis of 6-substitutedimidazo[1,2-*a*]pyridines via a microwave-assisted, Suzuki-Miyaura of 6-chloro imidazo[1,2-*a*]pyridines **3** . The reaction scope was investigated with a variety of aryl boronic acids. The desired products **4** were isolated in good overall yields ranging between 64% and 80% (Scheme 3).



Scheme 3

4. Direct *C*-arylation of1,2 dimethyl1-H imidazole under microwaves irradiation catalyzed by (SIPr)Pd(allyl)Cl complex

To show the capacity of our catalyst we made a direct arylation test on 1,2 dimethyl 1-H imidazole under the same conditions leading to the arylated product in position 3 with a yield of 63% (Schem 4)



Scheme 4

5. Conclusion

We have developed a novel and efficient palladium-catalyzed reactions for the functionalization of the imidazo[1,2-*a*]pyridine system by reactions developed in our laboratory such as Suzuki-Miyaura and direct *C*-arylation. The results obtained by using a relatively new technology provided promising opportunity in different fields of researches of biologically active molecules.

6. references

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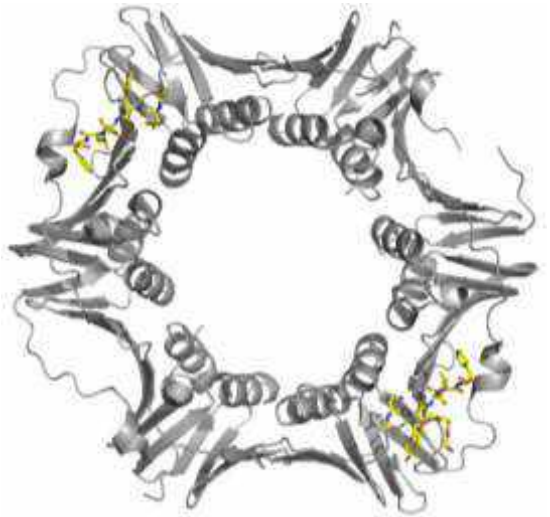
Structure-guided optimization of short peptides inhibiting the *E.Coli* sliding clamp	***FP 19***

Clément Monsarrat[(1)], Christophe André[(1)], Guillaume Compain[(1)], Jérôme Wagner[(2)], Dominique Burnouf[(3)] and Gilles Guichard[(1)]

(1) Chimie et Biologie des Membranes et des Nano-Objets, Université de Bordeaux - CNRS UMR 5248, Institut Européen de Chimie et Biologie 2 rue Robert Escarpit, 33600 Pessac
(2) Biotechnologie et Signalisation Cellulaire, CNRS UMR 7242, Université de Strasbourg, ESBS, 300 Boulevard Sébastien Brant, 67400, Illkirch
(3) Institut de Biologie Moléculaire et Cellulaire, Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, 2, Allée Konrad Roentgen, 67000, Strasbourg

The DNA sliding clamp (SC) or "β-ring" is a protein involved in bacterial DNA replication. This protein serves as an anchoring platform for the replicative DNA polymerases and other enzymes involved in DNA metabolisms.[(a)] The sliding clamp interacts with its partners through protein-protein interactions. This protein is thus essential for the viability of the bacteria and has emerged as a highly promising target for the development of new antibacterial compounds to fight against resistant bacteria.[(b)] In this poster, we will present our recent work aimed at optimizing short peptide sequences to bind to the sliding clamp of Gram negative bacteria. The original design of the peptides was based on the crystal structure of SC complexed to a binding sequence of the bacterial DNA polymerase IV.[(c)] By combining successive rounds of sequence optimization and crystallographic analysis of the complexes, we identified short peptides with much higher affinities for *E.coli* SC (in the nanomolar range) than the natural polymerase.



Crystallographic structure of E.Coli sliding clamp complexed with a lead peptide inhibitor

Bibliographic references:

(a) C. Indiana and M. O'Donnell, *Nat. Rev. Mol. Cell. Biol*, **7**, 751-761 (2006).
(b) A. Amanda S. and K. Zvi, *Front. Mol. Biosci.*, **5**, 87 (2018).
(c) W. Philippe *et al*, J. Med. Chem., **54**, 4627-4637(2011)

STRUCTURE – GUIDED OPTIMIZATION OF SHORT PEPTIDES INHIBITING THE *E.COLI* SLIDING CLAMP

Clément Monsarrat,[1] Guillaume Compain,[1] Christophe André,[1] Jérôme Wagner,[2] Dominique Burnouf[3] and Gilles Guichard[1]

[1] Institut de Chimie et de Biologie des Membranes et des Nano-objets, IECB, Université de Bordeaux, France, clement.monsarrat@u-bordeaux.fr

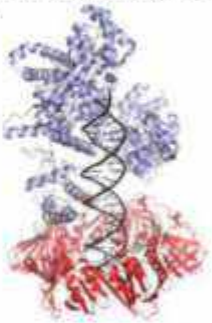
[2] Ecole Supérieure de Biotechnologie de Strasbourg, Université de Strasbourg, France

[3] Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire Université de Strasbourg, France

1- INTRODUCTION

Sliding clamp or « β-ring » : **A promising target for the development of new antibacterial compounds against Gram negative bacteria**

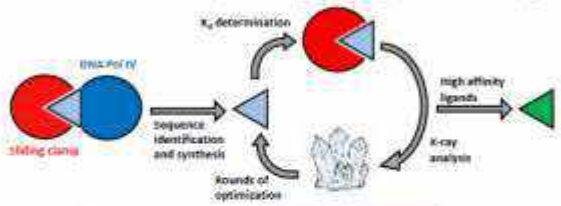
- Involved in **DNA replication** thanks to protein – protein interactions
- Fonctions: used as an anchoring platform for the replicative DNA polymerase (Pol III) and other enzymes involved in DNA metabolisms (Pol II, IV et V). [1,2]
- Sliding clamp is an essential protein for the viability of the bacteria [3]

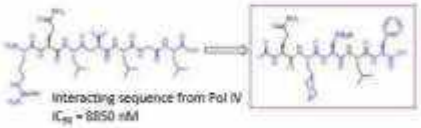


E. Coli sliding clamp (red) in complex with DNA polymerase III (blue) and DNA (black) [4] (PDB ID : 5FKW)

2 - STRATEGY & GOALS

Development of high affinity ligands for *E.Coli* sliding clamp





Synthesis the binding sequence of DNA Pol IV which interacts with the sliding clamp : the starting point for the development of inhibitors [5]

E.Coli sliding clamp with P1 (PDB ID : 6FVL)

➡ P1 = Starting peptide for rounds of optimization

3 - RESULTS

Three positions of peptides were modified : the acetyl group from the N-terminale position, the Asp residue and the Phe residue from the C-terminale position [6]
This study counts about 60 compounds

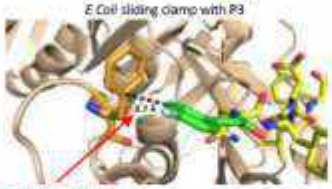
The affinity values (K_D) for the *E.Coli* sliding clamp were determined by isothermal Titration Calorimetry (ITC)

New modifications have been shown to significantly increase the affinity for sliding clamp

X-ray structure analyses

Aromatic group at N-terminale position

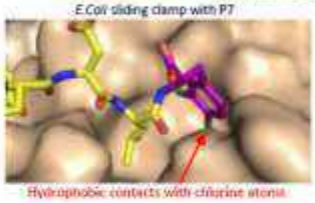
E.Coli sliding clamp with P3



π-stacking interaction

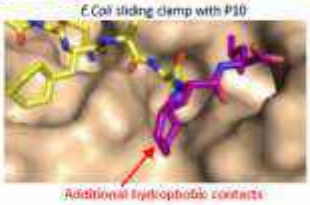
Hydrophobic group at C-terminale position

E.Coli sliding clamp with P7



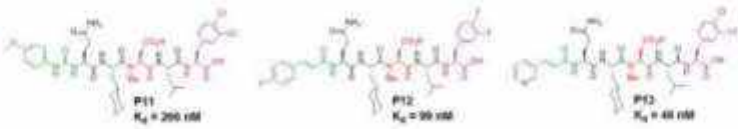
Hydrophobic contacts with chlorine atoms

E.Coli sliding clamp with P10



Additional hydrophobic contacts

Combinations of beneficial modifications



Conclusion :

Short peptides were identified with a much higher affinity (~200 times) for *E.coli* sliding clamp than the original Pol IV interacting sequence. Several single modifications show a significant beneficial effect on the affinity. The combination of beneficial modifications has been shown to increase the affinity in some cases, but was found to be detrimental for other combinations. Therefore, the increase in affinity when combining modifications could not be considered as a general rule.
The high affinity ligands identified with this study are promising compounds to find new antibiotics against Gram negative bacteria.

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Acknowledgements: This work is funded by National Defense Agence (Agence Innovation Défense) and the region Nouvelle Aquitaine

How to build α/β-chimera peptides containing furanoid sugar amino acid?

Kim Hoang Yen Duong[(1)], Viktória Goldschmidt Gőz[(2)], Dániel Horváth[(1)], Viktor Farkas[(2)], and András Perczel[*(1,2)]

[(1)] ELTE Eötvös Loránd University, Institute of Chemistry, Laboratory of Structural Chemistry and Biology - Pázmány P. stny. 1/A, 1117 Budapest, Hungary

[(2)] ELTE Eötvös Loránd University, MTA-ELTE Protein Modelling Research Group - Pázmány P. stny. 1/A, 1117 Budapest, Hungary

FP 20

Abstract

β-Sugar Amino Acids (β-SAAs)[(a)] is of a special interest in building blocks of chimera peptides, foldamers, glycomimetics and peptidomimetics. Fmoc-protected β-SAAs (like Fmoc-RibAFU(ip)-OH[(b)] or Fmoc-GlcAPU(Me,Bn)-OH[(c)]) as structural Lego-elements are ready to use for SPPS[(d)]. Previously we showed that 50% TFA is enough to remove the side chain protection from -RibAFU(ip)- residue[(e)]. Small chimera peptides containing the furanoid β-SAAs were prepared by manual and flow SPPS. Now we tested this new cleavage protocol with short sequences containing common protected, large side-chain protected and aromatic α-amino acids. This condition was sufficient to remove successfully the 1,2-*O*-isopropylidene protection from -RibAFU(ip)- residue as well as the various side chain protections from AA residues. The ratio of anomers of chimera peptide containing -RibAFU- was identified by 2D NMR measurements. Using the new modified cleavage protocol for chimera peptides incorporated the -RibAFU- or -GlcAPU(Me,Bn)- as hydrophilic or hydrophobic building blocks demonstrated that these β-SAAs are routinely used in SPPS.

Bibliographic references:

[(a)] Risseeuw M, Overhand M, Fleet GWJ, Simone MI (2013) A compendium of cyclic sugar amino acids and their carbocyclic and heterocyclic nitrogen analogues. Amino Acids 45:613-689

[(b)] Nagy A, Csordás B, Zsoldos-Mády V, Pintér I, Farkas V, Perczel A (2017) C-3 Epimers of sugar amino acids as foldameric building blocks: improved synthesis, useful derivatives, coupling strategies. Amino Acids 49:223-240

[(c)] Goldschmidt Gőz V, Pintér I, Harmat V, Perczel A (2018) Approaches to pyranuronic β-sugar amino acid building blocks of peptidosaccharide foldamers. Eur J Org Che. 3:355-361

[(d)] Nagy A, Goldschmidt Gőz V, Pintér I, Farkas V, Perczel A (2019) α/β-Chimera peptide synthesis with cyclic β-sugar amino acids: the efficient coupling protocol. Amino Acids 51:669-678

[(e)] Duong KHY, Goldschmidt Gőz V, Pintér I, Perczel A (2020) Synthesis of chimera oligopeptide including furanoid β-sugar amino acid derivatives with free OHs: mild but successful removal of the 1,2-*O*-isopropylidene from the building block. Amino Acids DOI: 10.1007/s00726-020-02923-3

* Correspondence: perczel.andras@ttk.elte.hu

How to build α/β-chimera peptides containing furanoid sugar amino acid?

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[1] ELTE Eötvös Loránd University, Institute of Chemistry, Hevesy György PhD School of Chemistry, Budapest, Hungary
[2] ELTE Eötvös Loránd University, Institute of Chemistry, Laboratory of Structural Chemistry and Biology, Budapest, Hungary
[3] ELTE Eötvös Loránd University, MTA-ELTE Protein Modeling Research Group, Budapest, Hungary
dkhyen@caesar.elte.hu, *perczel.andras@ttk.elte.hu

INTRODUCTION

β-Sugar Amino Acids (β-SAAs)[a] is of a special interest in building blocks of chimera peptides, foldamers, glycomimetics and peptidomimetics. Fmoc-protected β-SAAs (like Fmoc-RibAFU(ip)-OH[b] or Fmoc-GlcAPU(Me,Bn)-OH[c]) as structural Lego-elements are ready to use for solid phase peptide synthesis (SPPS)[d].

OBJECTIVE

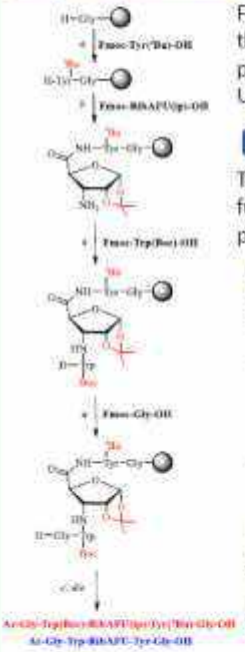
We tested 50% TFA[e] as the new modified cleavage protocol for short sequences containing -RibAFU(ip)- or -GlcAPU(Me,Bn)- and common protected, large side-chain protected and aromatic α-amino acids (AAs).

METHODS

Manual and flow SPPS[f]
Analytical approaches as HPLC, MS, UPLC-MS, NMR

RESULTS AND DISCUSSION

Peptide syntheses

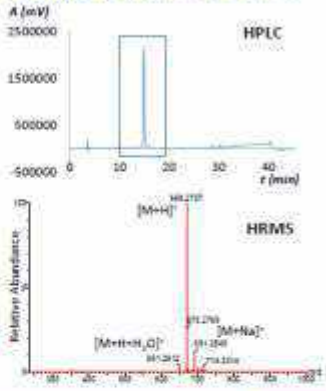


Flow SPPS was faster and resulted the same or better purity of peptides as manual one based on UPLC-MS measurements.

Analytical characterization

The fully protected peptide and fully unprotected peptide were purified and identified.

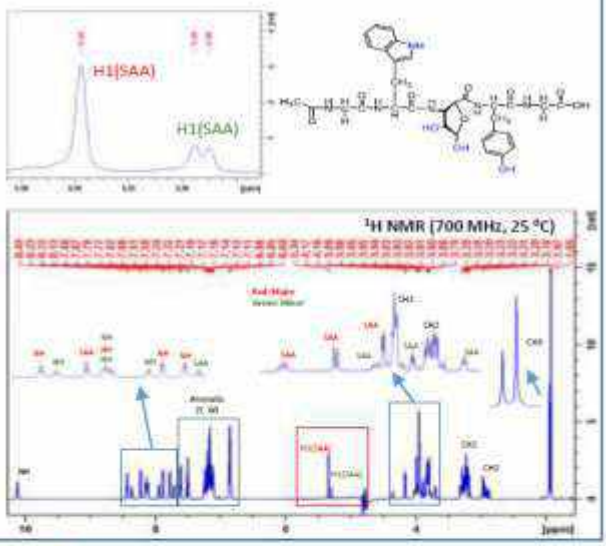
Ac-Gly-Trp-RibAFU-Tyr-Gly-OH



α/β-Anomer identification

The anomers are inseparable by HPLC. The structure of anomers was determined, with detecting ^{1}H signals of two anomeric components H1 sugar ring of peptide.

Ac-Gly-Trp-RibAFU(α/β,7:3)-Tyr-Gly-OH



CONCLUSIONS

The **50% TFA** was sufficient to **remove successfully the 1,2-*O*-isopropylidene protection** from -RibAFU(ip)- residue as well as the **various side chain protections** from AA residues.
The **structure** and **ratio of α/β-anomers** of chimera peptides containing -RibAFU- were identified by 2D NMR.

References

[a] M. Risseeuw, M. Overhand M, G. W. J. Fleet, M. I. Simone (2013) *Amino Acids* 45:613-689
[b] A. Nagy, B. Csordás, V. Zsoldos-Mády, I. Pintér, V. Farkas, A. Perczel (2017) *Amino Acids* 49:223-240
[c] V. Goldschmidt Gőz, I. Pintér, V. Harmat, A. Perczel (2018) *Eur. J. Org. Che.* 3:355-361
[d] A. Nagy, V. Goldschmidt Gőz, I. Pintér, V. Farkas, A. Perczel (2019) *Amino Acids* 51:669-678
[e] K. H. Y. Duong, V. Goldschmidt Gőz, I. Pintér, A. Perczel (2020) *Amino Acids* DOI: 10.1007/s00726-020-02923-3
[f] V. Farkas, K. Ferentzi, K. Horváti, A. Perczel (2021) *Org. Process Res. Dev.* DOI: 10.1021/acs.oprd.0c00178

Acknowledgement

This work was supported by Grant (VEKOP-2.3.2-16-2017-00014) and the purchase of the mass spectrometer was supported by Grant (VEKOP-2.3.3-15-2017-00020) from the European Union and the State of Hungary, co-financed by the European Regional Development Fund.

POSTER

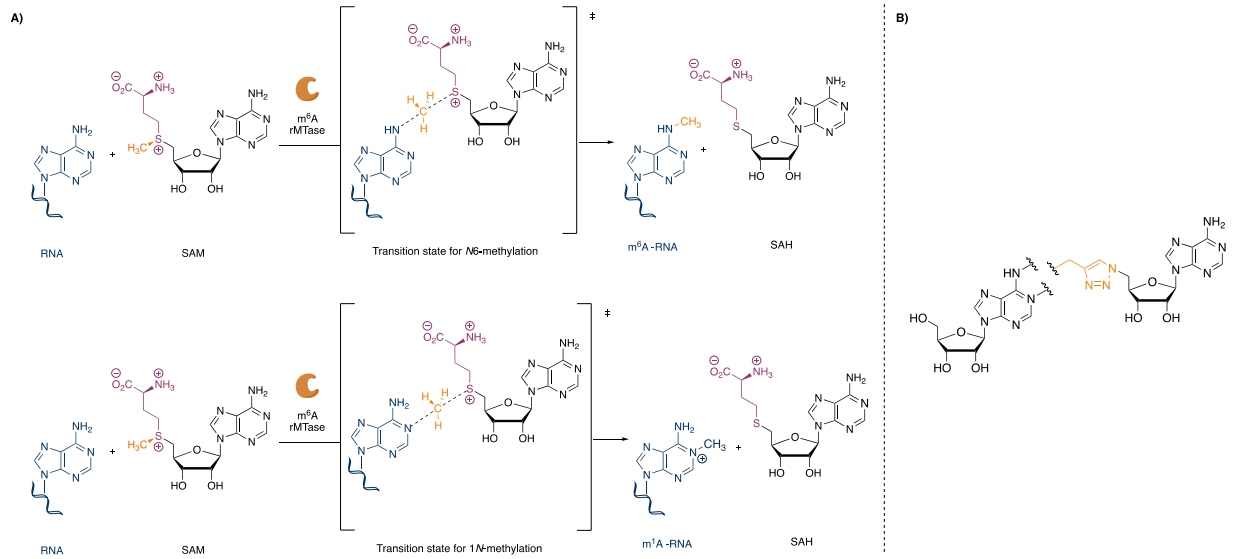
Synthesis of Triazole-Linked SAM-Adenosine Conjugates: Functionalization of Adenosine at N-1 or N-6 Position

Dylan Coelho(1)*, Colette Atdjian (1), Laura Iannazzo (1), Mélanie Ethève-Quelquejeu (1), Emmanuelle Braud (1).

(1) Chemistry of RNA nucleosides, peptides and heterocycles, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR CNRS 8601, Université de Paris, 45 rue des Saints-Pères, 75006 Paris, France

Restricted to organizers

RNAs undergo numerous post-transcriptional modifications regulating their fate and function at the cellular level. Among these modifications, methylation at the N6 position of adenosine (m^6A) in mRNA is crucial for RNA metabolism, stability and other important biological events. Methylation of the N1 position is found mainly in ncRNA and mRNA. This rearrangement plays an essential role in the stability and fidelity of translation. These modifications are installed respectively by m^6A and m^1A methyltransferase RNAs (rMtase). rMtases catalyze the transfer of the methyl group of the *S*-adenosyl-*L*-methionine (SAM) cofactor to the N6 or N-1 position of adenosine. However, rMTases remain little studied, due to a lack of structural data. The RNA recognition process and the molecular mechanism involved in methyl transfer, in particular, remain to be elucidated. In humans, the deregulation of m^6A rMTase activity is associated with many diseases including cancer, neurological or metabolic diseases. [(a)] Here, we report the synthesis of new SAM-adenosine conjugates containing a triazole linker branched at the N-1 or N-6 position of adenosine by CuAAC (Copper(I)-Catalyzed Alkyne-Azide Cycloaddition). The molecules described here were designed as potential bisubstrate analogues for the m^6A and m^1A RNA MTases that could be used for structural studies. [(b)]



Schema. A) rMTases-catalyzed methylation of adenosine at the N-6 and N-1 positions. B) Structure of SAM-adenosine conjugates with a 1,2,3-triazole linker

Bibliographic references:

(a) Batista, P. J. *Genomics Proteomics Bioinformatics* **2017**, *15,* 154-163.

(b) Atdjian, C.; Coelho, D.; Iannazzo, L.; Ethève-Quelquejeu, M.; Braud, E. *Molecules* **2020**, *25*, 3241.

* Correspondence: dylan.coelho@etu.u-paris.fr

Synthesis of Triazole-Linked SAM-Adenosine Conjugates: Functionalization of Adenosine at N-1 or N-6 Position

Dylan Coelho*, Colette Atdjian, Laura Iannazzo, Mélanie Ethève-Quelquejeu, Emmanuelle Braud.

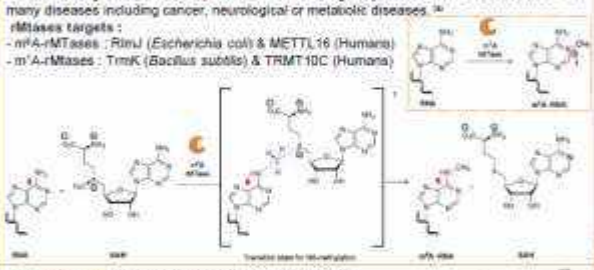
Chemistry of RNA, nucleosides, peptides and heterocycles, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR CNRS 8601, Université de Paris, 45 rue des Saints-Pères, 75006 Paris, France

**dylan.coelho@etu.u-paris.fr*

INTRODUCTION

I-Biological context: RNA methyltransferases (rMtases) catalyze the transfer of the methyl group of the *S*-adenosyl-*L*-methionine (SAM) cofactor to the N-6 or N-1 position of adenosine in RNA. Methylation has an impact on various biological processes and is associated with many diseases including cancer, neurological or metabolic diseases. [a]

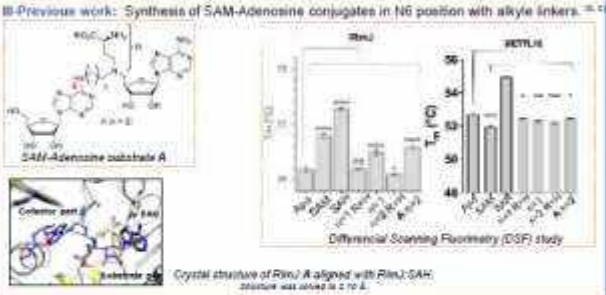
rMtases targets :
- m^{6}A-rMTases : RlmJ (*Escherichia coli*) & METTL16 (Humans)
- m^{1}A-rMtases : TrmK (*Bacillus subtilis*) & TRMT10C (Humans)



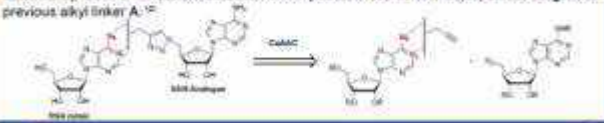
II-Objectives: Design and synthesis of transition state analogues of the methylation reaction to study the RNA recognition and catalytic mechanism of m^{6}A-rMtases or m^{1}A-rMtases.

→The target compounds are SAM-Adenosine conjugates composed of an analogue of the cofactor SAM and a mimic of the RNA substrate.

III-Previous work: Synthesis of SAM-Adenosine conjugates in N6 position with alkyle linkers. [b, c]



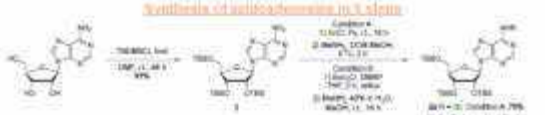
IV-Target molecules: SAM-Adenosine conjugates with triazole linker at N6 or N1 position obtained by CuAAC→ Possible utilization of N1 position, less flexible linker, similar length with previous alkyl linker A. [c]



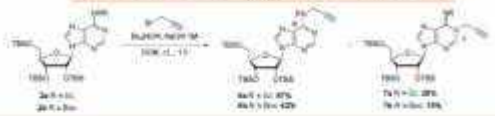
SYNTHESIS OF SAM-ADENOSINE CONJUGATES USING PROTECTING GROUPS

SYNTHESIS OF PRECURSORS FOR CuAAC

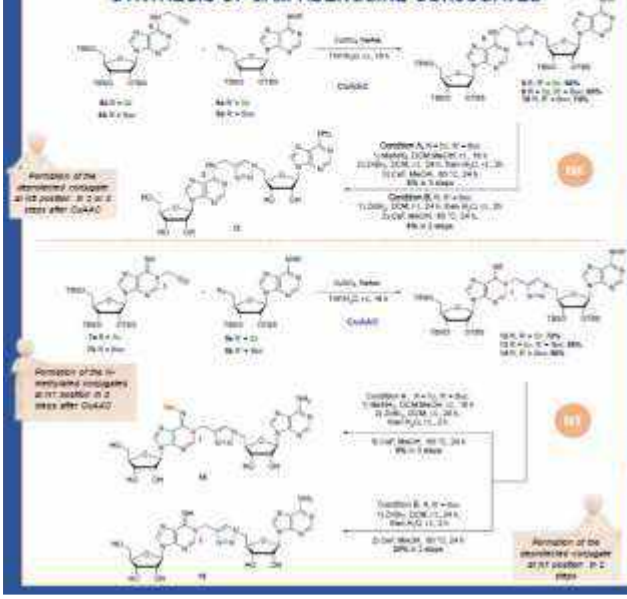
Synthesis of azidoadenosine in 3 steps



Synthesis of N6 or 1-N propargylated adenosine in 2 steps



SYNTHESIS OF SAM-ADENOSINE CONJUGATES



Formation of the alkynylated conjugate at N6 position in 2 steps after CuAAC

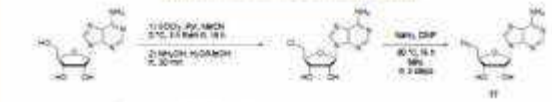
Formation of the alkynylated conjugate at N1 position in 2 steps after CuAAC

Formation of the azidoalkylated conjugate at N1 position in 2 steps

SYNTHESIS OF SAM-ADENOSINE CONJUGATES WITHOUT PROTECTING GROUPS

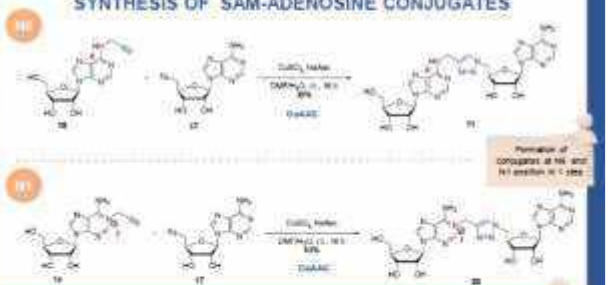
SYNTHESIS OF PRECURSORS FOR CuAAC

Synthesis of azidoadenosine in 3 steps



Synthesis of N6 or 1-N propargylated adenosine in 1 step

SYNTHESIS OF SAM-ADENOSINE CONJUGATES



Formation of conjugates at N6 and N1 position in 1 step

CONCLUSION

- ✓ Synthesis of new **SAM-adenosine conjugates** with a 1,2,3-triazole linker that covalently links the SAM analogue to the N-6 or N-1 position of the adenosine substrate.
- ✓ The **protecting groups strategy** requires numerous steps of protection and deprotection.
- ✓ The **strategy without protecting groups** is more straightforward and efficient. The N6 and 1-N conjugates were obtained in 2 steps with overall yield of 26% and 30%, respectively.
- ✓ These conjugated will be used in **different studies with rMtases** (DSF, ITC or X-ray crystallography) to increase our knowledge of the RNA recognition process and the molecular mechanism involved in methyl transfer.

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(b) Atdjian, C.; Iannazzo, L.; Braud, E.; Ethève-Quelquejeu, M. *Eur. J. Org. Chem.* **2019**, *32*, 4411-4425.
(c) Oerum, S.; Catala, M.; Atdjian, C.; Brachet, F.; Ponchon, L.; Barraud, P.; Iannazzo, L.; Droogmans, L.; Braud, E.; Ethève-Quelquejeu, M.; Tisné, C. *RNA Biol.* **2019**, *16*, 708-808.
(d) Atdjian, C.; Coelho, D.; Iannazzo, L.; Ethève-Quelquejeu, M.; Braud, E. *Molecules* **2020**, *25*, 3241.

The Microtubule Bench (MTBench): a novel technology to identify modulators of Protein-Protein Interaction (PPI) and Protein-Nucleic Acid Interaction (PNAI) in a cellular environment **Hélène Henrie[(1)*], Pierrick Craveur[(1)], Laurie Askenatzis[(2)], Jérôme Bignon[(2)], Widad Ghedamsi[(1)], Vandana Joshi[(3)], David Pastré[(3)], Guillaume Bollot[(1)*], Nicolas Babault[(1)]** *(1) SYNSIGHT, Genopole Entreprises, Evry France.* *(2) Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, Gif-sur-Yvette, France.* *(3) SABNP, Université d'Evry, INSERM U1204, Université Paris-Saclay, Evry, France*	***Restricted to organizers***

Drug discovery is in constant evolution to provide the most efficient and specific drugs for new therapies. To identify/optimize New Chemical Entities, the development of robust and sensitive bioassays is mandatory to complement the few gold standard techniques, notably those working in a cellular context. Here we present an innovative cellular bioassay to screen modulators of Protein-Protein Interaction (PPI) and Protein-Nucleic Acid Interaction (PNAI). This new technology, Microtubule Bench (MTBench), is based on the use of microtubules as intracellular nanoplatforms to visualize and quantify PPI or PNAI at the single cell level. We implemented the patented MTBench technology to quantify whether a prey is attracted onto microtubules by a bait, which is the readout of the assay, on High Content Screening (HCS) system at high resolution. We confirmed the robustness and the sensitivity of the MTBench to identify and qualify small molecule inhibitors on the well-known p53/MDM2 PPI system. As for PNAI, we detected and quantify the interaction of different RNA Binding Proteins (RBPs) with RNA. A PNAI system on MTBench to screen modulators of such interaction has already been implemented. **T**his novel cell-based assay, which is suitable for HCS, will facilitate the drug discovery in the fields of PPI and PNAI.

* Correspondence: helene.henrie@synsight.net, guillaume.bollot@synsight.net

The MicroTubule Bench (MTBench): a novel technology to identify modulators of Protein-Protein Interaction (PPI) and Protein-Nucleic Acid Interaction (PNAI) in a cellular environment

https://synsight.net

Hélène Henrie[1], Pierrick Craveur[1], Laurie Askenatzis[2], Jérôme Bignon[2], Widad Ghedamsi[1], Vandana Joshi[3], David Pastré[3], Guillaume Bollot[1], Nicolas Babault[1]

[1]SYNSIGHT Genopole Entreprises – 4, rue Pierre Fontaine 91058 EVRY
[2] Plateforme GIBI – ICSN - Centre de recherche de Gif - Bat. 27 – 1 avenue de la Terrasse 91198 GIF-SUR-YVETTE
[3]SABNP – Bat. Maupertuis – Université d'Evry, rue du Père Jarlan 91000 EVRY

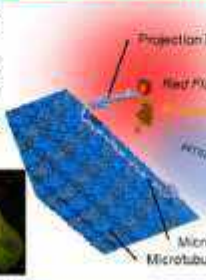
Microtubule Bench technology - MTBench

New method that uses the **Microtubule network** as an intracellular platform to detect protein interactions in living cells

Protein A (Bait) is fused to RFP and brought to the microtubule. RFP signal highlight the microtubule network.

Partner B (Prey) is fused to GFP. The presence of a prey on microtubules then reveals an effective interaction.

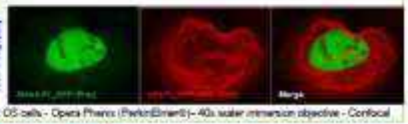
From Bosa et al., Scientific Reports 5, 2015



U-2 OS cells - Opera Phenix (PerkinElmer®) – 40x water immersion objective - Confocal

The **Microtubule Bench technology**:

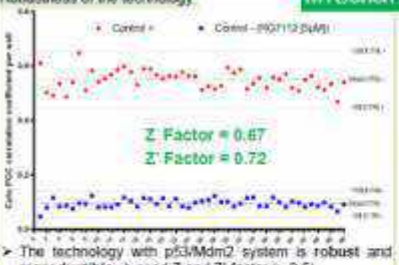
- Cellular based technology with a readout in High Content Screening (HCS)
- Direct vizualisation of Protein-Protein Interactions (PPI) as well as Protein-Nucleic Acid Interactions (PNAIs).
- Quantification and screen of modulators for these interactions



U-2 OS cells - Opera Phenix (PerkinElmer®)– 40x water immersion objective - Confocal

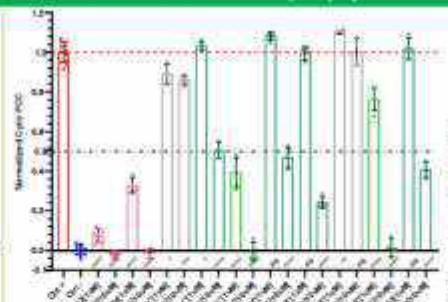
MTBench on Protein-Protein Interaction (PPI) : p53/Mdm2 system

Robustness of the technology



- The technology with p53/Mdm2 system is **robust** and **reproductible** → good Z and Z'-factor (> 0.5)
- System is ready for screening

PCC = Pearson Correlation Coefficient
Z-factor : CTRL+ = p53-FL/Mdm2-FL DMSO (0.1%) ; CTRL- = p53-FL/Mdm2-FL RG7112 [5µM]
Z'-factor : CTRL+ = p53-FL/Mdm2-FL ; CTRL- = p53ΔNΔC/Mdm2-FL



Hit identification

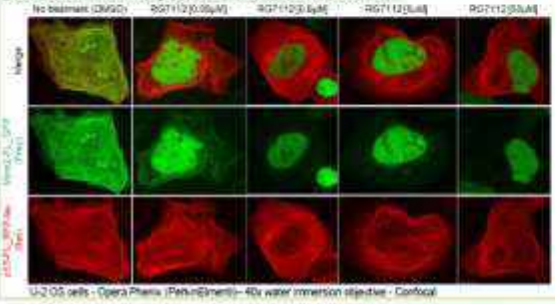
Identification of:

- Reference compounds: RG7112 & Nutlin-3a
- Interesting hits: Rac-4c' & (+)-8' (pure enantiomer)
- Potential hits (to a lesser extent): Rac-4a'', Rac-4d', Rac-8' & Rac-MG-1133
- No hits: Rac-7b'', (-)-8'

IC_{50} are calculated using the GraphPad Prism software
Student t-test paired vs normalized Ctrl+ (significantly $p < 0.05$) calculated using the R software

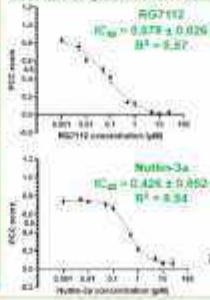
Ctrl+ DMSO [0.1%] / Ctrl- RG7112 [5µM]

Dose-effect inhibition of p53-Mdm2 interaction with RG7112



U-2 OS cells - Opera Phenix (PerkinElmer®)– 40x water immersion objective - Confocal

In Cellulo IC_{50} determination

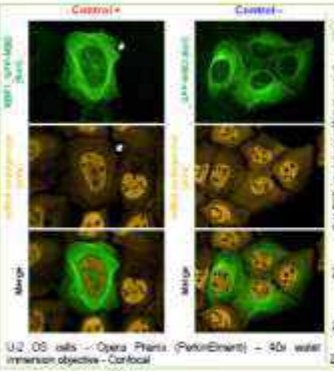


Compounds	Inhibitory activity (IC_{50} HTRF)*	Inhibitory activity (IC_{50} MTBench)	WB p53/p21
RG7112	++++ (IC_{50} = 0.018µM)	++++ (IC_{50} = 0.078µM)	++++/++++
Nutlin-3a	++++ (IC_{50} = 0.090µM)	++++ (IC_{50} = 0.426µM)	+++/+++
Racemic	++ (IC_{50} = 0.610µM)	++++ (IC_{50} = 0.614µM)	++++/++++
(+)-8'	++++ (IC_{50} = 0.003µM)	+++ (IC_{50} = 1.192µM)	+++/+++
Rac-MG-1133	- (IC_{50} > 5µM)	++ (IC_{50} = 2.059µM)	+/+
(-)-8'	++ (IC_{50} = 0.538µM)	- (IC_{50} > 25µM)	+/-
Rac-8'	++ (IC_{50} = 0.010µM)	+ (IC_{50} > 5µM)	+/+

- Identification of Rac-MG-1133 as **false negative**, (-)-8' as **false positive** compounds and effect of **enantiomers** on activity

*Vu et al., ACS Med. Chem. Lett. 4,2013 ; *Gloppel et al., J. Med. Chem. 25, 2018

MTBench on Protein-Nucleic Acid Interaction (PNAI)



U-2 OS cells – Opera Phenix (PerkinElmer®) – 40x water immersion objective - Confocal

Robustness of the technology

- Direct **visualisation** of the interaction between a RNA-Binding Protein (RBP) and mRNA endogenous in the cells
- The technology with RBP1/mRNA is robust and reproductible → good Z'-factor (> 0.5)

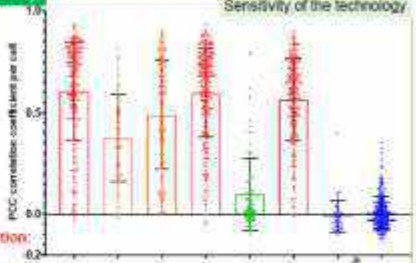
Z'-factor : CTRL+ = RBP1_GFP-MBD/mRNA ; CTRL- = GFP-MBD/mRNA

Protein-Nucleic Acid Interaction (PNAI) on 6 different RNA-Binding Proteins (RBPs) using the MTBench

Identification of:

- Strong interaction: RBP1/mRNA, RBP4/mRNA & RBP6/mRNA (PCC > 0.5)
- Moderate interaction: RBP2/mRNA & RBP3/mRNA (PCC between 0.3-0.5)
- Weak interaction: RBP5/mRNA (PCC between 0.1-0.3)
- No interaction: CTRL- (PCC < 0.1)

Sensitivity of the technology



Conclusions & Perspectives

- Confirmation of the **robustness** and **sensitivity** of the MTBench as a new bioassay in a cellular context
- **Identification** and **quantification** of Hit compounds on the well-known p53/Mdm2 PPI system
- Detection and quantification of 6 RBPs with mRNA endogenous
- Ready to screen in a 96- and 384-well plates format (Z'-factor = 0.63 > 0.5)
- Possibility to identify **hot-spots residues** (mutant) with the MTBench

Acknowledgments

- Virginie Georget and Benoit Bordignon at the MRI platform for the access to the Opera Phenix
- MEDETIA at the Imagine Institute for the access to the Opera Phenix
- Oliver Kepp at the Gustave Roussy Institute for the U-2 OS cells
- Genopole for the postdoc supporting grant of Hélène

IN VITRO ACTIVITIES OF A NEW BIOACTIVE FLUOROQUINOLONE DERIVATIVE AGAINST CHLAMYDIA TRACHOMATIS

T. H. Vu[(1) (2)], E. ADHEL[(1)*], N. T. Ha-Duong[(1)], G. Anquetin[(1)], A. Aubry[(3) (4)], E. Capton[(4)], P. Fechter[(5) (6)], P. Plésiat[(7)], P. Verbeke[(8)] and N. Serradji[(1)*]

[(1)] Université de Paris, ITODYS, UMR 7086, CNRS, 15 rue Jean Antoine de Baïf, Paris F-75205.
[(2)] University of Science and Technology of Hanoi (USTH), Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam;
[(3)] AP-HP, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, F-75013 Paris, France;
[(4)] Sorbonne Université, INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, équipe 13, F-75013, Paris, France;
[(5)] CNRS, UMR 7242, Biotechnologie et Signalisation Cellulaire, 67400 Illkirch-Graffenstaden, France;
[(6)] Université de Strasbourg, Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg, 67400 Illkirch-Graffenstaden, France;
[(7)] Centre National de Référence de la résistance aux antibiotiques, Hôpital Jean Minjoz, boulevard Fleming, 25030 Besançon, France;
[(8)] Université de Paris, INSERM U1149, Faculté de médecine Xavier Bichat, 16 rue Henri Huchard, F-75018 Paris, France.

Restricted to organizers

Chlamydia trachomatis is a human pathogen, a gram-negative and an obligate intracellular bacterium. *C. trachomatis* is responsible for 131 million cases of Chlamydiosis, the most common sexually transmitted bacterial infection worldwide, every year. *C. trachomatis* is also responsible for the development of trachoma, the most common infectious cause of blindness.
This bacterium needs iron in the medium to grow. In order to improve the antibacterial activity of a commercially available drug, the one-step coupling synthesis leading to a new Ciprofloxacin derivative is described in this work. The corresponding 8-hydroxyquinoline-ciprofloxacin conjugate obtained is more active than its broad-spectrum parent-antibiotic. Actually, it also has a significant antibacterial activity against pathogenic bacteria responsible for the development of nosocomial diseases such as from the ESKAPE group. At the same time, its iron (III)-chelating properties were studied using spectrophotometric titration.

* Correspondence: serradji@u-paris.fr; erika.adhel@etu.u-paris.fr

IN VITRO ACTIVITIES OF A NEW BIOACTIVE FLUOROQUINOLONE DERIVATIVE AGAINST *CHLAMYDIA TRACHOMATIS*

T. H. Vu[1,2], E. Adhel[1]*, N. T. Ha-Duong[1], G. Anquetin[1], A. Aubry[3,4], E. Capton[4], P. Fechter[5,6], P. Plésiat[7], P. Verbeke[8], and N. Serradji[1]*

[1] Univ de Paris, ITODYS-UMR CNRS 7086, 15 rue Jean-Antoine de Baif, 75205 Paris cedex 13, France.
[2] University of Science and Technology of Hanoi (USTH), Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.
[3] AP-HP, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, F-75013 Paris, France.
[4] Sorbonne Université, INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, équipe 13, F-75013, Paris, France.
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[6] Université de Strasbourg, Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg, 67400 Illkirch-Graffenstaden, France.
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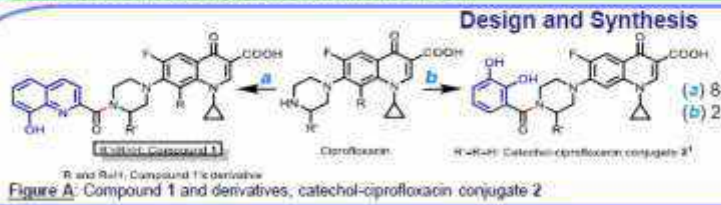
[8] Univ de Paris, INSERM U1149, Faculté de médecine Xavier Bichat, 16 rue Henri Huchard, F-75018 Paris, France.
*Correspondence : serradj@u-paris.fr; erika.adhel@etu.u-paris.fr

Abstract: *Chlamydia trachomatis (C. trachomatis)* is a human pathogen, a Gram-negative and an obligate intracellular bacterium. *C. trachomatis* is responsible for **131 million cases** of Chlamydiosis, the most common sexually transmitted bacterial infection worldwide, every year. *C. trachomatis* is also responsible for the development of trachoma, the **most common infectious cause of blindness**. This bacterium needs iron in the medium to grow. In order to **improve the antibacterial activity** of a commercially available drug, the **one-step coupling** synthesis leading to a new **Ciprofloxacin derivative** is described in this work. The corresponding 8-hydroxyquinoline-ciprofloxacin conjugate obtained is more active than its broad-spectrum parent-antibiotic. It also has a significant antibacterial activity against pathogenic bacteria responsible for the development of **nosocomial diseases** such as bacteria from the ESKAPE group. At the same time, its iron (III)-chelating properties were studied using spectrophotometric titration.

Design and Synthesis



(*a*) 8-hydroxyquinoline-2-carboxylic acid, TBTU, DIEA, DMF
(*b*) 2,3-dihydroxybenzoic acid, TBTU, DIEA, DMF

Figure A: Compound **1** and derivatives, catechol-ciprofloxacin conjugate **2**

Anti-chlamydia and other antimicrobial activity

- Inhibition of *C. trachomatis* growth in HeLa cells.
- The cells are infected by *C. trachomatis* serovar L2 strain, with or without the test molecules (0 - 50 µM) and with or without iron citrate (200 µM).
- 72 h post-infection, cell lysates were processed and used to infect new HeLa cells.
- The reinfection capacity is scored by Inclusion Forming Unit (IFU) of each cellular lysate.

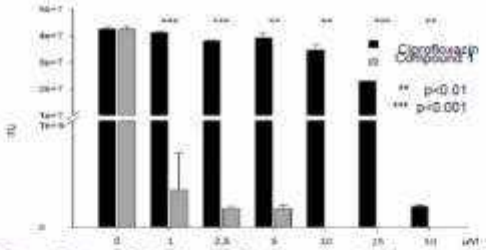


Figure B - Inhibitory effects on *C. trachomatis* infectious capacity of compound **1** and ciprofloxacin tested *in cellulo*.

Compound 1 is more active than the parent molecule, probably due to a gain in lipophilicity

Organism	Gram	Ciprofloxacin	1	2
M. tuberculosis	-	12-37[a,c]	>128[c]	>128[c]
E. coli	N	1-2.9	8-16	4-15
E. coli ATCC 25922	N	≤0.06[c]	≤0.06[c]	nd
K. pneumoniae ATCC 700603	N	0.25	4	nd

Table 1 – *In vitro* antibacterial activities (IC_{50} or MIC) of compounds **1**-**2**, ciprofloxacin.
nd: not determined.
[a] P/N: positive/negative.
[b] IC_{50} (µg/mL) against wild-type DNA gyrases of *M. tuberculosis* and *E. coli*.
[c] IC_{50}s (µg/mL) slightly higher than those previously determined[2].
[d] MIC (µg/mL).
* Similar MIC were obtained against *P. aeruginosa* PAOA, a laboratory strain (data not shown).

- Against the **ESKAPE** group
- **Compound 1 has a µM range antibacterial activity against these bacteria**
- **Less efficient than ciprofloxacin**

Iron-chelating properties: spectrophotometric titration

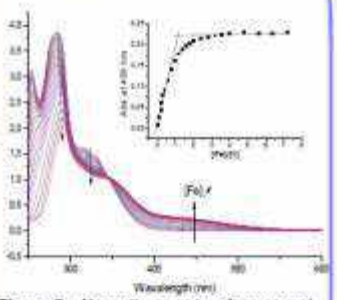


Figure C - Absorption spectra of compound **1** (10^{-4} M) in presence of increasing concentrations of $FeCl_3$ (0–10^{-3} M) at pH 2 and 25±0.5 °C. Absorbance at 450 nm plotted against [Fe(III)]/[1] in inset.

At pH 2, the addition of $FeCl_3$ to a solution of compound **1** leads to a red shift of the π-π* band (284 - 290 nm).

At pH 7, the addition of Fe-NTA to a solution of compound **1** leads to a decrease in absorbance at 282 and 319 nm and appearance a red shift from 319 to 350 nm.

- The fluoroquinolone part probably chelates iron at pH 2 and pH 7 with a stoichiometry of 1 metal : 1 ligand

- Iron chelation is not its main antibacterial mechanism

Conclusion: We report here the synthesis of a novel ciprofloxacin derivative by a single-step coupling of the parent antibiotic. This compound has notable antibacterial activity against Gram-negative and Gram-positive bacteria, including the obligate intracellular bacterium *C. trachomatis*. However, only its anti-chlamydial activity is higher than that of the parent antibiotic. This antibacterial effect is only partially reversed by the addition of iron(III), which is complexed by the fluoroquinolone part of the molecule. Thus, new compounds derived from compound **1** are currently under synthesis (**figure A**).

[1] Fardeau S *et al. Bioorg Med Chem.* 2014; 22: 4049-4060. [2] Aubry A *et al. Antimicrob. Agents Chemother.* 2004; 48: 1281–1288.

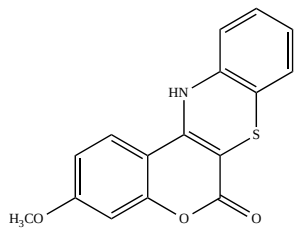
Pharmacomodulation on estrothiazine sulfone: synthesis of substituted 4-amino-3-phenylthiocoumarin dioxides.

Alexandre Acramel [1,2]*, Yves Jacquot [1]*.

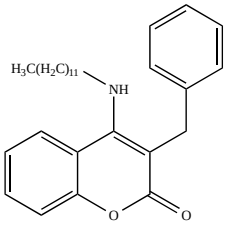
(1) Cibles Thérapeutiques et Conception de Médicaments, CNRS UMR 8038, INSERM U1268, Faculté de Pharmacie, Université de Paris, 4 avenue de l'Observatoire, 75270 Paris, France.
(2) Département de Pharmacie, Institut Curie, 26 rue d'Ulm, 75005 Paris, France.

Restricted to organizers

Estrogen receptor (ER) agonists are of clinical interest in the treatment of pathologies associated with endogenous estradiol deficiency, such as endometriosis, primary ovarian insufficiency and hypogonadism. We have previously synthesized ER agonists sharing a coumarinic motif, such as estrothiazine (**1**)[a-c] and the analogue **2**[d]. A rational pharmacomodulation on estrothiazine has allowed us to propose the sulfone function as a new pharmacophore for estrogenicity (see molecule **3**)[e]. Remarkably, demethoxyestrothiazine sulfone (**3**) displays estrogenic activity without inducing proliferation, when tested in various ER-positive breast cancer cell lines. This uncommon pharmacological profile shows that it is be possible to distinguish estrogenic activity from proliferation. Such molecules could be all the more interesting that estrogens are associated with cell proliferation and, therefore, with an enhanced risk of hormone-dependent cancer (e.g., breast cancer).
In this context, we have recently synthesized five-steps 4-amino-3-phenylthiocoumarin dioxide analogues (see molecule **4**) with good yields. These molecules will be tested on estrogen-dependent transcription and the proliferation of breast cancer cells.



1



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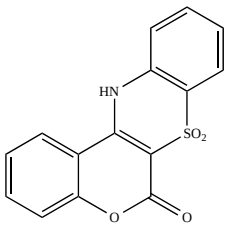

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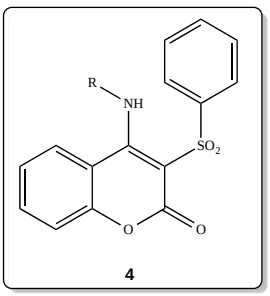

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[b] Jacquot et al. Biol. Pharm. Bull. (2002)
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* Correspondence: alexandre.acramel@gmail.com; yves.jacquot@parisdescartes.fr

Pharmacomodulation on estrothiazine sulfone: Synthesis of substituted 4-amino-3-phenylthiocoumarin dioxides

A. Acramel[1,2], **Y. Jacquot**[1,*]

[1] *Cibles Thérapeutiques et Conception de Médicaments, CNRS UMR 8038, INSERM U1268, Faculté de Pharmacie, Université de Paris, Paris, France*
[2] *Département de Pharmacie, Institut Curie, Paris, France*
*yves.jacquot@parisdescartes.fr

Introduction

Estrogen receptor (ER) agonists are of clinical interest for the treatment of pathologies that are associated with endogenous estradiol deficiency, such as endometriosis, primary ovarian insufficiency and hypogonadism. We have previously synthesized ER agonists that are derived form coumestrol and that share a coumarinic motif such as estrothiazine (**1**)[1-3] and the analogue **2**[4] (see Figure 1). A rational pharmacomodulation on estrothiazine has allowed us to propose the sulfone function as a new pharmacophore for estrogenicity (see molecule **3**, Figure 1).[5] Remarkably, the demethoxyestrothiazine sulfone **3** displays estrogenic activity without inducing proliferation, when tested in various ER-positive breast cancer cell lines. This uncommon pharmacological profile suggests that it would be possible to distinguish estrogenic activity from proliferation. Molecules **3** analogues could be all the more interesting that estrogens are associated with cell proliferation and, therefore, with an enhanced risk of hormone-dependent cancer (e.g., breast cancer). In this context, we have synthesized with five steps 4-amino-3-phenylthiocoumarin dioxide analogues (see molecule **4**). These molecules encompass a sulfone function in the same position as the molecule **3** and a potential thiazin ring, as for the molecule **2**.

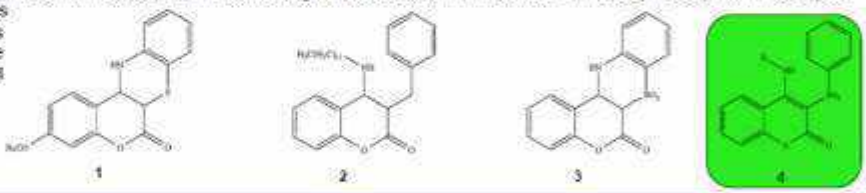


Figure 1. Chemical structures of estrothiazine (1), analogue 2 and demethoxyestrothiazine sulfone 3

Results and discussion

1. Physicochemical characteristics of estrothiazine

The bioactive molecule **1** (i.e., estrothiazine) is an orange powder (Figure 2) which is poorly soluble in acquous solution. Estrothiazine is, indeed, planar and highly hydrophobic (Figure 3).[1] The delocalization of π electrons, which also participate in its planarity, also confers to the molecule some chromophoric and absorption characteristics, with an aminophenyl chromophore at 272 nm, a cinnamoyl at 313 nm, and a coumarin at 430 nm (Figure 4). It is of note that the fluorescence spectrum of estrothiazine shows an excitation maximum at the wavelength of 381 nm (Figure 5).

Figure 2. Estrothiazine 1

Figure 3. Dynamic of estrothiazine 1 at 300K during 10 ps (NDIS/HT II)

Figure 4. UV-Vis spectrum of estrothiazine 1 (200 to 800 nm).

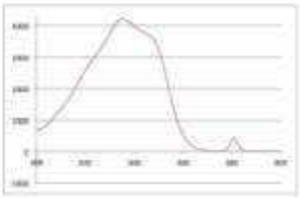
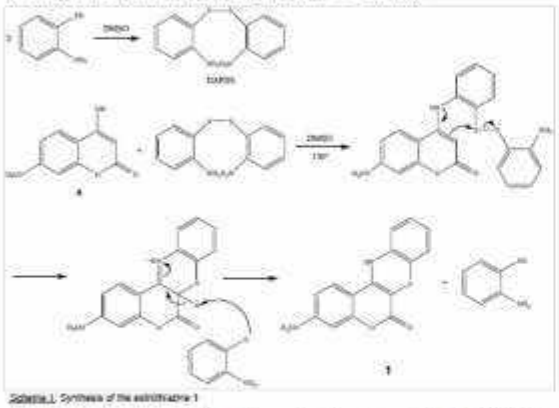


Figure 5. Fluorescence spectrum of estrothiazine 1 (200 to 800 nm).

2. Chemistry

Estrothiazine **1** was synthesized in one step by condensing 2-aminothiophenol on 4-hydroxy-7-methoxycoumarin **5** in DMSO at a temperature of 130° C. The yield is about 70% (Scheme 1). Briefly, the mechanism consists in the synthesis of DAPDS (Di-ortho AminoPhenyl DiSulfide) from amino-2-thiophenol. DAPDS reacts with 4-hydroxy-7-methoxycoumarin to afford the expected tetracyclic 3-methoxybenzopyrano[3,4-b][1,4]benzothiazin-6-one (**1**).

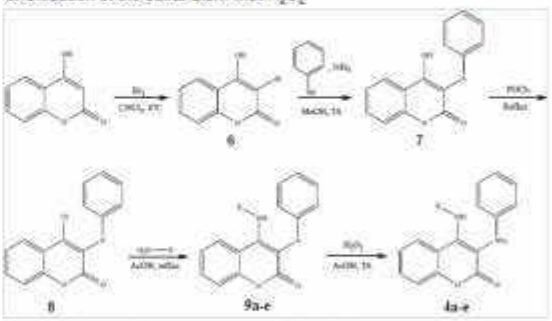


Scheme 1. Synthesis of the estrothiazine 1

It is of note that the oxidation in the sulfur atom is carried out with H_2O_2 in acetic acid (*e.g.*, molecule **3**).

Estrothiazine derivatives (compounds **4a-e**) were obtained in five steps, as followed:

1. Bromination in the position 3 of 4-hydroxycoumarin with Br_2 (yield ~ 75%)
2. Thioarylation in the position 3 with thiophenol (yield ~ 51%)
3. Chlorination in 4 with $POCl_3$ (yield ~ 74%)
4. Amination in 4 (yields, depending on the amine) : diethylamine (pKa~11,0, yield: 8%), pyrrolidine (pKa~11,3, yield: 10%), cyclopentylamine (pKa~10,6, yield: 14%), isobutylamine (pKa~10,7, yield 47%), phenylethylamine (pKa~9,8, yield 26%)
5. Oxidation of the sulfur atom with H_2O_2



Scheme 2. Synthesis of the estrothiazine derivatives 4a-e with a potential C ring

Figure 6. X-ray structure of coumarin 7

The 4-hydroxy (instead of the 4-keto) tautomeric form was preserved after the thioarylation in the position 3 (Figure 6). At least, amination of the coumarin **8** was carried out with low to modest yields, an observation that could be associated with the basicity of the amines and, therefore, to the opening of the coumarin moiety.

Conclusion

In conclusion, we have synthesized new 3,4-disubstituted coumarins sharing a thioaryl (sulfone) in 3 and an amine in 4. These estrothiazine derivatives will be tested with respect to their ability to modulate estrogenic pathways.

1. Y. Jacquot et al. Eur. J. Med. Chem. (2001)
2. Y. Jacquot et al. Bioorg. Med. Chem. Lett. (2002)
3. Y. Jacquot et al. Eur. J. Pharm. Sci. (2017)
4. G. Leclercq et al. J. Recept. Signal Transd. (2017)

We acknowledge Camille Pinter (Sorbonne University) for UV-Vis and fluorescence spectra.

Antibacterial and antibiofilm activity of non-steroidal anti-inflammatory drugs against *Escherichia coli* and *Staphylococcus aureus*

Anabela Borges [1,2]*, Cláudia Leão [1], Manuel Simões [1,2]

(1) Faculty of Engineering, University of Porto, LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

(2) Faculty of Engineering, University of Porto, DEQ - Department of Chemical Engineering, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal.

Restricted to organizers

Persistent infections, usually associated with biofilm-producing bacteria, continue to be a challenge for both medical and scientific communities. The potential interest in drug repurposing for biofilm control is growing due to the disinvestment in antibiotic R&D, reduced efficacy of the available panel of antibiotics and increased risks associated with new development plans[(a)]. In the present study, the antibacterial and antibiofilm activities of four non-steroidal anti-inflammatory drugs (NSAIDs), piroxicam (PXC), diclofenac sodium (DCF), acetylsalicylic acid (ASA) and naproxen sodium (NPX), were evaluated against *Escherichia coli* and *Staphylococcus aureus*. The minimum inhibitory/bactericidal concentrations (MIC and MBC) and the dose response curves from exposure to the selected NSAIDs were determined by the broth microdilution method and culturability, respectively. The potential of NSAIDs to eradicate preformed biofilms was performed using a microtiter plate assay and characterized in terms of biofilm mass removal (crystal violet staining), metabolic activity reduction (alamar blue staining) and biofilm cells culturability. Additionally, the ability of the selected NSAIDs combined with current in use antibiotics (kanamycin - KAN and tetracycline - TET) to eradicate 24-h old biofilms was also tested in order to evaluate their ability to potentiate the putative antibiofilm activity of antibiotics. MIC were found for PXC (800 µg/mL) and ASA (1750 µg/mL) against *E. coli*, and for DCF (2000 µg/mL) and ASA (2000 µg/mL) against *S. aureus*. No MBC were found (> 2000 µg/mL). Regarding biofilm eradication, ASA, DCF and PXC promoted significant reductions in metabolic activity (66.1% to 86.7%) and culturability (2.22 to 6.46 log CFU/cm^2). However, only PXC promoted biofilm mass removal (maximum of 28.6%). Additive interactions were obtained for most of the combinations between NSAIDs and KAN or TET. Overall, the results obtained suggest that NSAIDs appear to be a promising strategy to control biofilms as they demonstrated to be more effective than conventional antibiotics.

Bibliographic references:

(a) Leão, C., Borges, A. and Simões, M., 2020. NSAIDs as a Drug Repurposing Strategy for Biofilm Control. *Antibiotics*, *9*(9), p.591.

Acknowledgements: This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); Projects PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219 and PTDC/ASP-PES/28397/2017 - POCI-01-0145-FEDER-028397, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Anabela Borges thanks the Portuguese Foundation for Science and Technology (FCT) for the financial support of his work contract through the Scientific Employment Stimulus - Individual Call - [CEECIND/01261/2017].

* Correspondence: apborges@fe.up.pt

Antibacterial and antibiofilm activity of non-steroidal anti-inflammatory drugs against *Escherichia coli* and *Staphylococcus aureus*

Anabela Borges[1,2*], Cláudia Leão[1], Manuel Simões[1,2]

[1] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[2] DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal. *Correspondence: aiborges@fe.up.pt

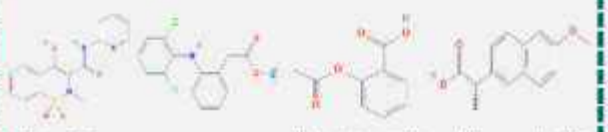
Introduction

Persistent infections, usually associated with biofilm-producing bacteria, continue to be a challenge for both medical and scientific communities. The potential interest in drug repurposing for biofilm control is growing due to the disinvestment in antibiotic R&D, reduced efficacy of the available panel of antibiotics and increased risks associated with new development plans[a]. In the present study, the antibacterial and antibiofilm activities of four non-steroidal anti-inflammatory drugs (NSAIDs), piroxicam (PXC), diclofenac sodium (DCF), acetylsalicylic acid (ASA) and naproxen sodium (NPX), were evaluated against *Escherichia coli* and *Staphylococcus aureus*.

(a) Leão, C., Borges, A. and Simões, M., 2020. NSAIDs as a Drug Repurposing Strategy for Biofilm Control. Antibiotics, 9(9), p.591.

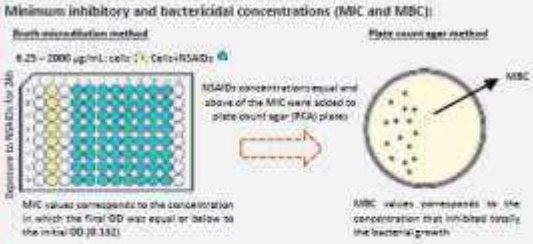
Methods

- Bacterial strain: *Escherichia coli* CECT 434 and *Staphylococcus aureus* CECT 976
- NSAIDS from 4 different families (Oxicams [1], Anilic acids [2], Salicylates [3], Propionic acids [4]):



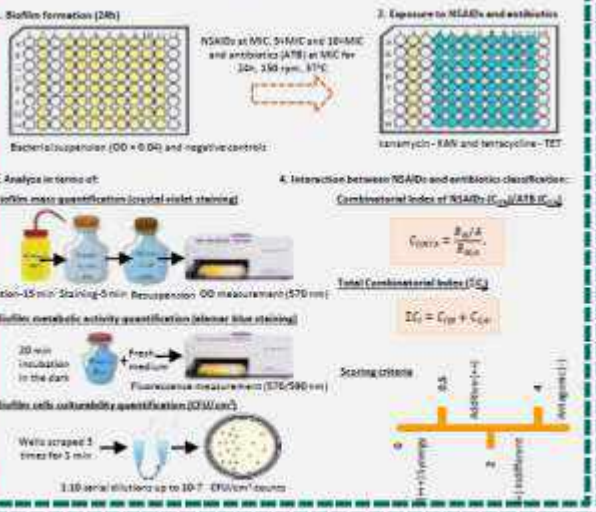
(1) Piroxicam (PXC) (2) Diclofenac sodium (DCF) (3) Acetylsalicylic acid (ASA) (4) Naproxen sodium (NPX)

- Minimum inhibitory and bactericidal concentrations (MIC and MBC):



- Dose response curves – culturability:

- Biofilm control – antibiotic-NSAID dual combinations:



Results

- Antibacterial activity of selected NSAIDs and antibiotics

- MIC were found for PXC (800 µg/mL) and ASA (1750 µg/mL) against *E. coli*, and for DCF (2000 µg/mL) and ASA (2000 µg/mL) against *S. aureus*. No MBC were found with any of the studied NSAIDs for both bacteria, in the range of tested concentrations (~2000 µg/mL) (Table 1). For ATB the MIC values were slightly higher than ones of the CLSI guidelines.

Table 1. MIC and MBC values for the selected NSAIDs (PXC, DCF, ASA and NPX) and antibiotics (KAN and TET) against *E. coli* and *S. aureus*.

	MIC (µg/mL)		MBC (µg/mL)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
PXC	800 ± 0	>2000	>2000	>2000
DCF	>2000	2000 ± 0	>2000	>2000
ASA	1750 ± 0	2000 ± 0	>2000	>2000
NPX	>2000	>2000	>2000	>2000
KAN	2.5 ± 0	5 ± 0	20	20
TET	5 ± 0	4 ± 0	64	48

- Effect of different doses of the selected NSAIDs on the culturability of *E. coli* and *S. aureus*

- The maximum of log CFU/mL reduction for PXC, DCF and ASA was 1.34, 3.68 and 1.08, respectively (Table 2). It was observed a dose-dependent effect.

Table 2. Log CFU/cm² reduction values for NSAIDs at 1/2 MIC, MIC and a concentration above MIC, after 8h.

	E. coli			*S. aureus*	
NSAID	C (µg/mL)	log reduction	NSAID	C (µg/mL)	log reduction
PXC	400	1.20	DCF	1000	2.50
	800	1.27		2000	3.86
	1600	1.34		2500	3.68
ASA	875	0.19	ASA	1000	0.09
	1750	0.83		2000	0.92
	2500	0.92		2500	1.08

- Effect of the selected NSAIDs and ATB on the *E. coli* and *S. aureus* biofilm eradication

- ASA, DCF and PXC promoted significant reductions in metabolic activity (66.1% to 98.7%) and culturability (2.22 to 6.48 log CFU/cm2). However, only PXC promoted biofilm mass removal (maximum of 28.8%) (Figure 1). Additive interactions were obtained for most of the combinations between NSAIDs and KAN or TET (Table 3).

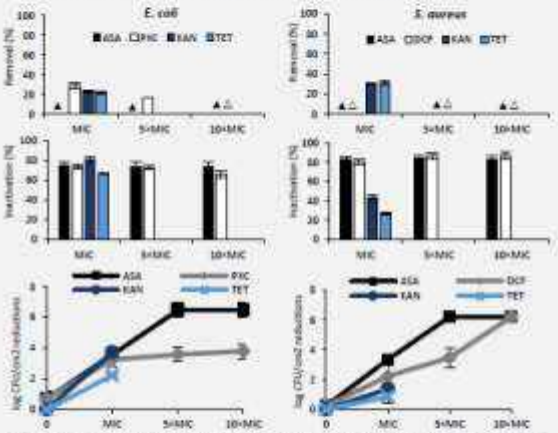


Figure 1. Effects of selected NSAIDs at three different concentrations (MIC, 5×MIC and 10×MIC) and ATB at MIC, against *E. coli* and *S. aureus* after 24 h of exposure in terms of: (a)(b) biofilm removal (R), (c)(d) biofilm inactivation and (e)(f) in the biofilm culturable cells - log CFU/cm2 reduction. ▲ (R) represents the biofilm removal. Mean values ± SD of three independent experiments are illustrated.

Table 3. TCI scoring of the selected NSAIDs (at MIC, ½MIC and 1/4 MIC) with TET and KAN (at MIC) in terms of biofilm removal/inactivation and culturability reduction. (+/-) - Additive; (+) - Indifferent; (-) - Antagonistic.

	E. coli				*S. aureus*			
Conc.	NSAID+ATB	Removal	Inactivation	Culturability	NSAID+ATB	Removal	Inactivation	Culturability
MIC+MIC	PXC+KAN	+	+	∞	DCF+KAN	+	∞	+
½MIC+MIC		+	+	∞		∞	∞	∞
1/4MIC+MIC		∞	+	∞		∞	∞	∞
MIC+MIC	PXC+TET	-	∞	∞	DCF+TET	∞	∞	∞
½MIC+MIC		-	∞	∞		∞	∞	+
1/4MIC+MIC		∞	∞	∞		∞	∞	∞
MIC+MIC	ASA+KAN	∞	∞	+	ASA+KAN	∞	∞	+
½MIC+MIC		∞	∞	∞		∞	∞	∞
1/4MIC+MIC		∞	∞	∞		∞	∞	∞
MIC+MIC	ASA+TET	∞	∞	∞	ASA+TET	∞	∞	∞
½MIC+MIC		∞	∞	∞		∞	∞	∞
1/4MIC+MIC		∞	∞	∞		∞	∞	∞

Conclusions

Overall, the results obtained suggest that NSAIDs appear to be a promising strategy to control biofilms as they demonstrated to be more effective than conventional antibiotics.

Acknowledgments

This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE – funded by national funds through the FCT/MCTES (PIDDAC). Projects PTDC/BII-BTI/30135/2017 - POCI-01-0145-FEDER-030135 and PTDC/ASP-PES/28507/2017 - POCI-01-0145-FEDER-028507, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Anabela Borges thanks the Portuguese Foundation for Science and Technology (FCT) for the financial support of his work contract through the Scientific Employment Stimulus - Individual Call - (CEECIND/01181/2017).

thesis of a new generation of potential anti-Influenza agents targeting the PA–PB1 Protein–Protein Interaction.	***Restricted to organizers***

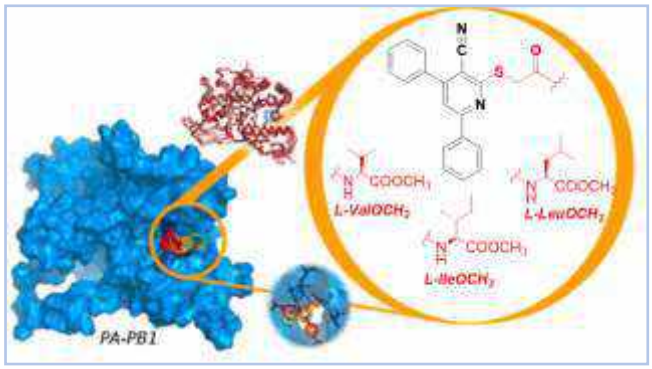
Annarita Cianciusi(1)*, Silvia Schenone(1) , Francesca Musumeci(1), Ilaria D'Agostino(2)

(1) University of Genoa, Department of Pharmacy, V.le Benedetto XV 3, I-16132 Genova-Italy

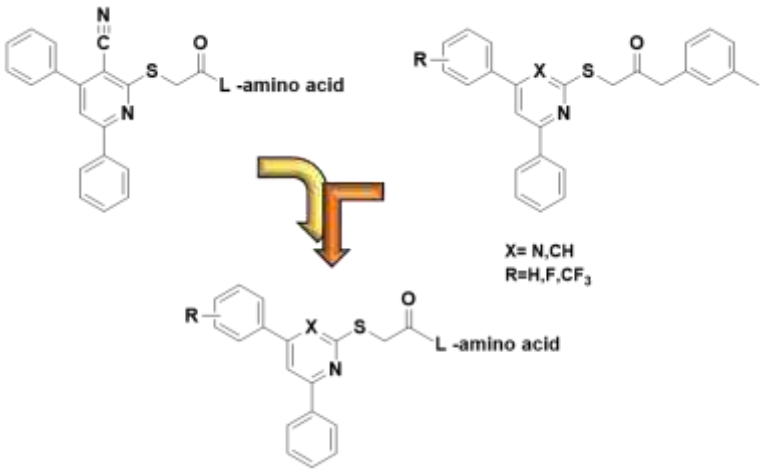
(2) *University of Siena, Department of Biotechnology, Chemistry and Pharmacy, V.A. Moro, I-53100 Siena-Italy*

Influenza is an infectious disease with high impact on the global public health.

The causative agents are enveloped viruses belonging to the *Orthomyxoviridae* family and classified in phylogenetically and antigenically distinct subtypes. Among these, especially influenza A virus represent a serious concern for humans[(a)], aggravated by its high trasmission rate and the limited antiviral therapeutic options. Consequently, the discover of novel antiviral strategies becomes essential[(b)].



In this regard, an attractive approach has been identified in the disruption of the viral RNA polymerase complex (RdRp) assembly through protein-protein interaction inhibition. In particular, among the interactions between the three different subunits of the RdRp, the one between PA and PB1 stands out as an interesting druggable target[(c)]. Starting from the previously identified *3-cyano-4,6-diphenyl-pyridines* bearing different aminoacidic portions in the C2 side chain[(d)], a *Mix & Match* synthetic approach was carried out to synthesize a small library of compounds, with the aim of obtaining a new generation of potential anti-flu agents.



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(b) A. Loregian et al. ; *Cell. Mol. Life Sci*. 71 (**2014**) 3659-3683.

(c) S. Massari et al. ; *J. Med. Chem*. 59 (**2016**) 7699-7718.

(d) I. D'Agostino et al. ; *European J. Med. Chem.* 157 (**2018**) 743-758

* Correspondence: annar.cianciusi@gmail.com

Synthesis of a new generation of potential anti-Influenza agents targeting the PA–PB1 Protein–Protein Interaction

Annarita Cianciusi(1)*, Silvia Schenone(1) , Francesca Musumeci(1), Ilaria D'Agostino(2)

(1) University of Genoa, Department of Pharmacy, V.le Benedetto XV 3, I-16132 Genova-Italy

(2) University of Siena, Department of Biotechnology, Chemistry and Pharmacy, V.A Moro, I-53100 Siena-Italy

Background & Project

Influenza is an infectious disease that represents an important public health burden because of the continuous antigenic evolution[1] of its causative agents. These mutations can lead to the onset of new aggressive viral subtypes. Reasonably, many efforts in search for antiviral drugs are directed towards the discovery of novel strategies and targets[2]. In this context, the disruptors of the viral RNA polymerase, especially those targeting PA-PB1 heterodimerization,[3] emerged as promising anti-flu agents.

In particular, my research group identified compounds possessing a 3-cyano-4,6-diphenylpyridine nucleus as effective inhibitors[4] against this target. Then, a hit-to-lead study allowed us to discover a second generation of more active derivatives, bearing a peptide side chain.[5]

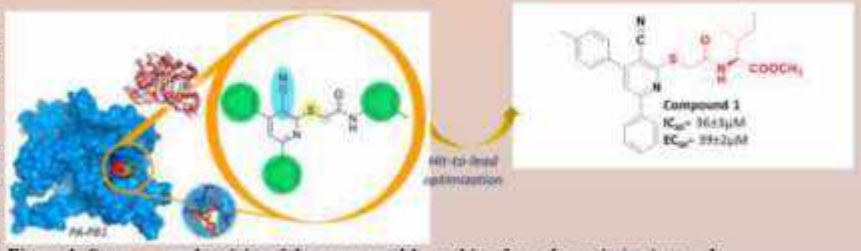


Figure 1: Structure and activity of the compound 1 resulting from the optimization study

Among these, compounds 1 stands out for the best biological profile. (Figure 1)

Based on these interesting results, we decided to synthesize a new family of potential anti-flu agents, combining the best pyridine or pyrimidine scaffolds of first generation compounds with the most promising amino acid portions of second generation ones (Figure 2).

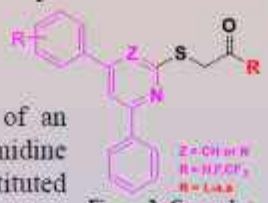


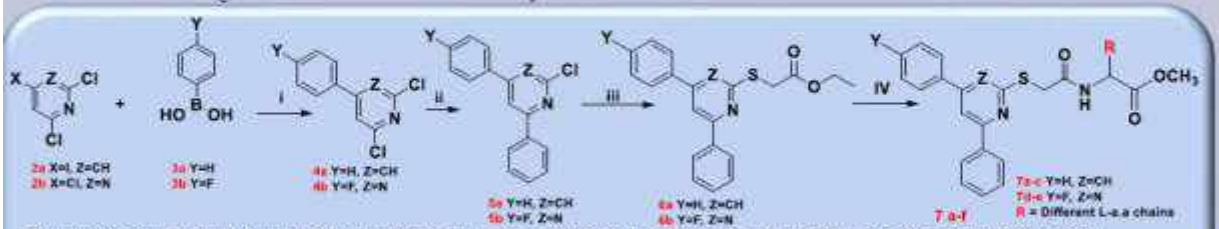
Figure 2: General structure of the new class of anti-flu agents

Chemistry & Conclusion

The first part of the synthetic work has been devoted to the setup of an optimized procedure for the preparation of the arylated pyridine and pyrimidine cores. The appropriate heterocycle **2a,b** and the properly substituted phenylboronic acids **3a,b** were reacted via Suzuki-Miyaura cross-coupling reaction affording the intermediates **4a,b.**

The latter were submitted to a second Suzuki coupling giving the diphenyl derivatives **5a,b**, that reacted with ethyl thioglycolate through an aromatic nucleophilic substitution giving the compounds **6a,b**.

To synthesize the desired final compounds **7a-f,** an amidation reaction was performed, using EDC and HOBt as coupling agents, dry DIPEA and the appropriate L-amino acid methyl ester in dry DMF. (**Schema 1**) . All the compounds are currently subjected to a biological evaluation, that together with new molecular modeling studies will orient future synthesis.



Schema 1. *Reagent and conditions*: i) $Pd[(PPh_3)]_4$, K_2CO_3, THF , N_2, reflux (to obtain 4a); $Pd(OAc)_2$, PPh_3, Na_2CO_3, THF , N_2, reflux; (to obtain 4b); ii) $Pd[(PPh_3)]_4$, K_2CO_3, DME, N_2, reflux (to obtain 5a); $PdCl_2(PPh_3)_2$, K_2CO_3, DCM, N_2, reflux (to obtain 5b) iii) ethylthioglycolate, K_2CO_3, DMF, N_2, reflux; iv) EDC, HOBt, dry DIPEA, dry DMF, N_2, 0°C-r.t.

Bibliographic references:

(1) F. Carrat, A. Flahault ; *Vaccine 25* (**2007**) 6852-6862.

(2) A. Loregian et al. ; *Cell. Mol. Life Sci.* 71 (**2014**) 3659-3683.

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(5) I. D'Agostino et al. ; *European J. Med. Chem.* 157 (**2018**) 743-758

* Correspondance: annar.cianciusi@gmail.com

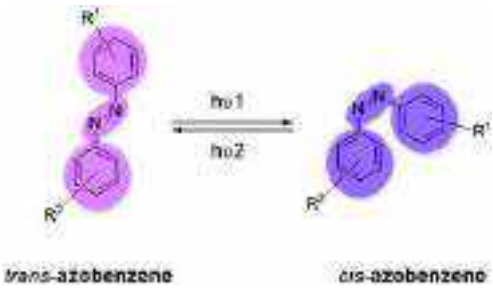
Photoswitchable ligands for the optical control of α7 nicotinic acetylcholine receptors: synthesis and photochromic characterization

Claudio Papotto (1,2), Rosalba Sortino (2), Hyojung Lee (2), Beatrice Preda (1,2), Carlo Matera (1,2), Marco De Amici (1), Pau Gorostiza (2,3,4) *, Clelia Dallanoce (1) *

(1) University of Milan, Dept. Pharmaceutical Sciences, Milan, Italy
(2) Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain
(3) Catalan Institution for Research and Advanced Studies (ICREA)
(4) Network Biomedical Research Center on Bioengineering, Biomaterials and Nanotechnology (CIBER-BBN)

Restricted to organizers

The α7 nicotinic acetylcholine receptor (α7 nAChR) is widely distributed in the human organism, where it mediates many physiological and pathological processes. In the central nervous system, it is expressed both on neuronal and non-neuronal cells, where it controls cognitive and mnemonic processes, sensory gating and neuroinflammation, and it is thus involved in the etiopathology of several diseases, such as Alzheimer's, depression, schizophrenia, attention-deficit/hyperactivity disorder (ADHD).[(a)] In the periphery, it is essential for the survival of α7 dependent tumours, which are the most predominant type of lung cancer in the population.[(b)] Therefore, alpha7 modulators have a huge therapeutic potential, but their clinical translation is hampered by the lack of adequate safety, generally because of low specificity. In addition, even though a vast pharmacological toolset to study α7 mediated functions has been developed, control of central α7 nAChRs with high spatiotemporal precision is missing. To address these issues, we have developed a small group of photoswitchable α7 nAChR ligands. Light is in fact an unparalleled tool as a noninvasive regulatory signal for pharmacological and biological applications, since it can be delivered with high spatiotemporal precision. An emerging light-based approach is photopharmacology, which relies on the use of reversibly photoswitching drugs.[(c)] Our photoisomerizable compounds were designed by "azologization" of selective α7 nAChR agonists. In this work, I will present the design, the chemical synthesis, and the characterization of the photochromic properties of these novel potential photoswitchable α7 ligands.



Bibliographic references:

[(a)] Baranowska U, Wiśniewska RJ. Postepy Hig Med Dosw 2017, 71(0), 633-648.
[(b)] Wang S, Hu Y. Oncol Lett 2018, 16(2), 1375-1382.
[(c)] Bregestovski P, Maleeva G, Gorostiza P. Br J Pharmacol, 2018, 175(11), 1892–1902.

* clelia.dallanoce@unimi.it, pau@icrea.cat

This project is co-funded by the Erasmus+ Traineeship Programme of the European Union.

Photoswitchable ligands for the optical control of α7 nicotinic acetylcholine receptors: synthesis and photochromic characterization

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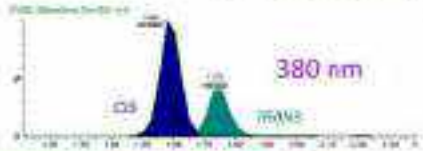
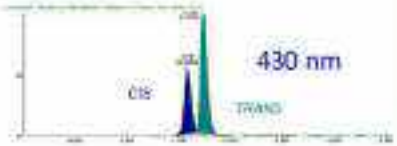
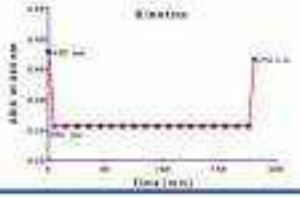
Introduction and aim

Photopharmacological approach

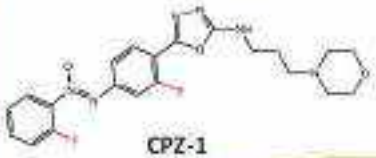
Photochemical characterization







Synthesis of the photoswitchable compounds



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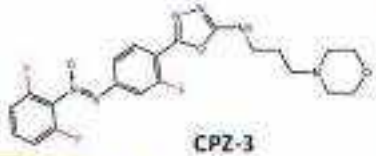
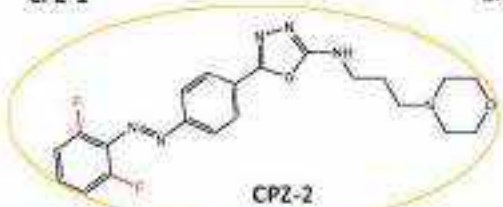


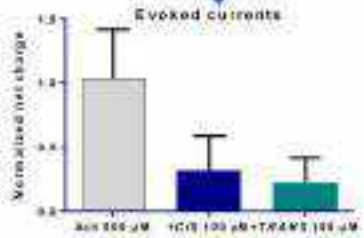
CPZ-3

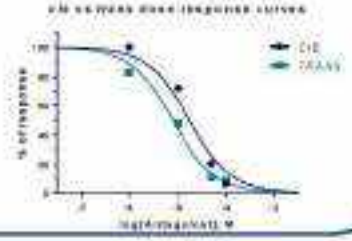


CPZ-2

Preliminary Biological Results

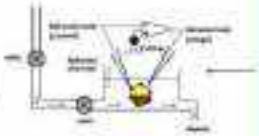
Antagonistic Activity





Conclusions and Future Perspectives

Confirmation of preliminary results on Xenopus Laevis Oocytes



Evaluation of two-photon absorption to allow control of the activity in near IR

Application of pharmacophore network and clustering approaches for structure-activity relationships

Damien Geslin[(1)(2)], Jean-Luc Manguin[(2)], Vinh Vo Nhat[(2)], Alban Lepailleur[(1)], Bertrand Cuissart[(2)], Ronan Bureau[(1)]

[(1)] Centre d'Etudes et de Recherche sur le Médicament de Normandie, Normandie Univ, UNICAEN, CERMN, 14000 Caen, France.
[(2)] Groupe de Recherche en Informatique, Image, Automatique et Instrumentation de Caen, Normandie Univ, UNICAEN, ENSICAEN, CNRS, GREYC, 14000 Caen France.

Restricted to organizers

We have recently described a new approach for the definition of pharmacophores starting from a large dataset called Norns.[(a)] With Norns, the main parameters for the extraction of pharmacophores are the size of the pharmacophores (number of nodes for the pharmacophoric graphs), the support of the pharmacophore (number of molecules associated to the pharmacophore) and the cut-off value for the growth rate (ratio of frequency for the number of active *vs* inactive molecules associated to a pharmacophore).

In this work, the same dataset as the initial publication[(a)] was considered, *i.e.* BCR-ABL tyrosine kinase (1492 compounds). With a biological cut-off at 1000 nM, the dataset was divided into two groups: active and inactive compounds (774 *vs* 718 compounds). The objective of this work is to analyse the potential to discriminate pharmacophores associated to active compounds towards pharmacophores associated to inactive compounds.

The poster presents this work with a visualization of the pharmacophore networks (see figure 1 for an example) and the clustering approaches.

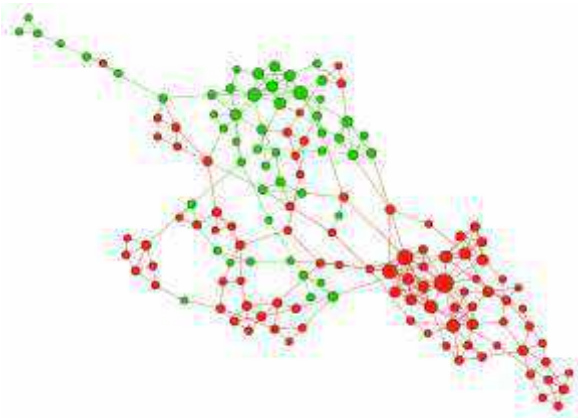


Figure 1. Separation between active and inactive pharmacophore with seven pharmacophoric features.

Bibliographic references:
[(a)] Métivier, J.-P.; Cuissart, B.; Bureau, R.; Lepailleur, A. The Pharmacophore Network: A Computational Method for Exploring Structure-Activity Relationships from a Large Chemical Data Set. Journal of Medicinal Chemistry 2018, 61 (8), 3551–3564

* Correspondence: damien.geslin@unicaen.fr

Application of pharmacophore network and clustering approaches for structure-activity relationships.

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* Correspondence: damien.geslin@unicaen.fr

Introduction:

We have recently described[1] a new approach called *Norns* for the automated detection of pharmacophores starting from a large chemical dataset. The main parameters for the extraction of the pharmacophores are the size of the pharmacophores (number of nodes of the pharmacophoric graphs, a.k.a. order of the pharmacophores), the support of the pharmacophores (number of molecules associated to the pharmacophore) and the cut-off value for growth-rate of the pharmacophores (imbalance between actives and inactives). For this experiment, we worked with a dataset of BCR-ABL inhibitors consisting of 1492 molecules (774 actives and 718 inactives).

Figure 1. A pharmacophore, its alignment on a molecule and the corresponding list of matching molecules

Problem:

With Norns, we have a large number of generated pharmacophores leading to a complexity for the search space. The objective of the work is to simplify this space and to answer the following question : Is it possible to differentiate active from inactive pharmacophores (generated from active and inactive compounds, respectively) by considering the similarities between them?

Pharmacophores:

A pharmacophore (see figure 2) represents potential chemical features (pharmacophoric functions) associated to the interactions with a biological receptor. In a pharmacophore, a node denotes a pharmacophoric function and an edge encodes the minimal distance between two nodes (number of chemical bonds). Each pharmacophore is labelled with a class (active or inactive) in relation with the chemical compounds (active and inactive) which fit it (see figure 3).

Figure 2. A pharmacophore with 5 pharmacophoric functions

Figure 3. Alignment of the pharmacophore with an active molecule.

The pharmacophores are defined on the basis of 6 pharmacophoric functions: HBA (Hydrogen Bond Acceptor) / HBD (Hydrogen Bond Donor) / Base / Acid / Hydrophobic group / Aromatic ring.
In the present study, we worked with different orders of pharmacophores (3 nodes called M3 to 7 nodes called M7).

Graph Edit Distance[2,3] (GED):

GED uses a branch-and-bound algorithm to define the similarity between graphs. The distance between two graphs (i.e pharmacophores) is calculated from the costs associated to the addition, the deletion and/or the substitution of nodes or edges.

Figure 4. example of graph edit distance with 3 pharmacophores

In Figure 4, you can see two close pharmacophores (A and B) and a different one (C). To switch from A to B, we only have to change the distances between the pharmacophoric functions. On the contrary, to go from A (or B) to C, we have to change both the distances and the functions between them. This leads to the following distances (by considering a cost of 20 for the substitution of a pharmacophoric feature).

$$dist(A,B) = 14 \; ; \; dist(A,C) = 40 \; ; \; dist(B,C) = 36$$

The GED between all pharmacophores were determined and distance matrices were generated for each order (M3 to M7) in function of the number of nodes).

Clusterings of pharmacophores:

Clustering is the process of grouping a set of objects so that the objects in the same group (called a cluster) are more similar to each other than to the objects in the other clusters. We tested four clustering methods in this work (hierarchical clustering, k-means, spectral, and DBSCAN)[4]. This clustering should complete an initial representation of the pharmacophore network carried out with a force atlas algorithm (see Figure 5).

Figure 5. Force-directed graph drawing of the two closest neighbours of the active (red) and inactive (green) pharmacophores of order 7. A zoom (right side) on some nodes was carried out with a view of the corresponding pharmacophores and the associated molecules.

The best clustering is obtained for M7 with an NMI score three times higher in comparison with M5 (see table 1). Starting from the eight defined clusters with M7 (see figure 6), complementary information could be added to the pharmacophore network (see figure 7) with ten closest neighbours for force atlas representation.

	M3	M4	M5	M6	M7
Number of clustering	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Average ... of clustering	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Best clustering	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
k	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
NMI	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 1. Clustering results from M3 to M7

Figure 6. Active clusters in red and inactive clusters in green with M7 (growth-rate value for each cluster)

Figure 7. The best clustering on a force-directed graph of the ten closest neighbours.
red: active cluster, green: inactive cluster, grey: mixted cluster, blue: outliers.

Conclusion:

We have succeeded in organizing active and inactive pharmacophores within a pharmacophore network. This network allows to better understand the activity of the molecules on the receptor. The best clusters were associated to M7 (seven pharmacophoric functions) and the worse to M4. In future works, we will study the potential of this method to analyze compounds acting on several receptors (comparison of pharmacophores in function of the couple receptors / ligands).

References:

(1) Métivier, J.-P.; Cuissart, B.; Bureau, R.; Lepailleur, A. The Pharmacophore Network: A Computational Method for Exploring Structure-Activity Relationships from a Large Chemical Data Set. *J. Med. Chem.* 2018, 61, 3551–3564.
(2) Garcia-Hernandez, C.; Fernández, A.; Serratosa, F. Ligand-based virtual screening using graph edit distance as molecular similarity measure. *J. Chem. Inf. Model.* 2019, 59, 1410–1421.
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(4) Saxena, A.; Prasad, M.; Gupta, A.; Bharill, N.; Patel, O. P.; Tiwari, A.; Er, M. J.; Ding, W.; Lin, C.-T. A Review of Clustering Techniques and Developments. *Neurocomputing* 2017, 267, 664–681.

Structure-activity-ADME properties relationship study of inhibitors of Insulin-Degrading Enzyme discovered by KTGS	***Restricted to organizers***

C.P. Dinh(1)*, R. Gealageas (1), P. Hermant (1), N. Kraupner (1), C. Lanthier (1), S. Warenghem (1), A. Biela (1), C. Lenglart (1), C. Piveteau (1), W.J. Tang (2), F. Leroux (1), B. Deprez (1), R. Deprez-Poulain (1), D. Bosc (1).

(1) Inserm U1177 Drugs and Molecules for Living Systems; Institut Pasteur de Lille, Université de Lille, Inserm, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE.

(2) Ben-May Institute, University of Chicago, IL, USA.

Insulin-Degrading Enzyme (IDE), a 110 kDa zinc metalloprotease, was first discovered in 1949 for its ability to degrade insulin but yet remaining poorly understood. It is involved in the clearance of numerous physiological peptides such as insulin, glucagon or amyloid-β. Moreover, IDE acts not only as a protease but also has a chaperone-like activity. With its multi-functional activity, IDE seems to be at the crossroad of several biological pathways.[(a)] The X-ray structure of IDE was reported by Tang *et al.* in 2006 and revealed unusual structural features such as a large catalytic chamber and an exosite involved in the recognition and positioning of the substrate (Figure 1). [(b)] In order to deeply understand the multiple roles of IDE, by using the kinetic target-guided synthesis, the molecule BDM_44768 was discovered as a potent IDE inhibitor.[(c,d)] Thanks to our synthesis effort to afford more than 140 analogues of BDM_44768, we were able to optimise the pharmacological profile of this chemical series in terms of IDE inhibition and ADME properties. These studies provide us the 7-quinolone (IC_{50} = 44 nM) and the 5-benzothiazole (IC_{50} = 150 nM) compounds as good probes to explore IDE's functions.

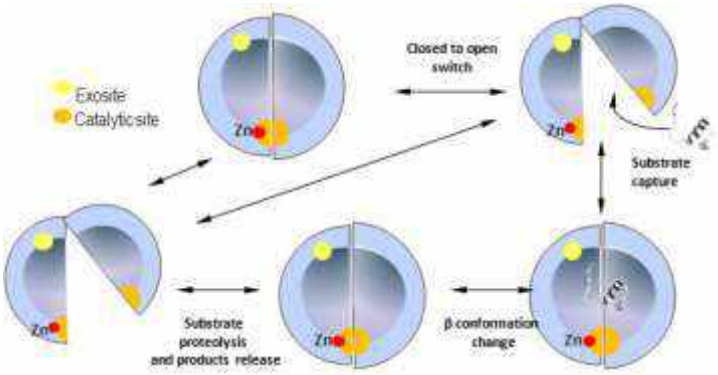


Figure 1: Catalytic mechanism of hIDE

Bibliographic references:

[(a)] Tundo, G.R. et al. *Crit. Rev. Biochem. Mol. Biol.* **2017**, 52, 554-582

[(b)] Shen, Y. et al. *Nature* **2006**, 443, 870-874

[(c)] Bosc, D. et al. *Future Med. Chem.* **2016**, 8, 381-404

[(d)] Deprez-Poulain, R. et al. *Nat Commun.* **2015**, 6, e8250

* Correspondence: chauphi.dinh@univ-lille.fr
damien.bosc@univ-lille.fr

Inserm U1177

Structure-activity-ADME properties relationship study of inhibitors of Insulin-Degrading Enzyme discovered by KTGS

C.P. Dinh[1,*], R. Geoffageas[1], P. Hermant[1], N. Kraupner[1], C. Lanthier[1], S. Warenghem[1], A. Biela[1], C. Lenglart[1], C. Piveteau[1], W.J. Tang[2], F. Leroux[1], B. Deprez[1], R. Deprez-Poulain[1], D. Bosc[1,*].

[1]Inserm U1177 Drugs and Molecules for Living Systems, Institut Pasteur de Lille, Université de Lille, Inserm, 1 rue du Professeur Laguesse, F-59000 LILLE, FRANCE. [2]Ben-May Institute, University of Chicago, IL, USA.

damien.bosc@univ-lille.fr
chauphi.dinh@univ-lille.fr
www.deprezlab.fr

1

Insulin-Degrading Enzyme (IDE), a 110 kDa zinc metalloprotease, was first discovered in 1949 for its ability to degrade insulin but yet remaining poorly understood.

The IDE is involved in the clearance of numerous physiological peptides such as insulin, glucagon or amyloid-β. Besides, IDE acts not only as a protease but also has a chaperone-like activity. With its multi-functional activity, IDE seems to be at the crossroad of several biological pathways. Noteworthy, the implication of IDE in neurodegenerative or metabolic diseases was confirmed by cellular, in vivo and gene polymorphism studies.[1]

The X-ray structure of IDE was solved by Tang et al.[2] in 2006 and revealed unusual structural features like a large catalytic chamber and an exosite involved in the recognition and positioning of the substrate (**Figure 1**).

In order to deeply understand the multiple roles of IDE, improved modulators of this enzyme that are potent, small, soluble and cell permeable are needed.

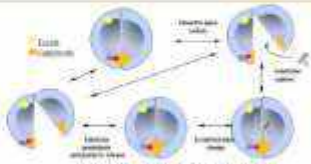


Figure 1: catalytic mechanism of hIDE

2

***In situ* multicomponent click-chemistry**[3] was used to access new inhibitors of IDE (**Figure 2**). 2 azides bearing a naphthyl or a phenyl group and 90 alkynes were used for this target-guided synthesis. TOF-HRMS analysis of the wells allowed the identification of ligands of hIDE among the 180 possible combinations.

All detected compounds were derived from naphthyl derivative. Among them, BDM_44768 was discovered.[4]

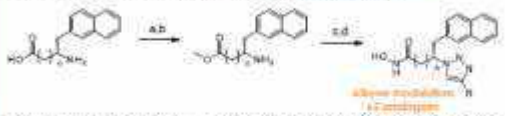
BDM_44768 displayed a submicromolar potency (IC_{50} = 250 nM) and acceptable but improvable physico-chemical properties especially the aqueous solubility (58 µM) and the mouse microsome stability ($t_{1/2}$ = 12 min).

Figure 2: Discovery of BDM_44768 by *in situ* multicomponent click-chemistry

3

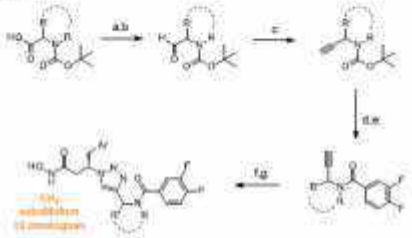
Synthesis of BDM_44768 analogs

To explore the role of the configuration and the distance between the amine and the carbonyl of the (*S*)-3-amino-4-(2-naphthyl)-butyric acid moiety, and of the 4-substituent of the 1,2,3-triazole, a convergent synthesis was set up to screen efficiently several functionalized alkynes (**Scheme 1**).



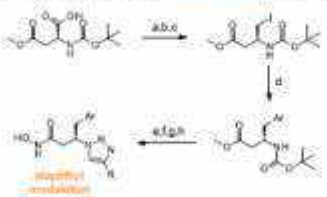
Scheme 1: Reagents and conditions: (a) $SOCl_2$, MeOH, rt, 2h; -16h; (b) $CuSO_4$,$5H_2O$, K_2CO_3, imidazole-1-sulfonyl azide hydrochloride or hydrogensulfate, MeOH, rt, 16 h; (c) alkyne, $CuSO_4$,$5H_2O$, sodium ascorbate, H_2O/DMF, rt, 1h-14h; (d) NH_4OH, KCN, H_2O, MeOH, rt, 16 h

We also synthesized alkynes from reduced unnatural or natural amino acids and Bestmann-Ohira reagent to understand the significance of the substitution between the triazole and the amide for the potency and ADME properties (**Scheme 2**).



Scheme 2: Reagents and conditions: (a) MeI-HOMe, HBTU, DMF, rt, 16h; (b) $LiAlH_4$, THF, 0°C, 20 min; (c) Bestmann-Ohira reagent, K_2CO_3, MeOH, 16h; (d) 4N HCl in dioxane, MeOH, 0°C, 1h; (e) RCOCl, Et$_3$N, MeOH, 0°C to rt; (f) azide, $CuSO_4$,$5H_2O$, sodium ascorbate, H_2O/DMF, rt, 1h-16h; (g) NH_4OH, KCN, H_2O, MeOH, rt, 16 h

The impact of the naphthyl moiety was also assessed by modulation thanks to a synthetic pathway involving a key Negishi coupling reaction (**Scheme 3**).



Scheme 3: Reagents and conditions: (a) HMS, DCC, EtOAc, 0°C to rt, 16 h; (b) $NaBH_4$, THF, H_2O, 0°C, 15 min; (c) I_2, PPh_3, imidazole, CH_2Cl_2, rt, 1.5 h; (d)[i] Zn*, iodine, DMF, rt, 30 min, [ii] $Pd_2(dba)_3$, PPh_3, aromatic bromide or iodide, rt, 16 h; (e) TFA, CH_2Cl_2, rt, 2 h or 4N HCl in dioxane, MeOH, rt, 16h; (f) $CuSO_4$,$5H_2O$, K_2CO_3, imidazole-1-sulfonyl azide hydrochloride or hydrogensulfate, MeOH, rt, 16 h; (g) alkyne, $CuSO_4$,$5H_2O$, sodium ascorbate, H_2O/DMF, rt; (h) NH_4OH, KCN, H_2O, MeOH, rt, 16 h

4

Potency and ADME properties of BDM_44768 analogs

More than 140 compounds were synthesized by these described syntheses and other strategies allowing us to map SAR on potency on IDE inhibition and SPR on ADME properties (**Figure 3**).

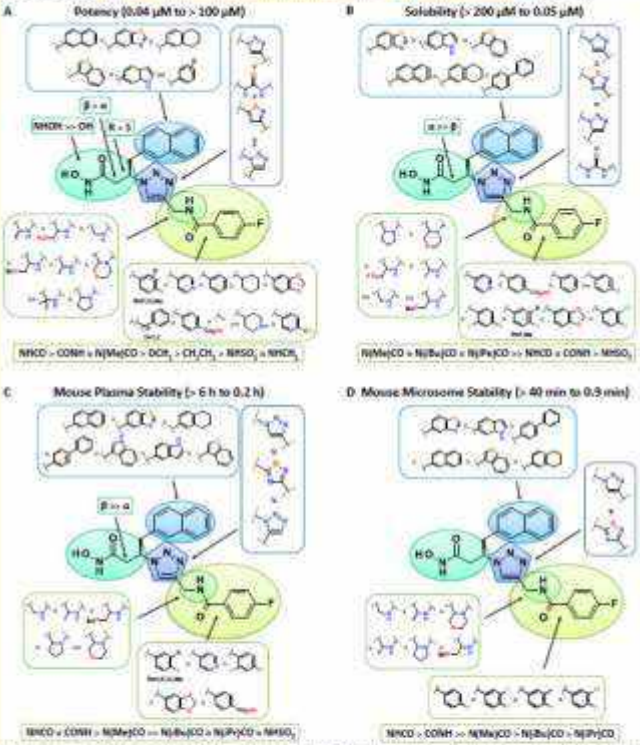


Figure 3: Summary of SAR on potency (A) and SPRs on solubility (B) and mouse plasma (C) and microsome (D) stabilities

These results show us that 1,4-triazole as central ring are appropriate to obtain a good profile in terms of potency and ADME properties. Potent and soluble molecules were particularly obtained by modifying the naphthyl group and/or by substituting the methylene between the triazole and the amide, especially by a methyl group. However, substitution of this methylene by larger moieties is detrimental for the stability in biologic fluids. High potency could also be obtained by introducing substituted 4-fluorophenyl amides.

Conclusion & Perspectives: Thanks to our synthesis effort to afford more than 140 analogues of BDM_44768, we were able to **optimise the pharmacological profile of this chemical series in terms of IDE inhibition and ADME properties**. These studies provide us a good probes to explore IDE's functions.

(1) Tundo, G.R. *et al. Crit. Rev. Biochem. Mol. Biol.* **2017**, *52*, 554-582
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gin-appended aza-porphyrinoids – synthesis and characterization	***Restricted to organizers***

Emil Kolasinski [1,2]*, Dariusz T. Mlynarczyk [1], Daniel Ziental [2], Lukasz Sobotta [2], Tomasz Koczorowski [1], Jadwiga Mielcarek [2], Tomasz Goslinski [1].

(1) Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland.
(2) Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland.

Aza-porphyrinoids, such as porphyrazines and phthalocyanines, keep arousing interest among scientists in the fields of medical, technical and chemical sciences. This is due to the fact that the exhibit interesting physicochemical, optical, photocatalytic and electrochemical properties[(a)]. Unfortunately, the planar and highly non-polar porphyrazine ring is prone to aggregation (by means of π-π stacking) and low solubility of the unsubstituted macrocyclic ring, functionalization is necessary.

In this study we synthesized a novel porphyrazine (**Pz**) and phthalocyanine (**Pc**) substituted in the periphery with 4-hydroxybenzoic moieties. The synthetic methodology was elaborated to optimize the reaction conditions, which resulted in over 14-fold yield increase, from initial 2.4% to 34% in case of the autocyclotetramarization leading to the novel phthalocyanine. All the new symmetrical macrocyclic derivatives, as well as all the intermediates, underwent physicochemical characterization (mass spectrometry, NMR and UV-Vis spectroscopy) and the porphyrinoids were subjected to additional spectroscopic and photochemical investigations, including the singlet oxygen generation quantum yield. Additionally, acute toxicity and photocytotoxicity of **Pc** and **Pz** was tested on *Aliivibrio fischeri* bacteria. Based on the results obtained, the phthalocyanine derivative was chosen as the most promising compound and was further modified by the exchange of methal ions in the central cavity, revealing interesting spectroscopic effects. The obtained results indicate the potential of these derivatives for their potential use in photodynamic therapy.

This study was supported by the National Science Centre – Poland under Grant No. 2016/21/B/NZ9/00783.

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[(a)] M. S. Rodriguez-Morgade, P. A. Stuzhin, The chemistry of porphyrazines: an overview, *J. Porphyr. Phthalocya*., 2004, **8**, 1129.

* Correspondence: emil.kolasinski95@gmail.com

NIPAGIN-APPENDED AZA-PORPHYRINOIDS – SYNTHESIS AND CHARACTERIZATION

Emil Kolasinski [1,2]*, Dariusz T. Mlynarczyk [1], Daniel Ziental [2], Lukasz Sobotta [2], Tomasz Koczorowski [1], Jadwiga Mielcarek [2], Tomasz Goslinski [1]

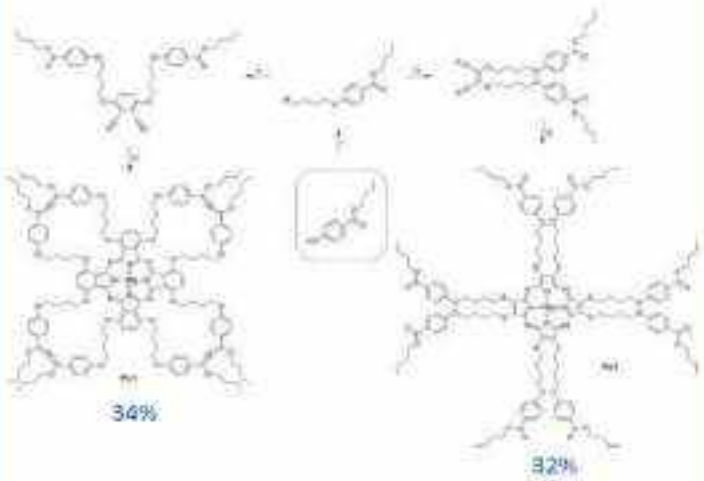
[1] Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland.
[2] Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland
* e-mail: emil.kolasinski93@gmail.com

Aza-porphyrinoids

Aza-porphyrinoids, such as phthalocyanines (Pcs) and porphyrazines (Pzs), are synthetic analogues of naturally occuring porphyrins. The change of methine bridges to aza-methine bridges that link the pyrrole moieties causes a shift in Q-band absorption maxima towards longer wavelengths. Unfortunately, due to high aggregation and low solubility of the unsubstituted macrocyclic ring, further functionalization is necessary.

Our approach was to combine macrocycles with 4-hydroxybenzoates, commonly known as nipagins, which exhibit by themselves strong antibacterial properties. Doing so, both macrocycles could be compared.

Synthetic approach



(i) 1,4-dibromobutane, K_2CO_3, DMF (*N,N*-dimethylformamide), rt, 72 h; (ii) dimercaptomaleonitrile disodium salt hydrate, K_2CO_3, DMF, 50°C, 20 h; (iii) $Mg(n\text{-}C_4H_9)_2$, $n\text{-}C_4H_9OH$, 120°C, 20 h.

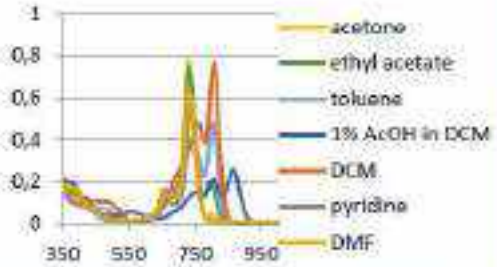
Spectral and photochemical properties

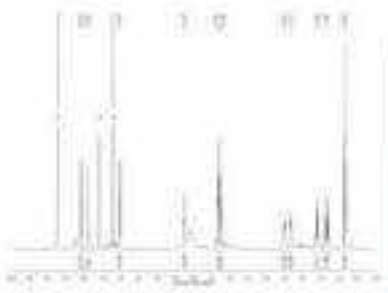
The synthesis of porphyrazine and phthalocyanine substituted in the periphery with the same substituents enabled to compare both molecules in terms of their spectral and photochemical properties. UV-Vis and NMR spectroscopy was applied.

To assess the potential usefullness of the synthesized macrocycles in photocatalysis, singlet oxygen generation quantum yields (Φ_Δ) were calculated. The method used was indirect chemical method with 1,3-diphenylisobenzofuran as singlet oxygen quencher and ZnPc as a reference.

Compound	$\Phi_{\Delta\ DMF}$
Pz1	0.02
Pc1	0.29

It was found that the phthalocyanine derivative can be treated as more efficient singlet oxygen generator, with moderate singlet oxygen quantum generation yield values.





^{1}H spectrum of **Pc1**.
(# - pyridine-d_5, ~ - water)

Solvatochromic effects observed for **Pc1**.

This study was supported by the National Science Centre, Poland, under grant No 2016/21/B/NZ9/00783.

Study of PHF6 aggregation process by molecular dynamics in Alzheimer's disease.

Charline Fagnen[(1)], Johanna Giovannini [(1)], Anne-Sophie Voisin-Chiret [(1)], Jana Sopkova de Oliveira Santos [(1)] *.

(1) Normandie University UNICAEN, EA 4258 CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie) FR CNRS 3038 INC3M SF4206 ICORE, Boulevard Henri Becquerel 14032 Caen

Restricted to organizers

Described in 1906 by Alois Alzheimer[(a)], Alzheimer's disease is a neurodegenerative disorder that represents 60 to 80% of dementia cases. Without treatment today, predictions reveal that its prevalence will be multiplied by four until 2050. This pathology is caused by the aggregations of Tau protein and amyloids that disrupt the neuronal signal. It is why we focus on Tau protein, more especially a short but crucial sequence called PHF6: ^{306}VQIVYK^{311} responsible for its own aggregation. Our goal is to understand this mechanism at the atomic scale.

To do it, 2 microseconds molecular dynamics (MD) simulations were performed, including 62 PHF6 peptides. Their analysis was realized with the PyEMMA[(b)] software package thanks to Markov State Model method. It permits the obtention of metastable structures, thermodynamics, kinetics, and making statistics on the different possible paths among states.
MD simulations showed that the PHF6 aggregation is initiated by a dimer aggregation, followed by a trimer, a tetramer… Moreover, the assembly of two multimers. The PHF6 aggregation mechanism is not known to an atomic level. A recent publication[(c)] describing the PHF6 dimerization, has given some clues about the beginning of its mechanism.

This work focuses on the different processes: i) dimerization, ii) trimerization, iii) tetramerization and iv) complexes' formation to gain a better of PHF6 aggregation. This knowledge is essential to understand how to prevent these phenomena for the treatment of Alzheimer's disease.

Aggregation of 9 PHF6 peptides.

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[(a)] Evolution in the Conceptualization of Dementia and Alzheimer's Disease: Greco-Roman Period to the 1960s. N. C. Berchtold, et al. *Neurology of Aging*, 1998

[(b)] PyEMMA 2: A Software Package for Estimation, Validation, and Analysis of Markov Models. Martin K. Scherer et al. *Journal of Chemical Theory and Computation*, 2015

[(c)] Disclosing the Mechanism of Spontaneous Aggregation and Template-Induced Misfolding of the Key Hexapeptide (PHF6) of Tau Protein Based on Molecular Dynamics Simulation. Liu and al. *ACS Chemical Neurosciences* 2020

* Correspondence: jana.sopkova@unicaen.fr

Study of PHF6 aggregation process by Molecular Dynamics in Alzheimer's disease

Charline Fagnen[1], Johanna Giovaninni[1], Anne Sophie Voisin-Chiret[1], and Jana Sopková-de Oliveira Santos[1*]

(1) Centre d'Etudes et de Recherche sur le Médicament de Normandie, Université de Caen Normandie, Caen, France, 14000

* Correspondence: jana.sopkova@unicaen.fr

Alzheimer disease (AD) established in 1906[1]
60-80% of dementia cases
Without treatment : prevalence x 4 in 2050

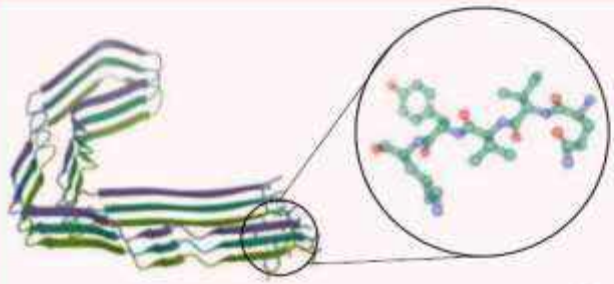
The Tau protein is one of the key elements responsible for the Alzheimer disease:
its aggregation causes the disrupt of neuronal signal.

Goal:
Understand the aggregation mechanism of the Tau protein to prevent this phenomena

Normal synaptic connection[2]

Tau protein aggregates

Synaptic connection in Alzheimer Disease



Aggregation of three tau proteins[3] → Extraction of PHF6[3] (from one tau protein) aa. 306-311

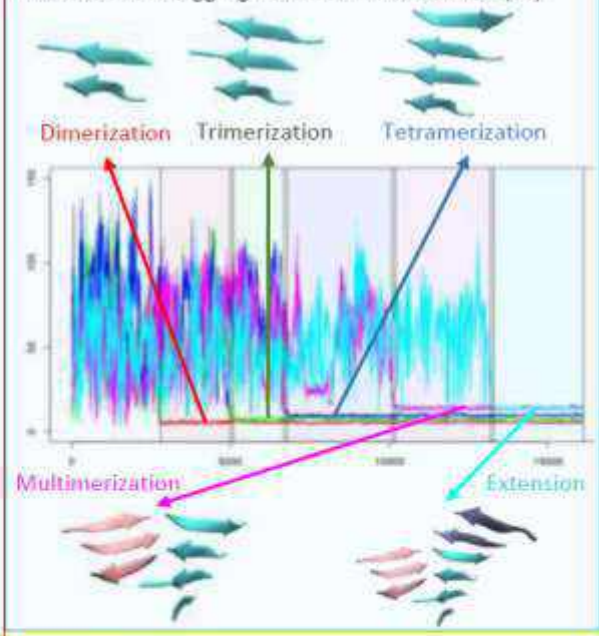
System is reduced to PHF6 because it was identified in the involvement in the aggregation process

Classical molecular dynamics simulations were launched on PHF6 during 2µs (with 62 PHF6)

0,25µs

1,50µs

The distances (Å) are computed between the center of mass of one or an aggregate of PHF6 and the center of mass of the next peptide/aggregate involved in the aggregation in function of time (ns)



Take Home:

- ✓ PHF6 correctly aggregates
- ✓ Different steps of this aggregation are identified

Future →

- ✓ Other kinds of multimerization observed
- ✓ Interaction networks of the multimers

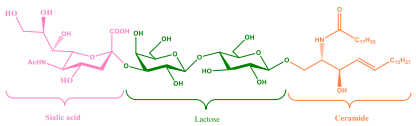
References:
[1] N. C. Berchtold, et al. *Neurology of Aging*, 1998, 19, 173-189
[2] Servier Medical Art
[3] B. Falcon, et al. *Nature*, 2019, 568, 420-423

Chemoenzymatic synthesis of oligomers of GM3 analogues and biological evaluation of their anticancer activities.	***Restricted to organizers***

Fang LIU (1)*, Matthieu SOLLOGOUB (1), Yongmin ZHANG (1).

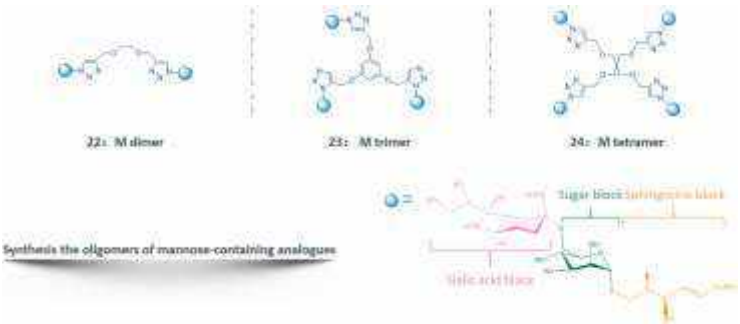
(1) IPCM, CNRS, Sorbonne University, 4 place Jussieu, 75005 Paris, France.

Glycosphingolipids (GSLs) are ubiquitous components on animal cell membranes and exposed on the outer surface of cell membranes. Ganglioside GM3, the first and simplest member in the metabolic series of a GSLs family, containing sialic acid, lactose and ceramide. GM3 has a strong impact on the occurrence and development of human cancer. GM3 is not only overexpressed in several types of cancer but also inhibits tumour cell growth through anti-angiogenesis or motility. In particular, the effect of GM3 on EGFR signalling is essential for cancer[(a)]. Previously our laboratory synthesised a series of GM3 analogues, among them, several compounds display interesting activity against tumour growth. In the present study, we based on the multivalent effect to improve the biological activity of the compound, we screened out mannose-containing analogues with better anti-tumor activity from the previously synthesized GM3 analogues[(b)], and further synthesized mannose-containing analogues oligomers， in order to study their antitumor activities and explore new leading compounds for cancer therapy.



Structure of ganglioside GM3

At first, the sialic acid was activated as sialyl xanthate form. Then the lipid precursor azidosphingosine was synthesized from the commercial D-(+)-galactose. Finally, the mannose bearing a free C6-OH residue was prepared by enzymatic hydrolysis, the enzyme CRL (lipase from Candida rugosa) can be specific to remove the c-6 acetyl group from per-acetylated mannose. further α-sialylation reaction and conjugation with lipid precursor, after several step manipulations, the mannose-containing analogues was synthesized. Next, conjugation of mannose-containing analogues to multivalent skeleton through the click reaction. At last, dimer, trimer and tetramer containing mannose analogues are synthesized.



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(b)C. Zheng, H. Guan, Y. Liu, Z. Li, T. Bavaro, M. Terreni, M. Sollogoub, J. Xu, Y. Zhang, Eur. J. Med. Chem. 2019, 165, 107-114.

* Correspondence: liufang85266@163.com

C-3 hemi-synthetic triterpenic esters as novel antitrypanosomal drugs : example of synthesis and biological studies

Schioppa L.[1]; Poupaert H. J.[2]; Quetin-Leclercq J.[1]

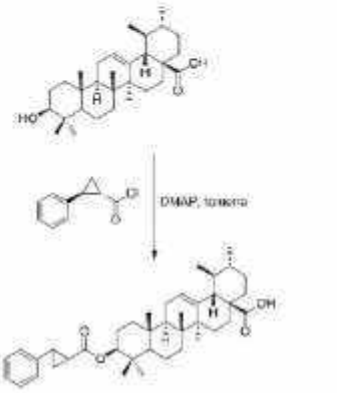
[1]Pharmacognosy Research Group (GNOS), Louvain Drug Research Institute, UCLouvain [2]Medicinal Chemistry, UCLouvain

Introduction

Human African trypanosomiasis, known as sleeping sickness and caused by the protozoan parasite *T. brucei*, is threatening low-income populations in sub-Saharan Africa[1]. Around 50 million people are estimated to live in the affected area.

In the late stage of the disease, parasite crosses the brain-blood barrier causing serious neurological disorders and usually leads to patient's death if left untreated. Despite the successful efforts of the last decades in controlling HAT, these regions are still at risk of large-scale epidemies.

In this context and in view of severe therapeutic limitations due to drugs toxicity or complex administration[2] and to increased parasite resistance[3], innovative drugs are urgently needed for HAT control and possibly eradication.



Scheme 1. Ursolic acid-O-cyclopropyl phenyl propionate one-step synthesis

Selectivity Index (SI)	
(1)	(2)
3.81	>20

Table 1. Selectivity indexes of tested compounds

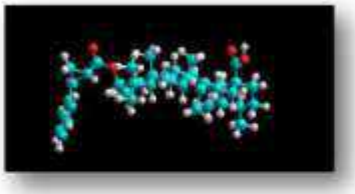


Figure 1. Ursolic acid-O-cyclopropyl phenyl propionate 3D molecular modeling

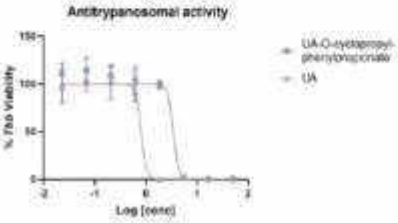


Figure 3. Dose activity relationship of tested compounds on Tbb

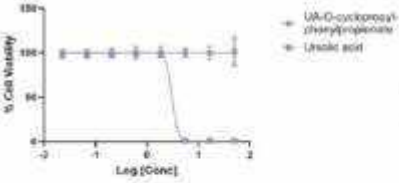


Figure 4. Dose activity relationship of tested compounds on WI38 cells

Materials and Methods

The hemi-synthesis of (2) was adapted from Tanachatchairatana, Tanud, et al[5]. respectively **(Scheme 1)**.

In vitro tests were performed at least in triplicate on bloodstream form of *Trypanosoma brucei brucei* (Tbb, strain 427) and mammalian cells (WI38) for cytotoxicity determination with the AlamarBlue assay.

The activities are defined with the IC_{50} values calculated on the inhibition sigmoidal curves. Selectivity indices (SI) were obtained as followed:

$$SI = \frac{IC_{50}\ mammalian\ cells}{IC_{50}\ parasite}$$

Acknowledgements

MS data were obtained at the MASSMET platform of UCLouvain. Laura Schioppa is a Research Fellow of the Fonds National de la Recherche Scientifique - FNRS. The authors wish to thank Maude Bourlet for her technical assistance.

Background

The approach towards the development of new antitrypanosomal drugs was based upon the known antitrypanosomal activity of pentacyclic triterpenic acids and their C-3 esters, which were previously shown to be active on *Trypanosoma brucei brucei*[4].

Underivatized free acids such as oleanolic and ursolic acid, are indeed endowed with interesting pharmacological profiles but suffer from rather important cytotoxicity.

The aim of this study is to generate triterpenic esters derivatives with improved efficacy and/or reduced toxicity to be used in the context of HAT.

Results

To improve the drug potential of ursolic acid, chemical modifications were implemented to generate a 3-ester to try to decrease the ursane cytotoxicity. A prototype was created with a one step-synthesis, namely ursolic acid-O- cyclopropyl phenyl propionate (2).

Characterization by 3D modeling **(Fig.1)** coupled with NMR data (not shown), Mass Spectrometry **(Fig.2)** and HPLC chromatography, confirmed the structure and the purity of the synthetized compound to be >95% (yield= 32%).

The synthetic compound showed an interesting profile on *Tbb*: despite its lower activity (IC_{50} = 5.4µM) compared to the correspondent acid (IC_{50} = 2.2µM), (2) it is still efficient in the micromolar range **(Fig.3)** with no cytotoxicity compared to usolic acid (UA) **(Fig.4)** so showed a highly improved selectivity **(Tab.1)**.

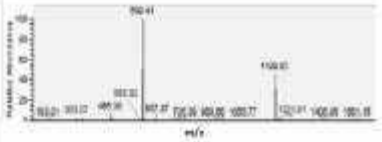


Figure 2. Ursolic acid-O-cyclopropyl phenyl propionate MS spectrum

Conclusion

Research for innovative drugs is crucial to contribute to HAT control and eradication. Through this study, the strategy of creating a new C-3 hemi-synthetic triterpenic ester as novel antitrypanosomal drug shows its interest and needs to be developed further.

Our effort to create a 3-O- derivatives library to have more hemi-synthetic derivatives should be pursued. We will also try to find a solution to overcome the overall low synthesis yield obtained and analyse its in vivo activity.

Contact

Laura Schioppa (MSc)
UCLouvain
laura.schioppa@uclouvain.be
www.linkedin.com/in/laura-schioppa
+32 (0)27647234

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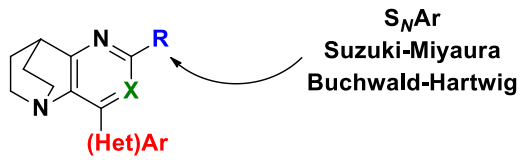
Design and functionalization of new heterocycles fused with a quinuclidine moiety as analogs of SK inhibitors

Flore CARE [(1)*], Mazarine LAURENT [(1)], Stéphane BOSTYN[(2)], Yves ROBIN[(3)], Sylvain ROUTIER[(1)], Frédéric BURON[(1)]

(1) Institut de Chimie Organique et Analytique (ICOA), Université d'Orléans, UMR CNRS 7311, rue de Chartres, BP 6759, 45067 Orléans, France.
(2) Institut de Combustion, Aérothermique, Réactivité et Environnement (ICARE), 1c avenue de la recherche scientifique, 45071 Orléans Cedex 2, France.
(3) PMC ISOCHEM, 4 rue Marc Sangnier, BP 16729, 45300 Pithiviers, France.

Restricted to organizers

Derivatives containing a quinuclidine scaffold are molecules of interest in medicinal chemistry. Indeed, this heterocycle is present in many natural molecules such as quinine, and have many different biological properties such as antimalarials, antipyretic or analgesic. Its high potential and its physicochemical properties have led chemists to develop synthetic molecules targeting, in particular, central nervous system receptors such as the α7 nicotinic acetylcholine receptor. [a]
Despite its potential, few fused polycyclic structures containing the quinuclidine scaffold are described in the literature.[b] Therefore, there is great interest in developing novel structures and associated synthetic methodologies to increase the molecular diversity of these families and to study their biological potentials.
To remove this lock, we have developed the synthesis of versatile platforms with a quinuclidine moiety fused with heterocycle containing nitrogen atom. We focused our research to create C-C or C-heteroatom bounds with several functionalization methodologies such as palladium-catalyzed cross-coupling or S_NAr using modern synthesis techniques, such as flow chemistry.
All of these methodologies will be applied to design original analogs of SK inhibitors.



X = C, N
R = OH, Cl

Bibliographic references:

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(b) i) W. S. Hamama, O. M. A. El-Magid, H. H. Zoorob, Z. Für Naturforschung B 2006, 61, 93-100; ii) R. Singh et al. ; WO2010/005879A1.

*** Correspondence:** flore.care@univ-orleans.fr

Modelization, application and transposition of a continuous flow chemistry process to pharmaceutical and cosmetic industries

Flore Caré[1]*, Mazarine Laurent [1], Stéphane Bostyn [2], Sylvain Routier [1], Frédéric Buron [1]

(1) ICOA, UMR 7311, rue de Chartres - BP6759, 45067 Orléans Cedex 2, France.
(2) ICARE, 1c, avenue de la recherche scientifique, 45071 Orléans cedex 2, France

Introduction

To respond to industrial challenges, continuous flow chemistry has risen as an innovative process alternative.
Its many advantages include enhanced heat and mass transfer, providing faster, safer and more efficient and reliable reactions, as well as easy scale up. [1]

It has therefore gained interest from pharmaceutical and cosmetic industries to employ this technology. Robust and compact processes coupling online functionalization and analysis can be designed and modulated with HPLC pump systems as presented in Fig. 1. Transposition to flow chemistry offers unlocking traditionally long or challenging reactions.

Figure 1: Device used to perform continuous flow reactions

Application to diazonium salt formation

- Formation of an unstable intermediate which must react very quickly
- Very exothermic reaction
- High risk of thermal runaway[4]

Challenges for transposition to flow chemistry

- Very corrosive environment → necessity of acid resistant pumps
- Suspension → evolution towards a homogeneous system
- Emission of N_2 → increase of pressure to handle

Implementation of the flow process in the lab

Semi-continuous process (Fig. 7) with neutralization of the diazonium salt in flask. Product is obtained with a good yield of 88% in 40 min with a production of 7,44g/h.

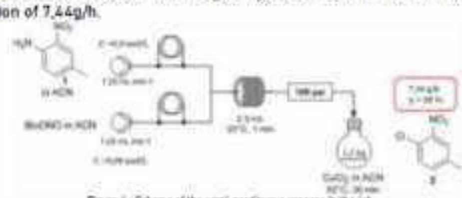


Figure 4: Scheme of the semi-continuous process in the lab

Transposition to industry

Transposition of the system to an industrial scale (Fig. 5).
Obtention of the expected product with a production of 120 g/h and a yield of 93%.

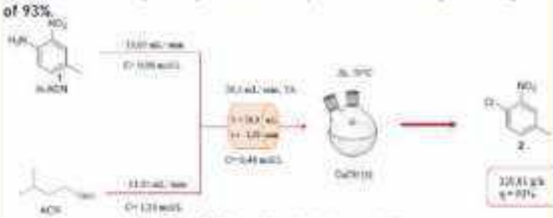


Figure 5: Scheme of the optimized process in industry

Increase of the security trough this process
Extrapolation possible to a production of R-Cl of 5 tones by year on the industrial site of Pithiviers.

Thermal study of the flow reactor

Figure 2: Coil reactor to model

Reactor description

The system studied (Fig. 2) is composed of :

- A coil reactor made of PTFE tubing (1 mm I.D.)
- A stainless steel cylinder with resistances ensuring coil reactor heating
- A glass lid for thermal insulation
- A Teflon disk (thermal insulation), not integrated to the model

Temperature distribution

Temperature and liquid hydrodynamics (water) distribution were modelized comparing :

- 2.5 and 20 mL reactors
- Entry speeds : 0.02 m/s and 0.42 m/s
- Heating temperature : 40, 80 and 120 °C

Results are presented in Fig. 3.

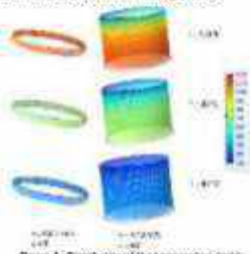


Figure 3: Distribution of the temperature inside the reactor

Temperature stabilizes but with a delay to reach set temperature. This delay increases when gradient is high or material such as PTFE offers low thermal conductivity. Taking into account this delay is necessary for study of reaction. Pre-heated reactants or metallic tubing can reduce this effect.

Application to aldol reactions

Aldolization - crotonization

3-quinuclidinone hydrochloride A and pyridine carboxaldehyde B react together to form the α,β-unsatured ketone C as described in Fig. 6.

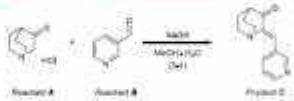


Figure 6: Reaction scheme

Impact of temperature

Modelization of a 5 mL PTFE reactor.
Input concentrations : 0.45mol/L (A); 0.46 mol/L (B).
Flow rates : 2 and 5 mL/min Temperature : 50 and 100°C

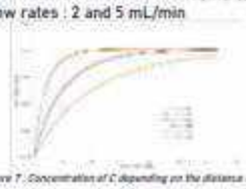


Figure 7: Concentration of C depending on the distance covered

- Limited reaction : maximum of 0.2 mol/L of C
- Weak impact of temperature
- Input rate results in important time saving.

Application of modelization

Variation of flow rate, input concentration and temperature in modelization to determine maximum yield and best operating parameters.

Temperature (°C)	50			
Input concentration in A (mol/L)	0.45			
Input flow rate (mL/min)	1			
Input concentration of B (mol/L)	0.45	0.90	1.35	1.80
Concentration of C at equilibrium (mol/L)	0.20	0.30	0.34	0.36
Time to reach equilibrium concentration (s)	48	43	31	24

Table 1: Results of the simulation

Thanks to modelization , a yield of 56 % was achieved in a minimum of experiments

Conclusion

This project allowed us to put in place a realistic modelization, which enabled the optimization of a chemical reaction in a minimum of experiments, thus limiting the cost.
On the industrial aspect, this modelization is a real asset since it will allow implementation of chemical reactions more quickly while gaining efficiency, speed, reliability, safety and environmental compliance. This was illustrated with the industrial transposition of diazonium salt formation thanks to continuous flow chemistry. Currently, we are studying flow chemistry technologies to modulate natural products for cosmetic application.

Acknowledgments

Centre-Val de Loire

Cosmetosciences

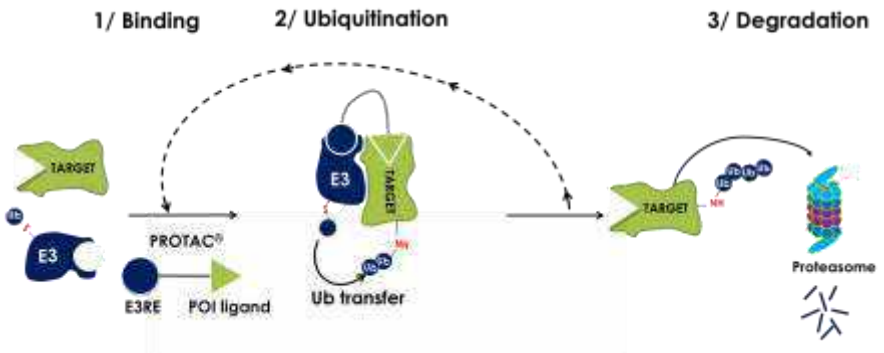
pmc isochem

flore.care@univ-orleans.fr

PROTAC® an emerging drug discovery technology for target validation **N. Kraupner*, L. Lesire, A. Herledan, S. Warenghem, V. Landry, F. Leroux, C. Piveteau, B. Deprez, R. Deprez-Poulain, D. Bosc.** *(1) Univ. Lille, Inserm, Institut Pasteur de Lille, U1177—Drugs and Molecules for Living Systems, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE*	***Restricted to organizers***

Thanks to its capability to recruit the cellular natural protein degradation system, PROTAC® (PROteolysis TArgeting Chimera) appears as a very promising technology in drug discovery for target validation. Indeed, PROTAC compounds are bifunctional small molecules that hijack the ubiquitin-proteasome system (UPS) to selectively degrade target protein in cells. In the last decades, new strategies have been proposed to modulate protein levels such as siRNA gene silencing and CRISPR-Cas9 gene-editing technology. Even if these strategies are very attractive, they cannot be used widely for example *in vivo*. With optimized properties, PROTACs® are new powerful tools for exploring biological pathways, target validation and development of therapeutic compounds.

Here, we will define PROTAC® and illustrate how we designed such compounds for an *in-house* protein of interest.



Bibliographic references:
(a): Churcher, I J Med Chem. 2018, 61,2 444–452.
(b) : Paiva, S.-L.; Crews, C. M. Gener. Ther. 2019, 50, 111–119.
(c) : Pettersson, M.; Crews, C. M. Drug Discov. Today Technol. 2019, 31, 15–27.

* Correspondence: nicolas.kraupner@univ-lille.fr

Inserm U1177




PROTAC® an emerging drug discovery technology for target validation

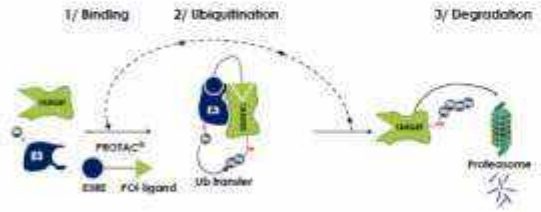
N. Kraupner*, I. Lesire, A. Herledan, S. Warenghem, C. Piveteau, V. Landry, F. Leroux, B. Deprez, R. Deprez-Poulain, D. Bosc.

Inserm U1177 Drugs and Molecules for Living Systems, Institut Pasteur de Lille, Université de Lille, Inserm, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE

nicolas.kraupner@univ-lille.fr
www.deprezlab.fr

1 Introduction to PROTAC®

PROTACs® (PROteolysis TArgeting Chimeras) are bifunctional small molecules that hijack the Ubiquitin Proteasome System (UPS) to selectively degrade target proteins within cells. They represent a new modality for small molecule ligands to achieve the selective degradation (knock-down) of a target protein.



PROTAC® compounds are a combination of three parts:

- **An E3 ligase recruiting element (E3RE)**
- **A warhead of the target protein**
- **A linker to gather the two moieties**

Churcher I. J Med Chem. 2018, 61,2 444-452.

2 Design of a PROTAC® compound

A PROTAC® compound, if correctly designed, is able to bind both E3 ubiquitin ligase and the target protein. The resulting ternary complex will allow the ubiquitin transfer of ubiquitin units from E3 to the target protein until the formation of a ubiquitin chain. As this chain is a degradation marker by the ubiquitin-proteasome system, the labeled protein will be degraded by this system.

Paiva, S.-L.; Crews, C. M. Genet. Ther. 2019, 35, 111–119.

3 Proteins compatibility and cooperativity

Proteins, including E3 ligase and the protein of interest, have a certain shape and polarity. The ligase and the protein of interest must be compatible regarding these features. Thus, in the context of a PROTAC® ternary complex (ABC), the binding affinity of the PROTAC® "B" to one protein partner "C" (binary binding) may be enhanced or reduced by the presence of the second protein partner "A" (ternary binding).

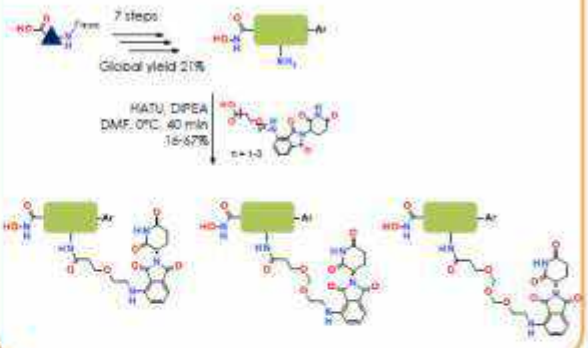
This effect can be quantified in terms of a "**cooperativity**" **(α)** factor, defined as below. This index can be either >1 when stabilizing PPIs between the two proteins promote ternary complex formation, or < 1 when interaction abrogates ternary binding. This index depends on the protein compatibility and the structure of the PROTAC®.

$$\alpha = \frac{K_D^{binary}}{K_D^{ternary}}$$

Pettersson, M.; Crews, C. M. Drug Discov. Today Technol. 2019, 31, 15–27.

4 Design and synthesis of a PROTAC® for our target

We want to explore and validate our target, a metalloprotease, which is targeted by our series of compounds possessing a **hydroxamate as ZBG** to coordinate the catalytic zinc in the binding pocket. A synthetic pathway was developed and allowed the obtention of 3 pomalidomide-based PROTACs®.



5 Preliminary results

Our new compound showed **nanomolar activity in enzymatic assay** on the target protein alone, showing evidence of target engagement in an in vitro experiment. Furthermore, permeability studies have been performed on these compounds and **all three are permeable in HeLa cells.**
During the development of degrader compounds, it is important to assess their cellular permeability and their affinity for the target protein. These preliminary results are very encouraging because target engagement and cell permeability are some hurdles encountered in the development of these type of compounds.

Conclusion & Perspectives: Thanks to our biological platform these compounds will be tested in western blot experiment in order to evaluate their ability to degrade our target of interest. If one of these compound is a proven PROTAC® and consequently capable to degrade the protein of interest, it should be a **good probe to explore the multiple roles of our target.**

(1) Churcher, I. J Med Chem. 2018, 61,2 444-452.
(2) Paiva, S.-L.; Crews, C. M. Genet. Ther. 2019, 35, 111–119.
(3) Pettersson, M.; Crews, C. M. Drug Discov. Today Technol. 2019, 31, 15–27.

Solid phase synthesis of Neurokinin receptor subtype specific agonists.

Maximilian Krisch, Kirtikumar B. Jadhav*.

Institute for biological chemistry, University of Vienna, Währinger strasse 38, 1090, Vienna, Austria.

Restricted to organizers

Tachykinins (TKs) are a family of endogenous peptides widely expressed in the central and peripheral nervous systems as well as in the GI tract featuring unique *C*-terminal amino acid sequence Phe-Xaa-Gly-Leu-Met-NH_2 [(a,b)]. They act as full agonists of *G*-protein coupled membrane receptors (neurokinin (NK) 1, NK2, and NK3)[(a,b)]. NK receptors are shown to be promising as druggable targets for eg. Tachykinin NK1 receptor antagonists for chemotherapy induced nausea and vomiting (rolapitant), as anticancer drugs, NK2 antagonist for irritable bowel syndrome (IBS), NK3 antagonists for treating schizophrenia [(b,c)].

However, because of wide presence of these tachykinin receptors, it is not surprising that systemic administration of their antagonists non-specifically targets gut receptors, leading to side effects and subsequent discontinuations from clinical trials. As a remedy, we aim to target gut receptors selectively with orally administered peptides to promote epithelial repair *via* interaction with gut receptors involved in the wound healing response such as for GI disorders (IBS & IBD).

Towards this goal, we report a successful synthesis of endogenous peptide agonists - Substance P, Neurokinin A and Neurokinin B; which display a very high selectivity towards NK1, NK2 and NK3 respectively. We synthesized these peptides by solid phase peptide synthesis using Fmoc strategy on an automated microwave peptide synthesizer. Peptides were purified by reversed phase preparative HPLC and characterized by UHPLC-MS. We obtained desired peptides in sufficient quantities (20-37 mg), good to very good yields (19-37%) and a very high purity (99%).

The synthesized agonists will serve as an in-house training set for biochemical, pharmacological, gut stability assays and for structural studies. In long term, this work will pave the way to identify TK analogues with anti-inflammatory effects as a novel treatment approach for IBD/IBS.

Bibliographic references:

(a) Goode, T., Shanahan F. *et al* Neurokinin-1 receptor expression in inflammatory bowel disease: molecular quantitation and localisation. *Gut*. **2000,** *47*, 387.

(b) i) Schöppe, J., Ehrenmann, J., Klenk, C. *et al*. Crystal structures of the human neurokinin 1 receptor in complex with clinically used antagonists. *Nat*. *Commun*. **2019**, *10*, 17. ii) Chen, S., Lu, M., Liu, D. *et al*. Human substance P receptor binding mode of the antagonist drug aprepitant by NMR and crystallography. Nat. Commun. **2019**, *10*, 638.

(c) Holzer, P., Holzer-Petsche, U. Tachykinin receptors in the gut: physiological and pathological implications. *Current Opinion in Pharmacology* **2001,** *1*, 583.

* Correspondence: kirtikumar.jadhav@univie.ac.at

Solid phase synthesis of Neurokinin receptor subtype specific agonists

Maximilian Krisch, Kirtikumar B. Jadhav*

Institute of biological chemistry, University of Vienna, Währinger Straße 38, 1090, Vienna, Austria

Overview

We report a successful synthesis of endogenous peptide agonists of NK-receptors Substance P, Neurokinin A and Neurokinin B. These Tachykinins (TKs) are widely expressed in the nervous systems as well as in the GI tract and act as full agonists of G-protein coupled membrane receptors (Neurokinin (NK) 1, NK2, and NK3)[1]. The NK-receptors are shown to be promising as druggable targets for e.g. NK2 antagonist for irritable bowel syndrome (IBS)[2,3]. However, because of wide presence of these tachykinin receptors, systemic administration of their antagonists leads to side effects and subsequent discontinuations from clinical trials. As a remedy, we aim to target gut receptors selectively with orally administered peptides to promote epithelial repair via interaction with gut receptors involved in the wound healing response such as for GI disorders (IBS & IBD).

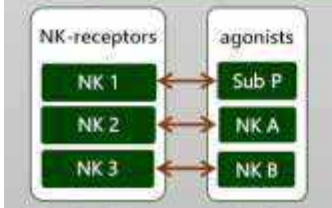


Fig. overview of NK-receptors and their specific agonists

Fig. NK1 (mint) with Substance P (pink)

Fig. NK-antagonists as possible therapy for GI disorders

Experimental

The peptides were synthesized by solid phase peptide synthesis on a 0.1 mmol scale using Fmoc strategy on an automated microwave peptide synthesizer with DIC and Oxyma as coupling reagents. We used MBHA rink amide resin and standard Fmoc and sidechain protected amino acids, except for one pseudo-proline in an Asp-Ser sequence in Neurokinin A to prevent aspartimide formation. Deprotection and cleavage of the peptides were performed in a one-pot reaction using reagent B (88% TFA, 5% phenol, 5% water, 2% triiso-propylsilane) with subsequent precipitation and washing in diethyl ether. The peptides were purified by reversed phase preparative HPLC, characterized by UHPLC-MS and finally dried via lyophilization to obtain the clean products.

Results

Substance P

19% yield (25mg)
pur. >99%

$[M+H]^+$ at 4.20min:
obs.: 1347.7m/z
calc.: 1347.7m/z

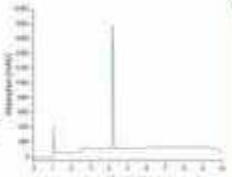


Fig. LC-MS spectrum of Substance P

Neurokinin A

33% yield (37mg)
pur. >99%

$[M+H]^+$ at 3.93min:
obs.: 1133.6m/z
calc.: 1133.3m/z

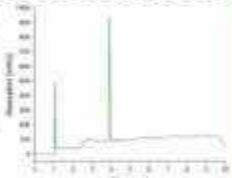


Fig. LC-MS spectrum of Neurokinin A

Neurokinin B

18% yield (20mg)
pur. >99%

$[M+H]^+$ at 5.63min:
obs.: 1210.1m/z
calc.: 1210.4m/z

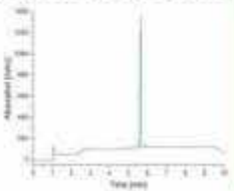
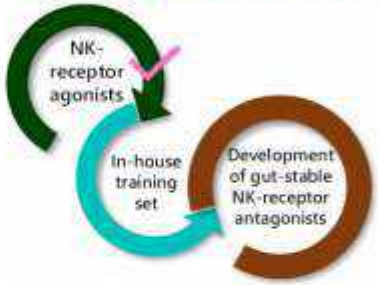


Fig. LC-MS spectrum of Neurokinin B

Future Prospects



The synthesized agonists will serve as an in-house training set for biochemical, pharmacological and gut stability assays as well as structural studies. In long term, this work will pave the way to identify TK analogues with anti-inflammatory effects as a novel treatment approach for IBD/IBS.

References

1) Goode, T., Shanahan, F. et al. Neurokinin receptor expression in inflammatory bowel disease: molecular quantitation and localization. Gut **2000**, 47, 367

2) a) Schäppi, L., Rheinwald, J., Klenk, C. et al. Crystal structure of the human neurokinin 1 receptor in complex with clinically used antagonists. Nat. Commun. **2019**, 10, 17. b) Chen, S., Lu, M., Liu, D. et al. Human substance P receptor binding mode of the antagonist drug aprepitant by NMR and crystallography. Nat. Commun. **2019**, 10, 638.

3) Holzer, P., Holzer-Petsche, U. Tachykinin receptors in the gut: physiological and pathological implications. Current Opinion in Pharmacology **2001**, 1, 563.

Acknowledgement: Support by the FWF-ZK (grant No. ZK-818 to K.B.J.) is gratefully acknowledged. We thank Assoc. Prof. Dr. Muttenthaler and Prof. Dr. Christian Becker for their generous support.

Design of FabZ inhibitors as potential antibiotics

Laurie Bibens*[(1)], Jean-Paul Becker[(1)], Nadine Lemaître[(1)], Nicolas Taudon[(2)], Alexandra Dassonville-Klimpt[(1)], Pascal Sonnet[(1)].

[(1)] AGIR, UR 4294, Université de Picardie Jules Verne, Amiens, France
[(2)] Unité de Développements Analytiques et Bioanalyse, IRBA, Brétigny-sur-Orge, France

Restricted to organizers

To date, antimicrobial resistance is one of the biggest public health challenges. Each year, at least 700 000 people die from antibiotic-resistance infections worldwide[(a)]. Among the most virulent pathogens, gram-negative bacteria such as *P. aeruginosa*, some mycobacteria as *Mycobacterium tuberculosis* are particularly worrying.

Therefore, it is urgent to identify and develop novel antibiotics with original antibacterial mechanisms of action. Amongst these, lipids are essential to maintain the bacterial membrane integrity and enzymes involved in their biosynthesis are potential antibiotic targets. These proteins are ubiquitous in the microbial pathogens and are not present in humans. Thus, risk of side effect with highly selective drugs is limited. FabZ is a β-hydroxyacyl-acyl carrier protein (ACP) dehydratase which plays a key role in the fatty acid elongation system (FAS II). So, the development of FabZ inhibitors could allow to design broad-spectrum antibiotics with limited side effects.

Very few FabZ inhibitors were described[(c), (d)] while several FabZ structures from different organisms such as *P. aeruginosa*, *P. falciparum*, *H. pylori* have been reported. Among FabZ inhibitors, a quinoline, NAS91-10 showed a good activity against *Pf*FabZ (IC_{50} (*Pf*FabZ)= 7.4 µM) [(c)]. Moreover, the structure of *Pf*FabZ in complex with NAS91-10 is available in the protein database (PDB code: 3AZA). Based on these, we have started a FabZ-based drug design study to propose new quinoline structures. The *in silico* study, the synthesis of the new quinolines and the first biological results will be exposed.

Bibliographic references:
[(a)] O'Neill, J., *Review on Antimicrobial Resistance*, **2016**, Final report.
[(b)] Kumar, A., Ghosh, D. K., Ali, J., Ranjan, A., *ACS Chemical Biology*, **2019**, 14, pp. 901–915.
[(c)] Maity, K., Venkata, B. S., Kapoor, N., Surolia, N., Surolia, A., Suguna, K., *Journal of Structural Biology*, **2011**, 176, pp. 238–249.
[(d)] Chen, J., Zhang, L., Zhang, Y., Zhang, H., Du, J., Ding, J., Guo, Y., Jian, H., Shen, X., *BMC Microbiology*, **2009**, 9, pp. 91–102.

* **Correspondence**: laurie.bibens@u-picardie.fr

Design of FabZ inhibitors as potential antibiotics

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[1] AGIR, UR 4294, Université de Picardie Jules Verne, Amiens, France
[2] Unité de Développements Analytiques et Bioanalyse, IRBA, Brétigny-sur-Orge, France
laurie.bibens@u-picardie.fr

INTRODUCTION

To date, antimicrobial resistance is one of the biggest public health challenges. Each year, at least 700 000 people die from antibiotic-resistance infections worldwide[1]. Among the most virulent pathogens, Gram-negative bacteria such as *P. aeruginosa*, some mycobacteria as *M. tuberculosis* are particularly worrying.

Therefore, it is urgent to identify and develop novel antibiotics with original and selective antibacterial mechanisms of action. Lipids are essential to maintain the bacterial membrane integrity and enzymes involved in their biosynthesis, particularly in the fatty acid elongation system (FAS II), are potential antibiotic targets. These proteins are ubiquitous in the microbial pathogens and are not present in humans, which limit the risk of side effects. Among these, FabZ, a β-hydroxyacyl-acyl carrier protein (ACP) dehydratase which plays a key role in the FAS II, is a suitable yet unexplored target to design broad-spectrum antibiotics with limited undesirable effects.

Very few FabZ inhibitors were described[2,3]. Among them, the **NAS91 family** with a quinoline core inhibits *Pf*FabZ with IC_{50} in a micromolar range (figure 1). Additionally, co-crystal structures of *Pf*FabZ in complex with members of **NAS91 family** were determined and are indexed in the Protein Data Bank (PDB) (3AZA, 3AZB, 3AZE). Based on these, we have started a FabZ-based drug design study to propose new potential antibiotic drugs with quinoline-based pharmacophore.

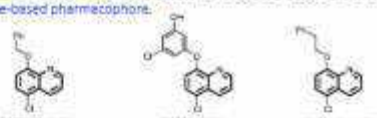

Figure 1: Known inhibitors of FabZ

AIM & STRATEGY

A FabZ-based drug design study was performed from FabZ-NAS91 family inhibitor complexes whose structures are available in the PDB. Thanks to the results of the structural analysis, three series 1-3 of potential inhibitors were designed. Herein, we will present the synthesis of the first quinoline-based inhibitors that belong to series 1 and 2. After synthesis, enzymatic assays on purified *Pf*FabZ will be carried out to check their ability to inhibit FabZ. In relation to these results, docking studies will allow us to better understand the mode of actions (MoA) and to establish the Structure-Activity Relationship (SAR).

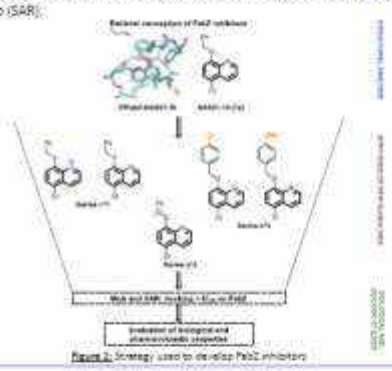

Figure 2: Strategy used to develop FabZ inhibitors

STRUCTURAL ANALYSIS and STRUCTURE-ACTIVITY RELATIONSHIP STUDY

STRUCTURAL ANALYSIS

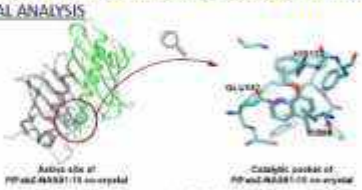

Figure 3: Structural analysis with the *Pf*FabZ-NAS91-10 co-crystal

The structure of *Pf*FabZ was solved in complex with three quinolinic compounds of the NAS91 family (PDB access code: 3AZA, 3AZB and 3AZE)[4]. These structures show three key interactions between the protein and its ligand (figure 3). First, HIS98 and HIS133 appear to create hydrogen bonds with an oxygen atom of NAS91 compounds. Second, GLU147 seems to interact with quinolinic nitrogen atom through another hydrogen bond.

SAR STUDY

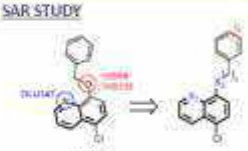

Figure 4: Pharmacomodulation of NAS91-10

	Series 1	Series 2	Series 3
Structural modification	X,Y: N or CH; X or NH; CO, O or CH_2	cf. 2 or 3	Choice of group R with QSAR method
Aim	Nature of interactions with HIS98, HIS133 and GLU147 ?	Creation of new interactions to stabilize the complex ?	Creation of new interactions to stabilize the complex ?

Table 1: Structural modification of NAS91-10 and aim for the SAR study

To identify the nature of the essential interactions for the complex stability, three series of molecules will be synthesized (table 1, figure 4). Alongside with *in silico* protein ligand docking studies (AutoDock Vina), this should allow the development of new compounds with increased affinity. In the meantime, the predicted binding mode of the synthesized molecules will be compared to those observed in the crystallographic structures and the computed binding affinities will be ranked. Here, we present the synthesis of **1a**,**1b**, **1d** (series 1) and **2a** (series 2).

SYNTHESIS of COMPOUNDS 1a, 1b and 2a

To synthesize **1a**, **1b** and **2a**, we have chosen to use reactional conditions described to synthesize NAS91-10[4]. CuI was used with Cs_2CO_3 as basis in a acetone-H_2O mixture (figure 5). But this reaction had to be optimized for compound **1a** (table 2, figure 5). Reaction time particularly interferes with the yield.

Figure 5: Synthesis of compounds **1a**, **1b** and **2a**

Entry	Reagents	T_1	T_2	CuX (x eq.)	Product	Yield
1	**4a**, **5a**	1 h	5 min	I- (0.04 eq.)	**1a**	0 %
2	**4a**, **5a**	30 min	30 min	I- (0.04 eq.)	**1a**	24 %
3	**4a**, **5a**	5 min	1 h30	I- (0.04 eq.)	**1a**	32 %
4	**4a**, **5a**	45 min	30 min	I- (0.04 eq.)	**1a**	60 %
5	**4a**, **5a**	45 min	30 min	I- (0.1 eq.)	**1a**	17 %
6	**4a**, **5a**	45 min	30 min	InBr (0.04 eq.)	**1a**	18 %
7	**4a**, **5b**	45 min	30 min	I- (0.04 eq.)	**1b**	40 %
8	**4b**, **5a**	45 min	30 min	I- (0.04 eq.)	**2a**	0 %

Table 2: Synthesis conditions of **1a**, **1b** and **2a**

1a was obtained in 60 % of yield (entry 4, table 2) and **1b** in a yield of 40 % (entry 7, table 2). These conditions did not lead to compound **2a** (entry 8, table 2). So, other conditions will be used. Bromoethylbenzene **4b** was reacted with 5-chloroquinolin-8-ol **5a** in presence of K_2CO_3 and KI in acetone at reflux (figure 6) to lead to **2a** in 42 % of yield.

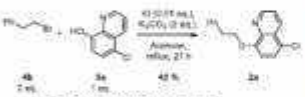

Figure 6: Synthesis of compounds **2a**

SYNTHESIS of COMPOUND 1d

Compound **1d** can be obtained in two steps via intermediate **8**: (i) a one pot synthesis of **8** through *N*-acylation and selective CS chlorination[5] (figure 7, Step 1) and (ii) a reductive reaction of secondary aryl amide function[6] (figure 8, Step 2). For the first step, the best yield was obtained with two equivalents of oxone as oxidant.

Figure 7: Step 1 of the synthesis of compound **1d**

For the second step, different reductive agents were assessed as hydride donor ($LiAlH_4$, $NaBH_3CN.NiCl_2$, $NaBH_4$, BH_3) with or without initial formation of triflic ether (reactional conditions not showed). Hydrogenolysis was also tried without success. Generally, the reduction failed except when $NaBH_4$ was used after the triflic ether formation (figure 8). However, **1d** was obtained in only 11 % yield. Soon, another reductive agent ($BiC_6F_5I_2$) will be tested[7].

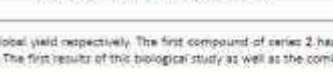

Figure 8: Step 2 of the synthesis of compound **1d**

CONCLUSION & PERSPECTIVES

Three compounds (**1a**, **1b** and **1d**) of the first series have already been synthesized in one or two steps with 3 %, 40 % and 60 % of global yield respectively. The first compound of series 2 has also been synthesized with 42 % yield. Currently, the enzymatic assays of the synthesized compounds against purified *Pf*FabZ are under progress. The first results of this biological study as well as the coming docking results should guide us to perform the design of quinoline-based FabZ inhibitors.

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20th Young Research Fellows Meeting
Paris (France) 11-12 February 2021

Design and synthesis of fluorescent probes as "Chemical Biology" tools to track bcl-2 dependency in breast cancer	***Restricted to organizers***

Hamid Marzag[(1)*], Karen Plé[(1)], Laurent Maillet[(2)], Sabine Sévère[(2,3)], Patricia Gomez-Bougie[(2)], François Guérard [(2)], Michel Chérel [(2)], Martine Amiot[(2)], Philippe Juin[(2,4)] and Sylvain Routier[(1)].

(1) ICOA - CNRS UMR 7311 - Université d'Orléans, Pôle de Chimie – Orléans. (2) CRCINA, UMR 1232 INSERM, Université de Nantes – Nantes. (3) Oniris, Nantes-Atlantic National College of Veterinary Medicine – Nantes. (4) ICO René Gauducheau – Saint Herblain.

Breast cancer remains the leading cause of death by cancer among women. In particular, triple negative breast cancers (TNBCs) and Luminal B (LumB) cancers are difficult to treat with standard chemotherapy although this is almost systematically used as a first line of treatment. For these cases, initial results may be promising, but progression and dissemination are not prevented in the long term.

Philippe Juin's team in Nantes currently studies the BCL-2 family of proteins which act at the core of the therapeutic response of cancer cells and significantly contribute to their adaptation to stress.[(a)] Anti-apoptotic members of this family, which include BCL-2, BCL-xL and MCL-1, exert a survival activity that relies on their ability to bind and antagonize pro-apoptotic members by engaging a network of intracellular interactions. The binding interfaces have been targeted through the use of small-molecules BH3 mimetics (ABT-737/navitoclax dual BCL-2/BCL-xL, ABT-199/venetoclax selective BCL-2). Their studies with breast tumors slices *ex vivo* suggests that BCL-2 inhibitors would be useful for the treatment of breast cancers that are refractory to the acute effects of chemotherapy.

There is thus a need to confirm that responsive tumors encompass chemosensitive ones and to define tools that could diagnose BCL-2 dependency to stratify patients. Sylvain Routier's team in Orleans has synthesized several fluorescent probes based on ABT-199 structure and using two chromophores (***Figure 1***) and the CRCINA has evaluated their efficacy to modulate the interaction between Bcl-2 and its intracellular partners. Work is in progress to develop new probes based on fluorescent and radiolabeled BH3 mimetics.

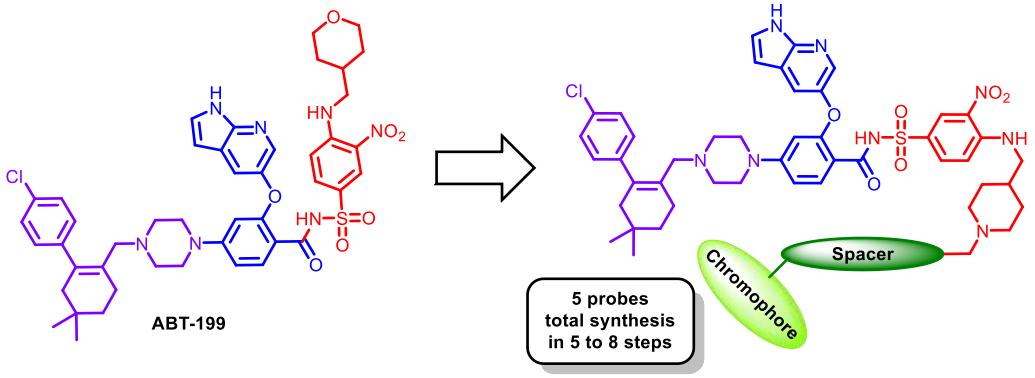


Figure 1: Design of fluorescent probes from ABT-199

Bibliographic references:

(a) Juin *et al.*, *Nature Rev. Cancer*, **2013**

*** Correspondence:** *hamid.marzag@univ-orleans.fr*

DESIGN AND SYNTHESIS OF FLUORESCENT PROBES AS "CHEMICAL BIOLOGY" TOOLS TO TRACK BCL-2 DEPENDENCY IN BREAST CANCER

Hamid Marzag [1], Karen Plé [1], Laurent Maillet [2], Sabine Sévère [2,3], Patricia Gomez-Bougie [2], François Guérard [2], Michel Chérel [2], Martine Amiot [2], Philippe Juin [2,4] and Sylvain Routier [1]

[1] ICOA – CNRS UMR 7311 - Université d'Orléans, Pôle de Chimie – Orléans. [2] CRCINA, UMR 1232 INSERM, Université de Nantes – Nantes. [3] Oniris, Nantes-Atlantic National College of Veterinary Medicine – Nantes. (4) ICO René Gaudducheau – Saint Herblain.

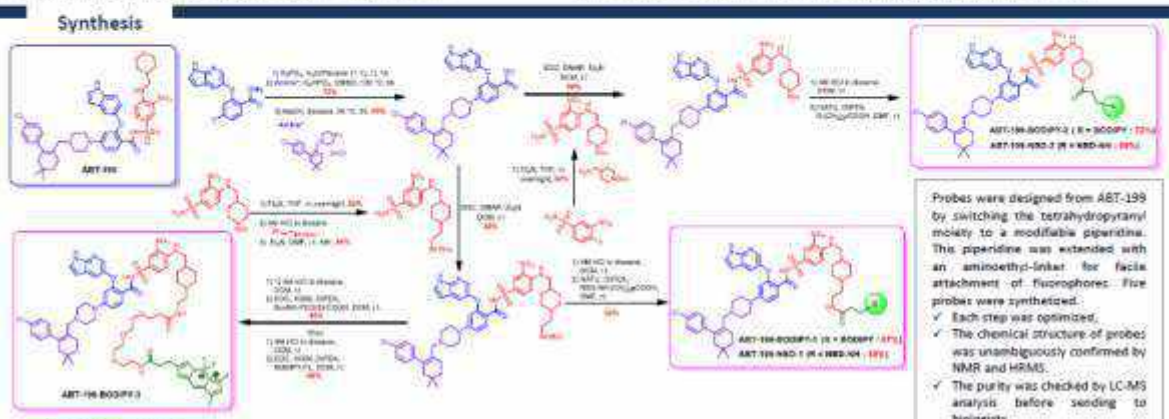
Introduction

Breast cancer remains the leading cause of death by cancer among women. In particular, triple negative breast cancers(TNBCs) and Luminal B (LumB) cancers remain difficult to treat with chemotherapy used as a standard first line treatment. Although initial results may be promising, progression and dissemination are not prevented in the long term. The BCL-2 protein family acts at the core of the therapeutic response of cancer cells and significantly contributes to their adaptation to stress. Anti-apoptotic members of this family, which include BCL-2, BCL-xL and MCL-1, exert a survival activity that relies on their ability to bind and antagonize pro-apoptotic members by engaging a network of intracellular interactions.

Small-molecule BH3 mimetics such as ABT-737/navitoclax (dual BCL-2/BCL-xL) and ABT-199 venetoclax (selective BCL-2) have been used to target binding interfaces. Studies with breast tumor slices ex vivo suggests that BCL-2 inhibitors would be useful for the treatment of breast cancers that are refractory to the acute effects of chemotherapy.

As part of a program to develop diagnostic tools a first fluorescent probe was synthesized. Biological tests are in progress which will help to confirm that responsive tumors encompass chemosensitive ones and diagnose BCL-2 dependency to stratify patients and better target cancer treatment. This probe has shown its efficacy in modulating the interaction between Bcl-2 and its intracellular partners, and its ability to trigger apoptosis in cancer cells.

Synthesis

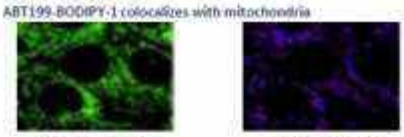


Probes were designed from ABT-199 by switching the tetrahydropyranyl moiety to a modifiable piperidine. This piperidine was extended with an aminoethyl-linker for facile attachment of fluorophores. Five probes were synthetized.

- ✓ Each step was optimized.
- ✓ The chemical structure of probes was unambiguously confirmed by NMR and HRMS.
- ✓ The purity was checked by LC-MS analysis before sending to biologists.

Cellular studies

ABT199-BODIPY-1 colocalizes with mitochondria



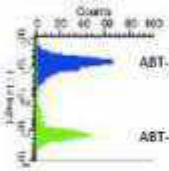
ABT-199-BODIPY-1 MITOCHONDRIA

MCF-7 cells were incubated with ABT-199-BODIPY (1 µM, 3 hours). ABT199-BODIPY-1 showed organelle staining consistent with the known localization of BCL-2. As expected it preferentially colocalizes with mitochondria.

ABT199-BODIPY spotlights BCL-2

MCF7 MCF7 + BCL-2 MCF7 + BCL-xL

Transiently expressed BCL-2, but not BCL-xL, specifically increased the fluorescence staining due to ABT199-BODIPY-1.

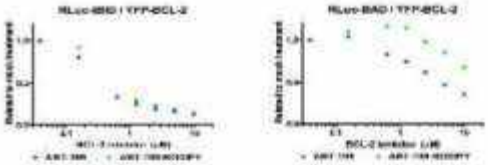


ABT-199

ABT-199-BODIPY

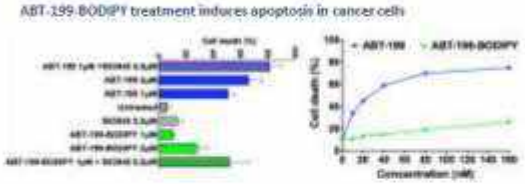
Flow cytometry analyses of canine cancer cells (TNBCs) treated with ABT-199-BODIPY during 4 days revealed an efficient and persistent labelling.

ABT-199-BODIPY inhibits the interaction between Bcl-2 and its pro-apoptotic BH3-only partners in live cells



A Bioluminescence Resonance Energy Transfert (BRET) assay allowed us to evaluate the ability of BH3 mimetics to inhibit the interactions between BCL-2 and its pro-apoptotic partners in live cells. ABT199-BODIPY-1 maintained efficacy against the BCL-2 /tBID interaction. However, it was less effective than ABT-199 to inhibit stronger interaction such as BAD/BCL-2, suggesting reduced affinity of ABT-199-BODIPY.

ABT-199-BODIPY treatment induces apoptosis in cancer cells



Primary culture of canine mammary tumors (TNBCs) were challenged with ABT-199, ABT-199-BODIPY and S63845 (a MCL-1 inhibitor) during 4 days (left). A human myeloma cell line (XG5) was treated by ABT-199 or ABT-199-BODIPY-1 for 24h followed (right). Then percentage of cell death was measured by APC Annexin-V staining and FACS analysis in flow cytometry. ABT-199-BODIPY induces apoptosis less effectively than ABT-199 alone or in synergy with S63845, suggesting reduced affinity of the ABT-199-BODIPY-1 probe.

Conclusion

We have synthesized a fluorescent BH3 mimetic probe specifically targeting BCL-2 from the ABT-199 molecule, an agent currently used in cancer treatment. This molecule makes it possible to visualize BCL-2, associated almost exclusively with cellular membranes. Our molecular and cellular analyzes suggest that it retains its activity in cellulo (release of pro-apoptotic partners and induction of apoptosis in tumor cells), although with a lower efficiency. This slight decrease in activity may stem from the reduced affinity of ABT-199-BODIPY-1 due to the presence of the additional linker and fluorescent moiety. Chemical optimization is currently underway to improve its affinity for BCL-2. The synthesis of fluorescent BH3 mimetic probes specifically targeting BCL-xL and MCL-1 is also envisaged. These molecules will offer new perspectives to determine dynamic tumor dependencies on individual anti-apoptotic proteins in circulating cancer cells ex vivo in order to predict clinical efficacy of BH3 mimetics and how this may evolve at different time-points of conventional therapies.

Acknowledgements

We are grateful for technical support from the Cellular and Tissular Imaging (MicroPICell) and from Cytometry (CytoCell) Core Facilities of Nantes University. The authors would also like to thank the Labex IRON (ANR-11-LABX-0018-01), the Ligue contre le Cancer and the Cancéropole Grand-Ouest for their financial support.

Blue light-enhanced cytotoxic activity of nitrogen-doped TiO_2 nanoparticles in human cervical cancer cells

Milica Matijević*, Marijana Petković , Milutin Stepić , Maja Nešić

Vinča Institute of Nuclear Science - National Institute of the Republic of Serbia, University of Belgrade, P.O. Box 522, 11001, Belgrade, Serbia

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We have studied the cytotoxicity and phototoxicity of nitrogen-doped TiO_2 (N-TiO_2) nanoparticles (NPs) of the anatase crystal form for a possible human cervical (HeLa) cancer therapy. TiO_2 is biocompatible with living structures [(a)] and its electrons in the valence band can be excited under ultraviolet (UV) light irradiation to produce reactive oxygen species and kill cancer cells. On the other hand, UV light is harmful and its penetration depth is short, which hinders its applications as a photosensitizer. Thus, we have chosen doping of TiO_2 with nitrogen, since it was already shown that N-doping shifts the absorbance towards the visible region and enables photoactivation of the TiO_2 with visible light, whereas the biocompatibility is preserved. [(b)] To assess the biocompatibility of N-TiO_2 with the cancer cells, we have treated the HeLa cell line with three different concentrations of N-TiO_2 NPs suspensions. After 24h of incubation in the dark, we have observed significant cytotoxicity in samples treated with concentration of 100 μg mL^{-1} of N-TiO_2 NPs, whereas no significant cytotoxicity of the concentrations ≤50 μg mL^{-1} was detected. Hence, we have selected the treatment of 50 μg mL^{-1} of the N-TiO_2 NPs for phototoxicity and cell imaging. Given that the N-TiO_2 absorbs in the region of 400-450 nm, we have examined the viability of the HeLa cancer cell line upon the N-TiO_2 treatment followed by continuous blue light irradiation (420 nm, 10 μW) for 15 minutes. The survival fraction of the cells treated with N-TiO_2 NPs decreased significantly upon blue light irradiation. The intracellular distribution of N-TiO_2 NPs was examined by laser scanning confocal microscopy. Most of the cells survived the treatment after 24h, and the agglomerates of N-TiO_2 NPs were distributed mainly inside the cancer cells. The results of viability in the dark show that N-TiO_2 NPs are not toxic for HeLa cells in the lower concentrations, and the evidence of cellular uptake points towards their biocompatibility. Furthermore, we have shown that it is feasible to induce cells' phototoxicity upon treatment with N-TiO_2 NPs and blue light irradiation.

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(a) López, T., Recillas, S., Guevara, P., Sotelo, J., Alvarez, M., Odriozola, J.A. Pt/TiO2 brain biocompatible nanoparticles: GBM treatment using the C6 model in Wistar rats. Acta Biomaterialia, 2008; 4: 2037–2044

(b) Li Z, Mi L, Wang PN, Chen JY. Study on the visible-light-induced photokilling effect of nitrogen-doped TiO2 nanoparticles on cancer cells. Nanoscale research letters. 2011; 6(1):356.

* Correspondence: milica.m@vin.bg.ac.rs

Blue light-enhanced cytotoxic activity of nitrogen doped TiO_2 nanoparticles in human cervical cancer cells

VINČA
INSTITUTE OF NUCLEAR SCIENCES
University of Belgrade
NATIONAL INSTITUTE OF THE REPUBLIC OF SERBIA

M. Matijević*, M. Petković, M. Stepić, M. Nešić

*milica.m@vin.bg.ac.rs

INTRODUCTION

We have studied the cytotoxicity and phototoxicity induced by blue light (BL) of the nitrogen-doped TiO_2 (N-TiO_2) nanoparticles (NPs) for a possible HeLa cancer therapy. TiO_2 is biocompatible with living structures [a] and under ultraviolet (UV) light irradiation can produce reactive oxygen species and kill cancer cells. On the other hand, UV light is harmful, and its penetration depth is short which hinders its applications as a photosensitizer. Thus, we have chosen doping of TiO_2 with nitrogen, since it was already shown that N-doping shifts the absorbance towards the visible region and enables photoactivation of the TiO_2 with visible light, whereas the biocompatibility is preserved.

METHODS

The crystalline phase of the N-TiO_2 NPs was determined by Raman spectra and X-ray diffraction (XRD). Their structure was examined by transmission electron microscopy (TEM), using a JEOL JEM-2100F. To assess the biocompatibility of N-TiO_2 NPs, the HeLa cells were incubated for 24h with three different concentrations of N-TiO_2 NPs suspensions. Similarly, we have conducted the phototoxicity test with additional incubation (48h) followed by continuous BL irradiation (420 nm, 10 μW) for 15 min. The intracellular distribution of N-TiO_2 NPs was examined by laser scanning confocal microscopy.

CHARACTERIZATION of N-TiO_2 NPs

Raman spectra exhibit five Raman peaks distinctive for the anatase phase around 154, 202, 390, 506, and 630 cm^{-1}. XRD patterns confirmed the anatase phase with strong diffraction peaks at 25° and 48°. [c]

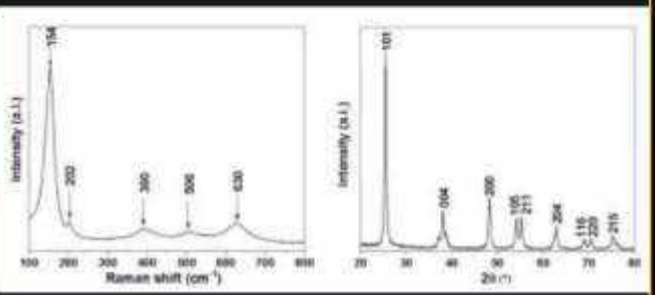
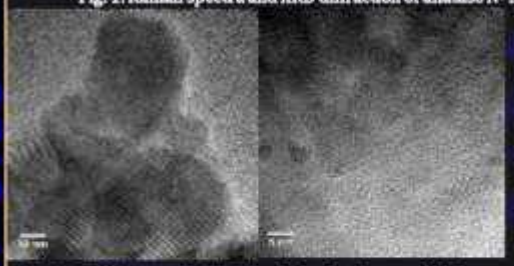


Fig. 1. Raman spectra and XRD diffraction of anatase N-TiO_2 NPs.



TEM images confirmed the expected size, shape, and appearance of the N-TiO_2 NPs.

Grain size of N-TiO_2 NPs Fig. 2, are in the range of 5–10 nm.

Fig. 2. TEM images of N-TiO_2 NPs obtained at lower and higher magnification.

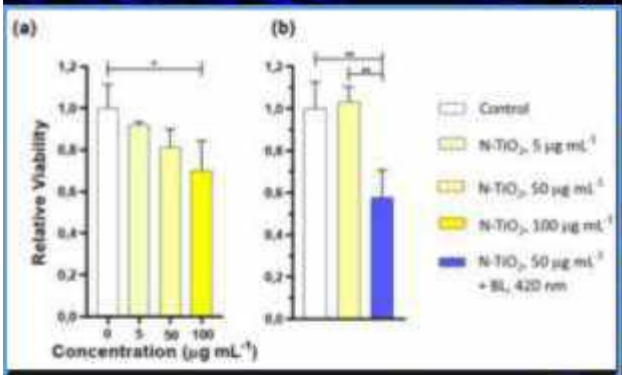


Fig. 3. The cytotoxicity of N-TiO_2 NPs as a function of the concentration (a). The cytotoxic effects of N-TiO_2 NPs (50 μg mL^{-1}), 48h after the treatment (yellow vertical bar), and phototoxic effect one additional hour after 15 min of continuous BL irradiation (blue vertical bar) (b).

RESULTS

Cytotoxicity

After 24h of incubation in the dark, we have observed significant cytotoxicity of the concentration of 100 μg mL^{-1} of N-TiO_2 NPs, whereas no significant cytotoxicity of the concentrations ≤50 μg mL^{-1} was detected, Fig. 3. (a). Hence, we have selected the treatment of 50 μg mL^{-1} of the N-TiO_2 NPs for cell imaging and phototoxicity.

Phototoxicity

The survival fraction of the cells treated with N-TiO_2 NPs decreased significantly upon BL irradiation, Fig. 3. (b).

Cell Imaging

Most of the cells survived the treatment after 24h, and the agglomerates of N-TiO_2 NPs were distributed mainly inside the cancer cells, Fig. 4.

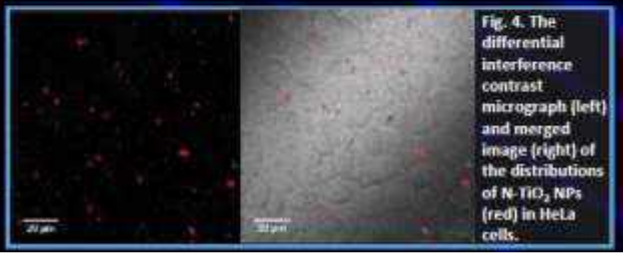


Fig. 4. The differential interference contrast micrograph (left) and merged image (right) of the distributions of N-TiO_2 NPs (red) in HeLa cells.

CONCLUSION

The results of viability in the dark confirmed that N-TiO_2 NPs are not toxic for HeLa cells in the lower concentrations, and the evidence of cellular uptake points towards their biocompatibility. Furthermore, we have shown that it is feasible to induce cells' phototoxicity upon treatment with N-TiO_2 NPs and blue light irradiation.

[a] Lopez T. et al. Acta Biomaterialia, 2008; 4: 2037–2044
[b] Li Z. et al. Nanoscale research letters, 2011; 6(1): 556.
[c] Thevenot X. et al. Nat. Sci. 2008; 2(9): 957-961.

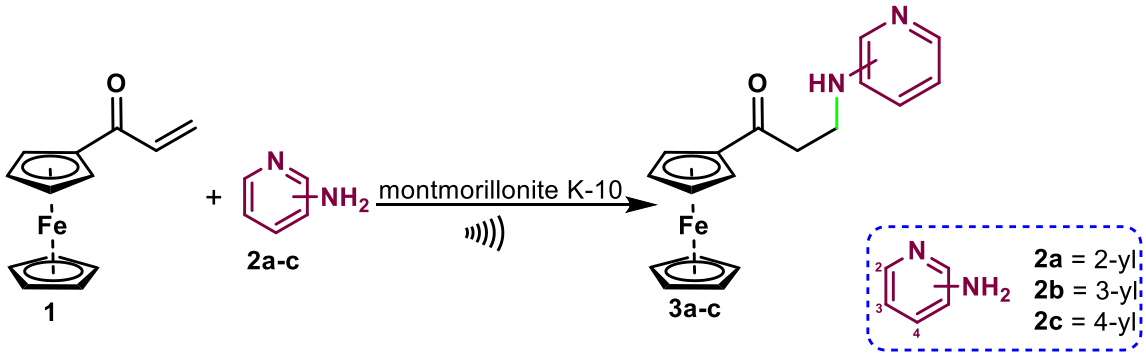
The preparation and spectral characterization of novel 3-(pyridinylamino)-1-ferrocenylpropan-1-ones

Aleksandra Minić[(1)]*, Danijela Ilić Komatina[(1)], Anka Todosijević[(2)], Dragana Stevanović[(3)].

(1) Faculty of Technical Sciences, University of Priština, Serbia.
(2) Faculty of Agriculture, University of Niš, Serbia.
(3) Faculty of Science, University of Kragujevac, Serbia.

Restricted to organizers

In continuation of our long-standing interest in the synthesis of novel ferrocene-containing heterocyclic compounds,[a] of potential biological interest,[b] in this work we will report an easy performable synthesis of novel 3-(pyridinylamino)-1-ferrocenylpropan-1-ones. A fruitful aza-Michael addition of pyridinamine moiety to a conjugated enone, 1-ferrocenylpropenone, has been accomplished by an ultrasonic irradiation of the mixture of these reactants (reaction protocol previously reported by our research group).[c] As the catalyst montmorillonite K-10 has been used and the reaction has been carried out as solvent-free, yielding ferrocene containing Mannich bases, compounds considered as important precursors in organic synthesis. The reaction score has been evaluated on three examples. The prepared products have been purified by column chromatography and a detailed characterization of the obtained 3-(pyridin-2-ylamino)-1-ferrocenylpropan-1-on and 3-(pyridin-3-ylamino)-1-ferrocenylpropan-1-on has been completed by IR and NMR spectroscopy. In addition, this synthetic approach gives rise to favorable starting materials for further biological evaluation.



Acknowledgement: *This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2020-14/200122).*

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[(b)] A. Minić *et al., Eur. J. Med. Chem.* **187** (2020) 111963; A. Pejović *et al., Polyhedron,* **155** (2018) 382; *A. Pejović et al., J. Organomet. Chem.* **869** (2018) 1.

[(c)] I. Damljanović *et al., J. Organomet. Chem.* **696** (2011) 3703; b) A. Pejović *et al., Helv. Chim. Acta*, **95** (2012) 1425.

* Correspondence: aleksandra.minic.989@gmail.com and aleksandra.minic@pr.ac.rs

The preparation and spectral characterization of novel 3-(pyridinylamino)-1-ferrocenylpropan-1-ones

Aleksandra Minić[(1)*], Danijela Ilić Komatina[(1)], Anka Todosijević[(2)], Dragana Stevanović[(3)]

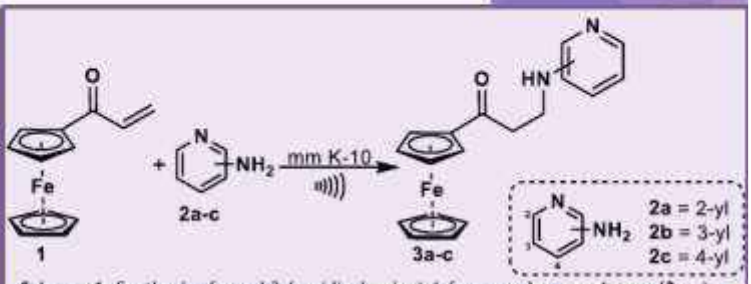
(1) Faculty of Technical Sciences, University of Pristina, Serbia.

(2) Faculty of Agriculture, University of Nis, Serbia.

(3) Faculty of Science, University of Kragujevac, Serbia.

Introduction

Ferrocene has been discovered in 1951,[1] and since then its derivatives have been found applications in many areas, among which the most important are in material science, asymmetric catalysis, bioorganometaltic chemistry, medicinal chemistry, and organic synthesis. Thus, the ferrocene moiety was recognized as an attractive pharmacophore in drug design and a multitude of reports dealing with derivatives of this metallocene have been appeared in the literature. In continuation of our long-standing interest in the synthesis of novel Fc-containing (Fc – ferrocene) heterocyclic compounds, of potential biological interest,[2] and in design and optimizations of reactions conditions, we reported synthesis of bioactive 2-ferrocenoyl ethyl aryl amines and 1-ferrocenyl-3-(quinolinylamino)propan-1-ones.[3] These *Mannich* bases have been proved to be an excellent starting material for the synthesis of Fc derivatives. Hence, the synthesis and spectral characterization of novel *Mannich* bases bearing ferrocenyl group and pyridinamine ring gained in this manner could be of great interest.



Scheme 1. Synthesis of novel 3-(pyridinylamino)-1-ferrocenylpropan-1-ones (**3a-c**)

Results and discussion

Synthesis: For required synthesis within this study we applied already know reaction conditions (see Scheme 1). First, we found optimal conditions based on test reaction. A test tube containing a well homogenized mix of 1-ferrocenylpropenone (240 mg, 1 mmol), the corresponding pyridinamine (**2a**, 2 mmol) and montmorillonite K-10 (100 mg, 0.42 m-eq.) has been placed in the ultrasonic cleaner for 1h irradiations in the absence of solvent, at ambient temperature. Later, the crude product has been purified by column chromatography (SiO_2/n-hexane-MeOH, 9 : 1, v/v) to give 3-(pyridin-2-ylamino)-1-ferrocenylpropan-1-on (**3a**) in only 10% yield. Therefore, we set reaction again, but this time reaction outcome has been monitored by TLC. Indeed, based on TLC plate, for the reaction to be fully done it was necessary much more time, around 8 hours. Usual workup of the reaction and column chromatography (SIO2/n-hexane-MeOH, 9 : 1, v/v), provided compound **3a** in 60% yield based on 1-ferrocenylpropenone. With optimal conditions, reaction score has been evaluated on two additional examples and we discovered very interesting results (see Table 1).

Table 1. Substrate scope for the synthesis of 3-(pyridinylamino)-1-ferrocenylpropan-1-ones (3a-c)

Entry	Starting substrate	Time (h)	Product	Yield (%)
1	pyridin-2-amine (**2a**)	8	**3a**	60
2	pyridin-3-amine (**2b**)	6	**3b**	75
3	pyridin-4-amine (**2c**)	10	**3c**	/

Spectral characterization: The structure of newly obtained compounds **3a** and **3b** has been validated by detailed spectral (IR, ^{1}H and ^{13}C NMR) characterization. The IR spectra of compounds **3a** and **3b** contained characteristic vibrations of the N-H bonds at 3234 cm^{-1}. The strong band at 1670 cm^{-1} relating to absorptions of the C=O bond. Three sets of signals have been observed in the ^{1}H NMR spectra. The first belongs to protons of the methylene groups, the second to protons of the ferrocene moiety and the third to the aromatic protons (see Figure 1). Supplementary, signals assigned to the corresponding carbons of the synthesized compounds **3a** and **3b** appear in the expected regions of the ^{13}C NMR spectra (see Figure 2).

Conclusion

- ✓ First time prepared 3-(pyridinylamino)-1-ferrocenylpropan-1-ones.
- ✓ Proposed structures of synthesized molecules were undoubtedly confirmed by spectroscopic techniques (IR and NMR).
- ✓ The synthesized molecules correspond to be interesting starting material for biological evaluation.

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[3] I. Damljanović *et al., J. Organomet. Chem. 696* (2011) 3703; A. Pejović *et al., Helv. Chim. Acta, 95* (2012) 1425; A. Minić *et al., Eur. J. Med. Chem. 187* (2020) 111963.

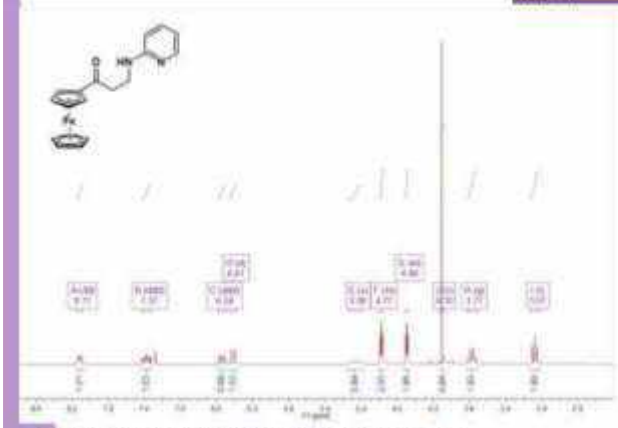

Figure 1. ^{1}H NMR (200 MHz, $CDCl_3$) spectrum of **3a**

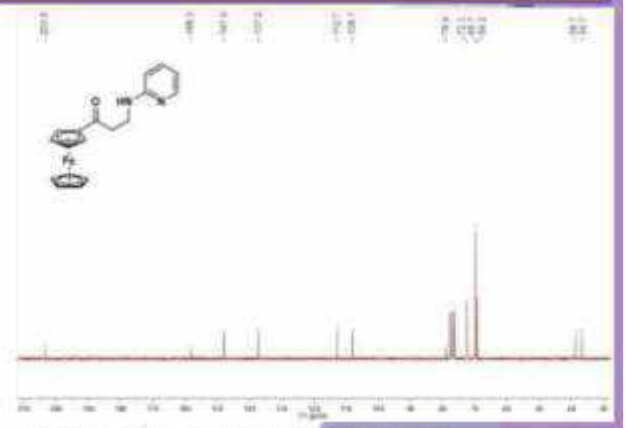

Figure 2. ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of **3a**

Acknowledgement: *This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2020-14/200122).*

* Correspondence: aleksandra.minic.989@gmail.com or aleksandra.minic@pr.ac.rs

Macrocycles in drug discovery: A promising scaffold for enhancing ADME properties and target selectivity

N. Bou Karroum*, A. Biela, C. Piveteau, B. Deprez, R. Deprez-Poulain, D. Bosc.

(1) Univ. Lille, Inserm, Institut Pasteur de Lille, U1177—Drugs and Molecules for Living Systems, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE

Restricted to organizers

Macrocycles in drug discovery have been defined as a ring system consisting of 12 or more atoms. These compounds with properties outside Lipinski's rule of 5 have raised particular interest in the field of medicinal chemistry[(a)]. In fact, they can modulate novel targets that have difficult, large, and featureless binding sites. The macrocycle allows a molecule to achieve a degree of structural pre-organization, such that key functional groups can interact across extended binding sites in proteins without a major entropic loss upon binding. This can result in high affinity and selectivity for protein targets, while preserving sufficient bioavailability to reach intracellular locations[(b)]. Despite the molecular weight of the macrocycles which doesn't respect the "lipinski's rule of 5", the macrocycles have shown favorable physicochemical and pharmacokinetic properties such as good solubility, lipophilicity, metabolic stability and bioavailability[(c)]. A recent survey lists 70 marketed macrocycle drugs and 35 macrocycles in clinical development, and these drugs belong to different classes including peptidic and nonpeptidic natural products[(b)] such as cyclosporine A and Tacrolimus respectively, non-natural (synthetic) peptides and non-natural (synthetic) macrocycles such as the dual JAK2/FLT3 inhibitor pacritinib[(a)], now in advanced Phase III trials. Given the drug-like physicochemical and pharmacokinetic properties of macrocycles, the macrocyclization has found a lot of application in the field of kinase, protease and polymerase inhibitors and other biological targets[(d)]. In this poster, we describe the impact of macrocyclization on ADME properties as well as its role in improving target selectivity, based on the various scientific research carried out in latest years in this field.

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(b) Driggers *et al. Nature.* **2008**, *7,* 608-624.

(c) Whitty *et al. Drug Discov. Today.* **2016**, *21,* 712-717.

(d) Cummings *et al. J. Med. Chem.* **2019**, *62*, 6843-6853.

* Correspondence: E-mail : nour.boukarroum@univ-lille.fr

Inserm U1177

Macrocycles in drug discovery : A promising scaffold for enhancing ADME properties and target selectivity

N. Bou Karroum*, A. Biela, C. Piveteau, R. Deprez-Poulain, B. Deprez, D. Bosc.

Inserm U1177 Drugs and Molecules for Living Systems, Institut Pasteur de Lille, Université de Lille, Inserm, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE

nour.boukarroum@univ-lille.fr
www.deprezlab.fr

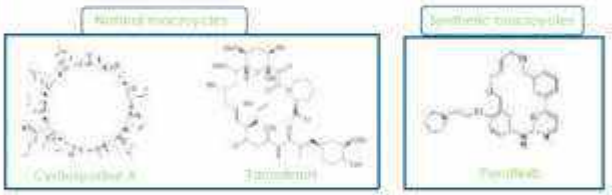
Macrocycles in drug discovery

Macrocycles in drug discovery have been defined as a ring system consisting of 12 or more atoms. These compounds with properties outside Lipinski's rule of 5 have raised particular interest in the field of medicinal chemistry. Macrocycles are not only larger versions of small molecules, but can be considered as the smallest examples of biomolecules that exhibit functional subdomains.

Terrett, N.K. Drug Disc. Today: Technologies **2010**, 7, e97-e104

Macrocyclic drugs

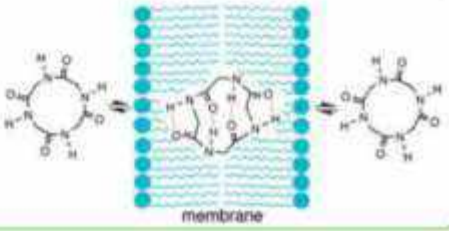
A recent survey listed 70 marketed macrocyclic drugs and 35 macrocycles in clinical development. These drugs belong to different classes including peptidic and nonpeptidic natural products such as cyclosporine A and tacrolimus respectively, and also synthetic peptidic macrocycles and non-peptidic macrocycles such as the dual JAK2/FLT3 inhibitor pacritinib, now in advanced Phase III trials.



Driggers et al. Nature. **2008**, 7, 608-624 — Marsion et al. Future Med. Chem. **2012**, 4, 1409-1438

Peptidic macrocycles

Cyclosporine (CSA) is the only orally administered cyclic peptide among the 30 approved macrocyclic peptide drugs. Despite its molecular weight, PSA and LogP, which violate Lipinski's rule, CSA has shown good ADME properties. In fact, CSA penetrates the cell membrane by passive diffusion and this membrane permeability is due to the ability of cyclosporine to adopt different conformations depending on the nature of the environment (polar or non polar). This behavior is due to "chameleonic properties" of some peptidic macrocycles, which can internalize in a non-polar media (lipophilic plasmatic membrane) or expose in a polar media (water, blood) their hydrogen bond acceptors and donors.

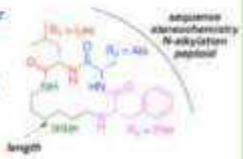


Rezai et al. J. Am. Chem. Soc. **2006**, 128, 2510-2511

Semi-peptidic macrocycles

Macrocyclic peptides are often poorly permeable and mostly administered parenterally, with the exception of CSA. In order to enhance the cell permeability and ADME properties, the use of less peptidic macrocycles has proven to be advantageous. Recently Le Roux et al. have synthesized a wide variety of semi peptidic macrocycles in order to elucidate the influence of structural characteristics on permeability :

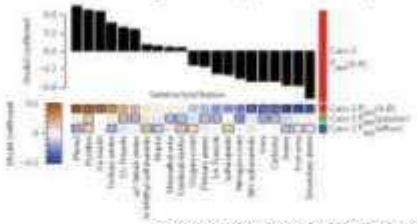
- ✓ Cellular permeability governed by efflux: Larger rings decreased efflux and contribution of P-gp
- ✓ Permeability: Best correlation with ensemble-based 3D-PSA
- ✓ NMR or Molecular Dynamics failed to find the prevalence of a conformation maximizing intramolecular hydrogen bonds



Le Roux et al. J. Med. Chem. **2020**, 63, 6774-6783

Non-peptidic macrocycles

In order to better understand the molecular properties that control the cellular permeability of non-peptidic macrocycles Over et al. carried out a very large study on about 200 non-peptidic macrocycles in order to identify the molecular characteristics which provide favorable physicochemical properties and good cell permeability. In this interesting study, Over et al. provide to the chemists the structural and conformational determinants that impact cell permeability and help researchers in the design of non-peptidic macrocycles.

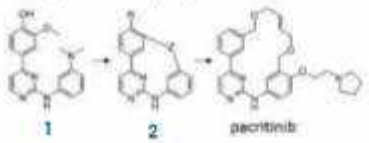


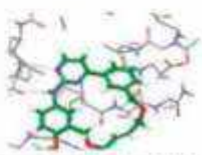
Over et al. Nature Chem. Bio. **2016**, 12, 1065-1074

Non-peptidic macrocycles and target selectivity

Macrocyclization has found several applications in different fields of medicinal chemistry including kinase inhibitors field. For example in the discovery of pacritinib, the researchers found that phenylaminopyrimidine (compound **1**) exhibit a very strong inhibitory activity and adopts "U-shaped conformation" in kinase pocket. In order to improve the inhibitory activity of compound **1**, researchers thought about macrocyclization and the introduction of a linker (compound **2**).

Linker introduction was very useful to modulate kinase affinity and selectivity, and improve ADME properties.





William et al. J. Med. Chem. **2011**, 54, 4638-4658

Conclusion

There is growing interest in the use of macrocycles in drug discovery for several reasons:

- ✓ Macrocycles offer an opportunity to develop small-molecule drugs which violate Lipinski's rule to explore a new chemical space.
- ✓ Macrocyclization could be beneficial for pursuing difficult targets such as protein-protein interactions, and can be an approach to overcoming property challenges in small-molecule drug.

Cummings et al. J. Med. Chem. **2019**, 62, 6843-6855

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(3) Madrison et al. Future Med. Chem. **2012**, 4, 1409-1438
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(8) Cummings et al. J. Med. Chem. **2019**, 62, 6843-6855

Inserm U1177
Drugs and Molecules for Living Systems
3 rue du Professeur Laguesse
F-59006 LILLE Cedex

Synthesis of potential HDAC selective inhibitors

Ozdarska Katarzyna[(1,2)*], Bourguet Erika [(1)], Wroczyński Piotr [(2)].

(1) Institut de Chimie Moléculaire de Reims, UMR 7312-CNRS, UFR Pharmacie, Université de Reims Champagne-Ardenne , 51 rue Cognacq-Jay , 51096 Reims Cedex , France.

(2) Department of Bioanalysis and Drugs Analysis , Medical University of Warsaw , S. Banacha 1 , 02-097 Warsaw , Poland.

Restricted to organizers

Epigenetics represents a modification of gene expression without altering the nucleic sequence of DNA. One of the main mechanisms of regulation of gene expression is chromatin remodeling *via* histone modification which is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively.[a] Abnormal increased and decreased expression of HDACs is correlated with many diseases. All current HDAC inhibitors are characterized by a common pharmacophore with three key-elements for inhibitor-enzyme interactions: a metal binding domain that interacts with the active site, a linker domain and a surface recognition group interacting with the residues on the rim of the active site.[b] To design isoform selective inhibitors we were concentrated on the pharmacomodulations of capping region and the zinc binding group (ZBG) (Fig. 1). The aim was to obtain a molecule with the chain similar to that of SAHA and TSA (C5 or C6), with strong inhibitory activity against HDACs. Thus, the surface recognition groups in our compounds are phenylamine, trifluoromethylamine, trifluoromethylbenzylamine or difluorophenylamine with the chain of four or five carbon atoms, and with or without double bond. (Fig. 2). Chen *et al*. described the guaiacolate group as a potential ZBG using a DFT (M05-2X) method.[c] Besides the cap, we decided to incorporate 4-hydroxy-3-methoxyphenyl, 2-hydroxy-3-methoxyphenyl, 4-difluoromethoxy-3-methoxyphenyl as a ZBG. The incorporation of a CF_3 group to our compounds can improve liphophilicity and the inhibitory activity against HDACs. A trifluoroacetanamide group can be potent and selective class IIa histone deacetylase inhibitors as described by Lobera et al. The compounds displayed about 10-fold selectivity for class II over class I HDACs, the results were confirmed by molecular modeling.[d,e]

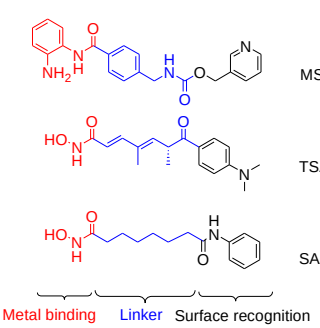

Fig.1. Pharmacophore model.

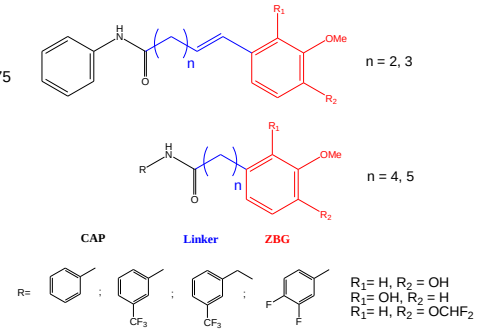

Fig. 2. Structures of molecules envisioned as HDAC inhibitors.

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* Correspondence: katarzyna.ozdarska@gmail.com

SYNTHESIS OF POTENTIAL SELECTIVE HDAC INHIBITORS

Ozdarska, K.[1, 2]; Bourguet, E. [1]; Wroczyński, P.[2]; Wu, K.C.[3]; Reid, R. [3]; Fairlie, D.P.[3]

[1] Institut de Chimie Moléculaire de Reims, UMR 7312-CNRS, UFR Pharmacie, Université de Reims Champagne-Ardenne, Reims, France
[2] Department of Bioanalysis and Drugs Analysis, Medical University of Warsaw, Warsaw, Poland
[3] Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia

INTRODUCTION

Epigenetics represents a modification of gene expression without altering the nucleic sequence of DNA. One of the main mechanisms of regulation of gene expression is chromatin remodeling via histone modification which is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively.[1] Abnormal increased and decreased expression of HDACs is correlated with many diseases. All current HDAC inhibitors are characterized by a common pharmacophore with three key-elements for inhibitor-enzyme interactions: a metal binding domain that interacts with the active site, a linker domain and a surface recognition group interacting with the residues on the rim of the active site.[2] To design isoform selective inhibitors, we were concentrated on the pharmacomodulations of capping region and the zinc binding group (ZBG).

OBJECTIVES

The aim was to obtain a molecule with the chain similar to that of **SAHA** and **TSA** (C5 or C6), with strong inhibitory activity against HDACs. Thus, the surface recognition groups in our compounds are phenylamine, trifluoromethylamine, trifluoromethylbenzylamine or difluorophenylamine[4,5] with the chain of four or five carbon atoms, and with or without double bond. Besides the cap, we decided to incorporate 4-hydroxy-3-methoxyphenyl, 2-hydroxy-3-methoxyphenyl, 4-difluoromethoxy-3-methoxyphenyl as a ZBG. Chen et al.[3] described the guaiacolate group as a potential ZBG using a DFT (M05-2X) method. We were interested to test it.

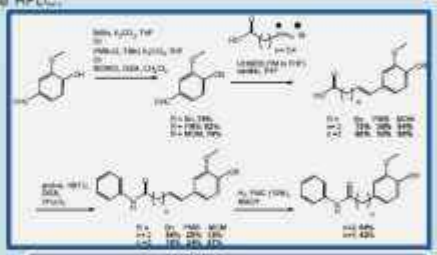
RESULTS

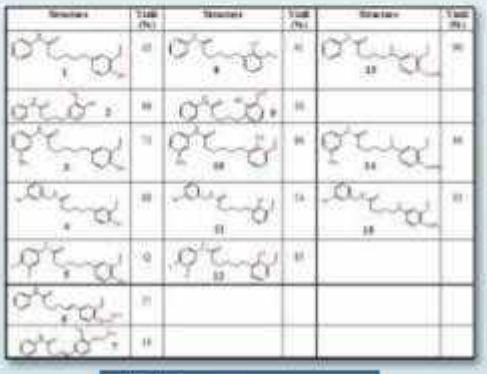
• Docking

Among the selective class IIa HDAC inhibitors, **TMP269** is a highly potent identified selective HDAC7 inhibitor (IC_{50}= 43 nM). Superposition of one of our compounds and **TMP269** indicates that the phenyl group from the aniline moiety is nearly in the same place. The compound **11** adopts counterclockwise direction comparing to that of **TMP269**. The best pose for compound **11** was with score ΔG= -8.7 kcal mol^{-1} and the distance between Me-O and Zn was 2.38 Å which is in order to a good interaction with the zinc ion.

• Synthesis

The interesting docking prompted us to synthesize the series of compounds with vanillin and o-vanillin starting material. One synthesis is described below in the scheme. The results are combined in the Table. All compounds were purified by reverse phase HPLC.





• HDAC assays

The NMR pulcon experiment has been done to quantify the concentration of the compound in assay solutions. All compounds were tested in HDAC1 and HDAC7 enzyme assay in the IMB (Australia). **AR42** and **TMP195** were used as the control compounds for HDAC1 and HDAC, respectively. None of our molecules shows inhibitory activity towards HDAC1 and HDAC7.

CONCLUSION

The hypothesis based on molecular modeling and other researchers' studies[5] didn't confirm that compounds with incorporated 4-hydroxy-3-methoxyphenyl or 2-hydroxy-3-methoxyphenyl groups as potential zinc binding groups are efficient. It suggests that the preview DFT method is not sufficient to predict the activity of a new ZBG.

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Contact: katarzyna.ozdarska@gmail.com

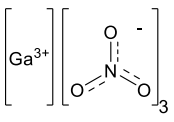
Synthesis and study of gallium piperazine-based complexes as potential antipseudomonal agents.

Pauline Loupias[(1)]*, Isabelle Dechamps-Olivier[(2)], Laurent Dupont[(2)], Nicolas Taudon[(3)], Alexandra Dassonville-Klimpt[(1)], Pascal Sonnet[(1)].

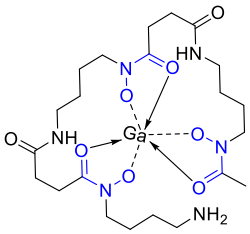
(1) AGIR, UR 4294, UFR de Pharmacie, Université de Picardie Jules Verne, 1 rue des Louvels, 80037 Amiens, France.
(2) ICMR, UMR 7312, Université de Reims Champagne-Ardenne, 3 Avenue du Maréchal Juin, 51100 Reims, France.
(3) Unité de Développements Analytiques et Bioanalyse, Institut de Recherche Biomédicales des Armées, 91223 Brétigny-sur-Orge, France.

Restricted to organizers

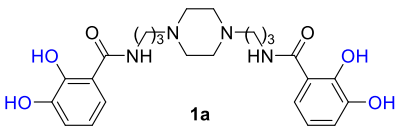
Resistance to conventional antibiotics of Gram-negative bacteria such as *Pseudomonas aeruginosa* is an increasing phenomenon and a major medical problem which leads to therapeutic failure and requires new antibiotic therapies. Using bacterial iron transport systems is a promising Trojan Horse strategy to overcome these issues. TonB-dependent receptors allow specific recognition of ferric siderophore complexes in order to transport iron within bacteria[(a)]. According to their kind, bacteria express different types of receptors that recognize their endogenous siderophores but also xenosiderophores. These specific systems may allow the introduction of antibacterial agents by forming antibiotic-siderophore conjugates or toxic complexes such as gallium complexes[(b)]. Indeed, gallium has similar atomistic characteristics to iron and can be internalized by bacteria as siderophore-Ga(III) complexes which compete with the corresponding siderophore-Fe(III) complexes. Once inside the bacteria, gallium, which cannot be reduced, blocks the bacteria's iron-dependent biological mechanisms. An FDA-approved citrate-buffered **GaN** solution has been investigated in phase II and has shown promising results for clinical application in the treatment of *P. aeruginosa* in patients with cystic fibrosis. It is used as a low flow intravenous treatment to prevent the formation of a precipitate causing nephrotoxicity[(c)]. The use of siderophore-Ga(III) complexes might overcome its toxicity. A proof of concept has already been established with the desferrioxamine-gallium **DFO-Ga(III)** complex active against *P. aeruginosa*[(d)]. Recently, we have synthesized two 1,4-disubstitued piperazines bearing catechol and hydroxypyridinone moieties (**1a** and **1b**). Their physicochemical properties (pFe and coordination mode of the metal) as well as the effective transport of the corresponding siderophore-Fe(III) complexes through iron uptake pathways have been evaluated by the ICMR and AGIR laboratories[(e)]. These two chelators were also exploited to form and study the corresponding toxic complexes **1a-Ga(III)** and **1b-Ga(III)**.

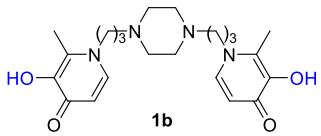


GaN



DFO-Ga(III)





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* Correspondence: pauline.loupias@u-picardie.fr

Synthesis and study of gallium piperazine-based complexes as potential antipseudomonal agents

Pauline LOUPIAS[a], Isabelle DECHAMPS-OLIVIER[b], Laurent DUPONT[b], Nicolas TAUDON[c], Alexandra DASSONVILLE-KLIMPT[a], Pascal SONNET[a]

[a] AGIR, UR 4294, UFR de Pharmacie, Université de Picardie Jules Verne, 1 rue des Louvels, 80037 Amiens, Cedex 1
[b] ICMR, UMR 7312, Université de Reims Champagne-Ardenne, 3 Avenue du Maréchal Juin, 51100 Reims, France
[c] Unité de Développements Analytiques et Bioanalyse, Institut de Recherche Biomédicales des Armées, 91223 Brétigny-sur-Orge

pauline.loupias@u-picardie.fr

Introduction & Aims

Resistance to conventional antibiotics of Gram-negative bacteria such as *Pseudomonas aeruginosa* is an increasing phenomenon and a major medical problem which leads to therapeutic failure. Using bacterial iron transport systems is a promising Trojan Horse strategy. Indeed, iron is a micronutrient necessary for the survival of bacteria and essential to many biological processes such as respiration, DNA synthesis... Under iron limited conditions, many bacteria synthesize molecules of low molecular weight called siderophores able to chelate the surrounding iron. According to their kind, bacteria express different types of receptors that allow specific recognition of their endogenous siderophores but also xenosiderophores or synthetic siderophores[1]. These specific systems may allow the introduction of antibacterial agents by forming antibiotic-siderophore conjugates or toxic complexes such as gallium complexes[2].

Gallium has similar atomistic characteristics to iron and can be internalized by bacteria as siderophore-Ga(III) complexes which compete with the corresponding ferric-siderophore complexes. Once inside the bacteria, gallium, which cannot be reduced, blocks the bacteria's iron-dependent biological mechanisms. Used as a low flow intravenous treatment, an FDA-approved citrate-buffered GaN solution has shown promising results for clinical application in the treatment of *P. aeruginosa* in patients with cystic fibrosis[3]. The use of siderophore-Ga(III) complexes might overcome its nephrotoxicity such as the desferrioxamine-gallium DFO-Ga(III) complex active against *P. aeruginosa*[4].

Recently, we have synthesized two 1,4-disubstituted piperazines bearing catechol and hydroxypyridinone moieties (1a and 1b). Their physicochemical properties (pFe and complex stoichiometry) as well as the effective transport of the corresponding analogs of siderophore-Fe(III) complexes through iron uptake pathways have been evaluated on *P. aeruginosa* strains[5]. These two chelators were also exploited to form and study the corresponding potential toxic complexes 1a-Ga(III) and 1b-Ga(III). Their potential antipseudomonal activity has been evaluated (Figure 1).

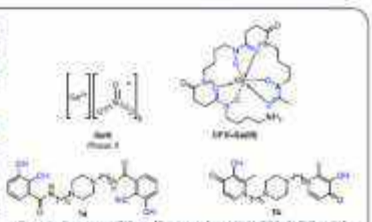

Figure 1. Structures of the gallium complexes GaN, DFO-Ga(III) and the 1,4-disubstituted piperazines 1a and 1b.

1,4-Disubstituted Piperazines Synthesis

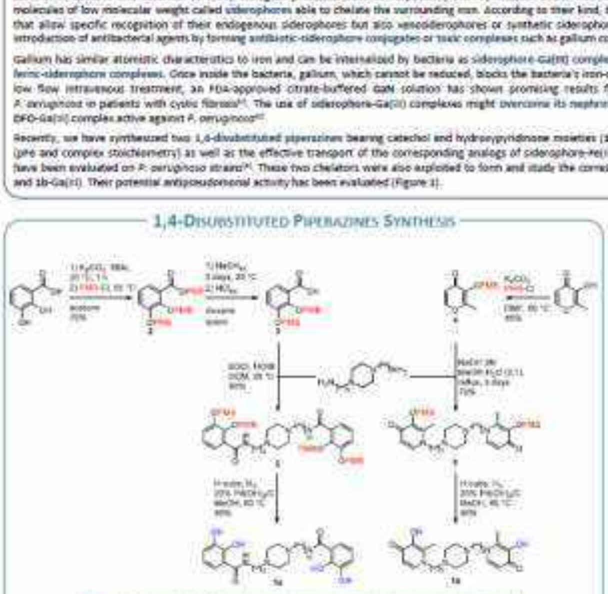

Figure 2. Synthesis of the 1,4-disubstituted piperazines based siderophore analogs 1a and 1b.

The bidentate ligands precursors 3 and 4 were synthesized with PMB as a protective group in order to be coupled with the 1,4-bis(3-aminopropyl)piperazine. The common hydrogenation step was optimized and carried out using a H-cube system generating hydrogen by electrolysis of water in presence of Pearlman's catalyst at 60 °C.

1a and 1b were, respectively, obtained in four and three steps with a 38% and 41% yield (Figure 2).

Physicochemical Studies of 1a and 1b

The products obtained are poorly soluble in aqueous media. Their physicochemical parameters were studied in a H_2O/DMSO 50/50 mixture (v/v), bringing the molar fraction of DMSO to x_{DMSO} = 20. The neutralization curve was converted as a z vs pH curve and acidity constants of 1a and 1b were estimated on the basis of the global protonation constants. Resulting pKa were assigned as shown Figure 3.

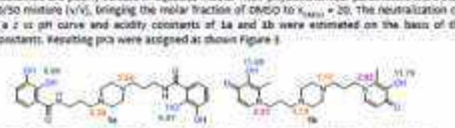

Figure 3. pKa values for 1a and 1b (I=0.1 mol.L-1 (KCl), T=298 K, H_2O/DMSO media (v/v 50:50)).

The distribution curves of the various species were plotted as a function of pH (Figure 4). At physical pH, with 1a, the $Fe_2(1a)_3$ complex predominates whereas two species ($Fe_2(1b)_2(OH)_2$ and $Fe_2(1b)_2(OH)_4$) were found with 1b. The most suitable way to compare the Fe(III) chelating ability between ligands 1a and 1b was to determine pFe. Under our conditions, taking into account the extended pH range due to DMSO, the free Fe(III) concentration was calculated at pH 8.44, the pFe values obtained for 1a and 1b were respectively 28.04 and 24.38, meeting the criteria for an iron chelation therapy agent (pFe >20).

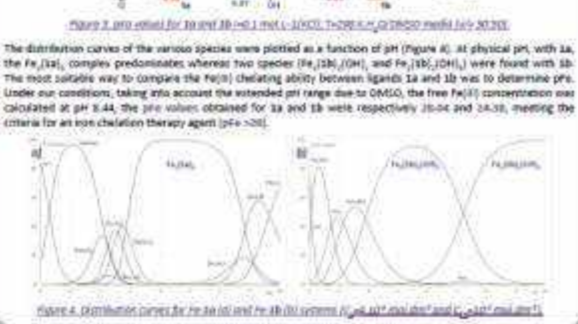

Figure 4. Distribution curves for Fe-1a (a) and Fe-1b (b) systems.

Biological Activity and « Siderophore-like » effect of 1a and 1b on *P. aeruginosa*

The antipseudomonal activities of the siderophore analogs 1a and 1b were evaluated on *P. aeruginosa* DSM 1117 in two mediums : the Mueller-Hinton (MH) and the Succinate Minimum Medium (SMM), a medium virtually deprived of iron.

1a and 1b have no intrinsic antipseudomonal activities since the MICs measured are greater than 512 mg.L-1, convenient for a "siderophore-like" activity.

The cytotoxicity was also evaluated on the Hep-G2 cell line, a human liver cancer cell line. 1a and 1b did not show toxicity at concentrations below 100 µM.

Figure 5. "Siderophore-like" activities of the ligands 1a and 1b on *P. aeruginosa* PAO7 (a,c) and PAO1 (b,d) * p<0.05 (vs control, Test Mann–Whitney).

Growth experiments were carried out with *P. aeruginosa* PAO1 as a reference strain and its pyoverdine and pyochelin-double deficient mutant PAO7. SMM was used to evaluate the "siderophore-like" activity of 1a and 1b without and with the addition of a known amount of $FeCl_3$. Controls, without ligands, show the natural bacterial growth under these latter conditions.

In presence of 1a, PAO1 bacterial growth is promoted suggesting a competition with the siderophore of *P. aeruginosa*. The growth of PAO7 shows that 1a can be internalized by the bacteria. Bacterial growth were lower in the presence of 1b which could by explained by a lower recognition by the bacteria.

Preparation and Study of Gallium Complexes

Table 1. Conditions for gallium complexes formation.

	Ligands	Gallium salts	Temperature Time	Buffer (pH 7)	Results (Mass Spectroscopy)
1	1a	$Ga(NO_3)_3$	20 °C 1 h	No	1a only
2		$Ga(acac)_3$			
3		$Ga(NO_3)_3$	40 °C 3 h	Tris. HCl	
4		$Ga(acac)_3$	20 °C 21 h		
5	1b	$Ga(NO_3)_3$	20 °C 1 h	No	Ga(1b), $Ga(1b)_2$, $Ga_2(1b)_3$
6		$Ga(acac)_3$			
7		$Ga(NO_3)_3$	40 °C 3 h	Tris. HCl	$Ga(1b)_2$, $Ga_2(1b)_3$
8		$Ga(acac)_3$	20 °C 21 h		

In order to form the toxic gallium complexes, several conditions have been carried out using two gallium salts ($Ga(NO_3)_3$ or $Ga(acac)_3$) at room temperature without buffer (Table 1, issues 1,2,5,6) or at 40 °C in presence of Tris.HCl (Table 1, issues 3,4,7,8).

No complex formation has been observed with 1a. The characterization by mass spectroscopy showed several complexes with 1b. Physicochemical studies have been carried out to determine the gallium affinity of 1b.

The Figure 6 represents the distribution curves of the various species plotted as a function of pH. At physical pH, two species ($Ga_2(1b)_3H_2$ and $Ga_2(1b)_3H$) were found with 1b corresponding to the complex below and matching the mass spectroscopy observations.

The pGa value obtained for 1b was 20.85.

Unfortunately, the 1b-Ga(III) has no antipseudomonal activity. This might be due to a lower recognition of the complex by the bacteria.

Figure 6. Distribution curves for the Ga-1b system.

Conclusion & Prospects

Two 1,4-disubstituted piperazines 1a and 1b have been obtained in four and three steps synthesis and global yields in an average 40%. Their pFe values were respectively 28.04 and 24.38, meeting the criteria for an iron chelation therapy agent. These promising results showed the interest of the piperazine core to synthesize new efficient siderophore. As a Trojan Horse strategy, gallium salts have been used in order to form the corresponding toxic complexes 1a-Ga(III) and 1b-Ga(III). Unfortunately, no complex has been formed in presence of 1a and 1b-Ga(III) did not show antipseudomonal activity. This could be explained by the lower recognition of this ligand by *P. aeruginosa*. However, the 1,4-disubstituted piperazine plateform could be used to vectorize antibiotics, 1a and 1b being recognized by *P. aeruginosa*.

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[5] Loupias, P. et al., *Pharmaceuticals*, 12, (2019). [6] Allam A. et al., *Inorganica Chimica Acta*, 366, 310-319 (2011). [7] Nurchi V.M et al., *J. Inorg Biochem.*, 102, 684-692 (2008).

PD-L1 protein fragments as potential immunomodulators

Piotr Ciura[(1)], Magdalena Bojko[(1)], Marta Spodzieja[(1)], Adam Sieradzan[(2)], Katarzyna Węgrzyn[(3)], Sylwia Rodziewicz-Motowidło[(1)]

(1) University of Gdańsk, Faculty of Chemistry, Department of Biomedical Chemistry
(2) University of Gdańsk, Faculty of Chemistry, Department of Theoretical Chemistry
(3) University of Gdańsk, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and MUG, Institute of Biotechnology of the University of Gdańsk

The PD-1 (Programmed Death Receptor 1) protein is one of the immune system checkpoint receptors found on the surface of activated T lymphocytes. It regulates the immune system's immune response, interacting with its ligand - the Programmed Death protein – Ligand (PD-L1) on the surface of antigen presenting cells such as B lymphocytes or dendritic cells. The formation of a complex between PD-1 and PD-L1 proteins leads to a decrease in the activity of lymphocytes. This feature is intended to prevent the appearance of autoimmune diseases (a). However It has been proven that in some types of cancer (eg melanoma, pancreatic cancer) the PD-L1 protein is overexpressed, which means that the immune system response is impaired, leading to the development of the cancer (b). Due to the fact that cancer is often considered the "Plague of the 21st century", the subject of a number of studies has been the synthesis of compounds that can block the formation of the protein complex.
In my work, I focus on the synthesis and study of the PD-1 protein affinity of peptides designed on the basis of the structure of the PD-L1 protein, which can block the formation of the PD-1 / PD-L1 complex (c). The peptides used in my research were designed on the basis of the crystal structure of the PD-1 / PD-L1 complex (PDB 4ZQK) and the MM-GBSA analysis. The synthesis of the above compounds was carried out on a solid support using the SPPS technique. As a result of this research, it is planned to obtain inhibitors of the PD-1 / PD-L1 complex, potential immunomodulators that can be used in the treatment of selected neoplasms.

Work financed by the Polpharma Scientific Foundation as part of the 17th Edition of the Competition for Research Projects No. 1 / XVIII / 19: "Peptide immunomodulators as inhibitors of the next generation PD-1 / PD-L1 checkpoints".

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[(a)] N. L. Syn, The Lancet Oncology, 2017, vol. 18, no. 12, 731–741,
[(b)] X. Wang, OncoTargets and Therapy, 2016, vol. 9, 5023–5039,
[(c)] J. Weber, Seminars in Oncology, 2010, vol. 37, no. 5, 430–439.

* Correspondence: ciurapiotr96@gmail.com

PEPTIDE INHIBITORS OF PD-1/PD-L1 PROTEIN COMPLEXES AS POTENTIAL NEW GROUP OF ANTI-CANCER DRUGS

Piotr Ciura[1], Magdalena Bojko[1], Marta Spodzieja[1], Adam Sieradzan[2], Małgorzata Kogut[2], Katarzyna Węgrzyn[3], Sylwia Rodziewicz-Motowidło[1]

[1] University of Gdańsk, Faculty of Chemistry, Department of Biomedical Chemistry
[2] University of Gdańsk, Faculty of Chemistry, Department of Theoretical Chemistry
[3] University of Gdańsk, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and MUG, Institute of Biotechnology of the University of Gdańsk
Contact: ciurapiotr96@gmail.com

INTRODUCTION

- The Programmed Death Receptor 1 (PD-1) is one of the immune checkpoint receptors, which is found on the surface of activated T cell lymphocytes. It is a member of a co-inhibitory receptors family responsible for negative regulations of the immune system (Fig. 1).
- The formation of a complex between the PD-1 and PD-L1 proteins leads to a decrease in the activity of lymphocytes, inhibiting the immune response to a given cell.
- In some types of cancer (e.g., melanoma, non-small cell lung cancer) expression of the PD-L1 on a cell surface leads to a disturbance of the immune system response and tumour development. Therefore, work is underway to find peptidic immunomodulators blocking the PD-1/PD-L1 complex.

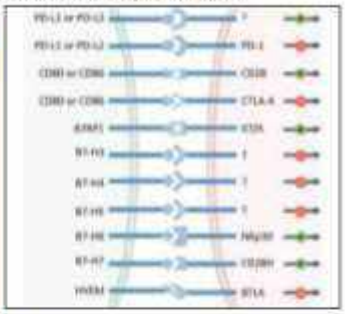


Fig. 1. Immune checkpoint receptors (left) and their ligands (right) taking part in regulation of immune system's functions.

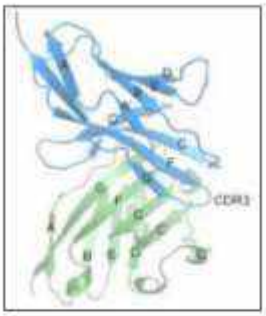


Fig. 2. Structure of PD-1/PD-L1 complex (PDB: 4ZQK).

RESULTS AND DISCUSSION

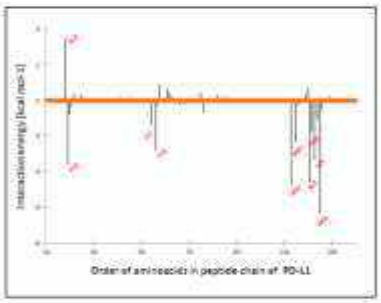
- Peptides were designed based on the crystal structure of the PD-1/PD-L1 complex (PDB: 4ZQK) (Tab. 1A) and MM-GBSA analysis (Tab. 1B).
- Two peptides with the best dissociation constant were chosen for further modifications - PDL1 (113-126) peptide and its analogue with an intramolecular disulfide bridge at the C114-C124 position (Tab. 2).
- The dissociation constant was determined using the surface plasmon resonance (SPR) technique. The PDL1 (113-126) C114-C124 peptide was selected for further research, due to its high stability in the RPMI 1640 medium, which will be used in further tests.
- In the next step, we decided to introduce changes in the sequence of the PDL1 (113-126) C114-C124 peptide in order to increase interaction with the PD-1 protein.
- Based on the MM-GBSA simulation, we decided to replace the rest of the glycine at position 120, giving a positive energy contribution to the formation of the PD-1/PD-L1 complex. These three peptides (with glycine replaced with serine, phenylalanine and glutamic acid) are a subject of further research on their stability and dissociation constants (Tab. 2).

Tab. 1. Aminoacids taking part in PD-1 i PD-L1 complex creation designated by: A. Crystalic structure (PDB 4ZQK), B. MM-GBSA analysis of PD-L1 protein.

A.

PD-1	PD-L1	Bond type
Ile 134	Tyr 123	Alkyl-π
Tyr 68	Tyr 123	π-π
Glu 136	Arg 113	Salt bridge
Asn 66	Ala 121	Hydrogen bond
Tyr 68	Asp 122	Hydrogen bond
Gln 75	Arg 125	Hydrogen bond
Gln 75	Asp 26	Hydrogen bond
Thr 76	Tyr 123	Hydrogen bond
Thr 76	Lys 124	Hydrogen bond
Lys 78	Phe 19	Hydrogen bond
Ala 132	Gln 66	Hydrogen bond
Ile 134	Tyr 54	Hydrogen bond
Ile 134	Glu 58	Hydrogen bond
Glu 136	Tyr 123	Hydrogen bond

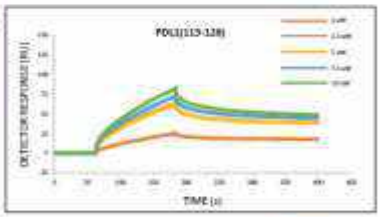
B.



Tab. 2. Synthesized peptides and their dissociation constant (K_D) with PD-1 protein.

Peptide designation	Peptide sequence	K_D (pM)
PDL1(113-126)	Ac-RAbuNISVGGADYKRI-NH$_2$	4.5×10-7 ± 2.8×10-7
PDL1(113-126) C114-C124	Ac-RCNeISVGGADYCRI-NH$_2$	8.83×10-7 ± 4.8×10-7
PDL1(113-126) C114-C124; G120S	Ac-RCNeISVGSADYCRI-NH$_2$	-
PDL1(113-126) C114-C124; G120F	Ac-RCNeISVGFADYCRI-NH$_2$	-
PDL1(113-126) C114-C124; G120E	Ac-RCNeISVGEADYCRI-NH$_2$	-

C.



D.

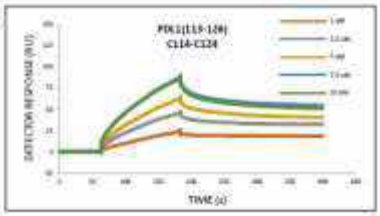


Fig. 4. Summary of sensograms for C. PDL1 (113-126), D. PDL1 (113-126) C114-C124 peptides titrated with PD-1 protein.

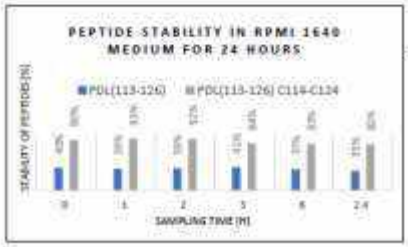


Fig. 3. PDL1 (113-126) and PDL (113-126) C114-124 peptides stability in RPMI 1640 medium for 24 hours.

1) N. L. Syn, The Lancet Oncology, 2017, vol. 18, no. 12, pp. e731–e741.
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CHEMIA UG

Nanoformulations of Curcumin and (−)-Epigallocatechin-3-*O*-Gallate in Bladder Cancer Treatment

Ludwika Piwowarczyk[(1)*], Małgorzata Kucinska[(2)], Jaroslaw Piskorz [(3)], Dariusz T. Mlynarczyk[(4)], Marek Murias[(3)], Tomasz Goslinski[(4)], Anna Jelińska[(1)]

(1) Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland
(2) Chair and Department of Toxicology, Poznan University of Medical Sciences, ul. Dojazd 30, 60-631 Poznań, Poland.
(3) Chair and Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland.
(4) Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland

Restricted to organizers

Bladder cancer (BC) is one of the most common urological diseases. Its incidence continues to increase despite the development of various treatment regimens, and it has become necessary to search for new therapeutic approaches and pharmacological agents. Curcumin (CUR) and epigallocatechin gallate (EGCG) represent new options in the treatment of cancers, including BC. Their availability, safe use, and low production costs drive their increased implementation in therapy either alone or in combination with other drugs[(a)]. However, their efficiency is hampered by low solubility in water and low stability in solutions.

Nanoformulations are a promising tool for efficient drug substance delivery to the target site. Among these formulations, liposomes offer an effective way to increase bioavailability, improve solubility, and enhance intracellular absorption of the carried compounds, including CUR and EGCG [(b,c,d)].

In the presented study, CUR and EGCG were entrapped in various liposomes, of different composition, to determine the optimal formulation for their most potent action on cancer cells. What is more, the combination of the two molecules in one formulation was also assessed *in vitro* on cancer cell lines to evaluate the potential synergism in action.

This research was funded by National Science Centre, Poland, grant number 2019/35/B/NZ7/01165.

Bibliographic references:

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* Correspondence: ludwika.piwowarczyk@student.ump.edu.pl

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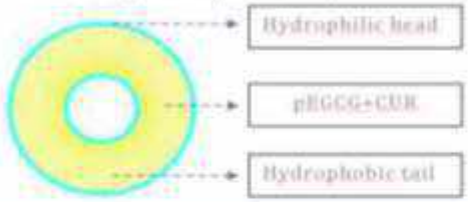
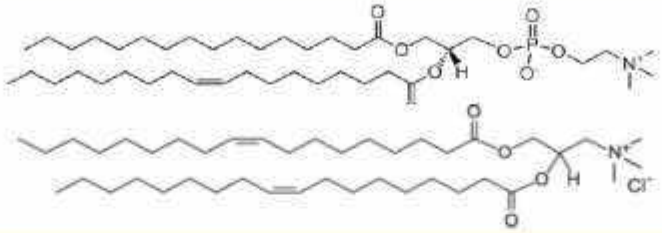
*ludwika.piwowarczyk@student.ump.edu.pl

Structure of liposomes (POPC:DOTAP):





POPC – 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine

DOTAP – 1,2 Dioleoyl-3-Trimethylammonium-Propane (Chloride Salt)

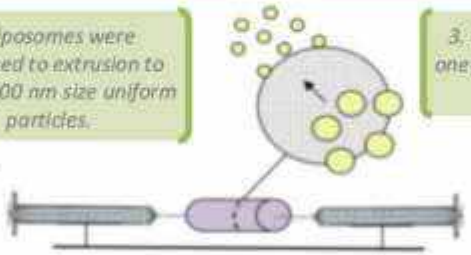
Nanoformulations are a promising tool for efficient delivery of the drug substance to the target site. Among these formulations, liposomes offer an effective way to increase bioavailability, improve solubility, and enhance intracellular absorption of the carried compounds, including curcumin (CUR) and peracetyl-(−)-epigallocatechin-3-*O*-Gallate (pEGCG).

1. In the presented study, CUR and pEGCG were entrapped in various liposomes (usingthin film hydration method), of different composition, to determine the optimal formulation for their most potent action on cancer cells.

1.

2. Liposomes were subjected to extrusion to obtain 100 nm size uniform particles.

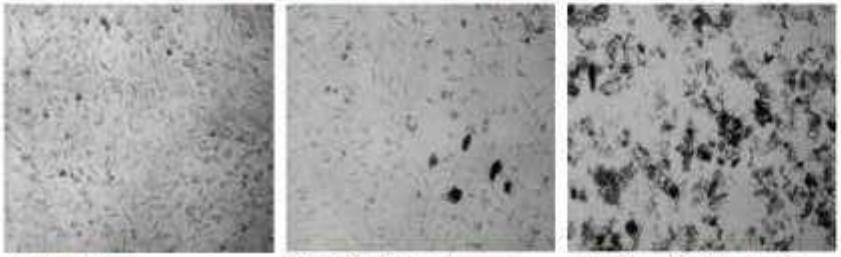
2.



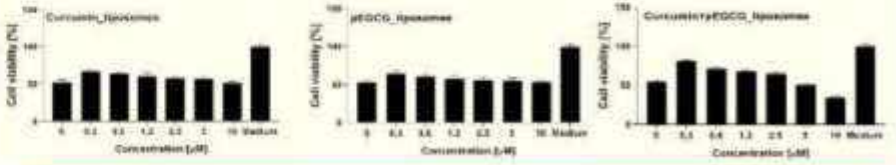
3. CUR and pEGCG and the combination of these two molecules in one formulation was assessed in vitro on cancer cell lines to evaluate the potential synergism in action.

3.

Micrographs of the cells treated with the liposomal formulations:



Control culture | CUR+pEGCG 0.30 µM liposomes | CUR+pEGCG 10 µM liposomes



CONCLUSION

Based on the performed experiments it can be concluded that:

- Liposomes can be effectively used for delivery of natural polyphenols.
- The efficacy of such formulations was proven more successful in decreasing cancer cell viability *in vitro*.
- CUR and pEGCG exhibit synergy in anticancer activity.

Influence of different anchoring groups on photocatalytic activity of hybrid materials based on titanium(IV) oxide and porphyrins.

Rafał Krakowiak (1)*, Joanna Musiał (2), Robert Frankowski (3), Agnieszka Zgoła-Grześkowiak (3), Beata Stanisz (2), Dariusz T. Mlynarczyk (1), Tomasz Gośliński (1)

(1) Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland
(2) Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland
(3) Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland

Restricted to organizers

Emerging contaminants are a group of chemical substances that have been detected at increasing concentrations in the environment in recent years [(a)]. Long-term exposure to low concentrations of these substances has unknown effects but is predicted to be harmful. Usually, such contaminants are degraded in remediation facilities, but these new contaminants, such as pharmaceutics or cosmetics, demonstrate increased stability, when compared to regular contaminants. It is often impossible to eliminate them with the aid of conventional remediation techniques. As such, new methods of degradation are being sought for.

Recently, we demonstrated the use of hybrid materials (HM) prepared by chemical adsorption of phthalocyanines on TiO_2 nanoparticles' surface [(b)]. Prepared HMs showed an average photodegradation efficiency of 90% of initial ibuprofen after 6 h of irradiation with UV light. However, no photodegradation was observed when the same HMs were exposed to red light (665 nm). Ideally, this kind of photocatalysts would benefit from photoexcitation by visible light. Thus, new HMs were prepared by employing anchoring groups, which are specific functional groups that covalently bind to the carrier's surface, allowing for efficient energy transfer [(c)]. Operating on HMs that utilize anchoring groups we expected to achieve photocatalytic activity under visible light.

Herein, we present preparation of described HMs and obtained results of photocatalytic studies performed on HMs where metal-free porphyrins were bound to TiO_2 surface through three different anchoring groups.

This study was supported by the National Science Centre, Poland under grant number 2016/21/B/NZ9/00783.

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[(a)] D. Kanakaraju, B. D. Glass, and M. Oelgemöller, 'Advanced oxidation process-mediated removal of pharmaceuticals from water: A review', *J. Environ. Manage.*, vol. 219, pp. 189–207, Aug. 2018, doi: 10.1016/j.jenvman.2018.04.103.

[(b)] R. Krakowiak *et al.*, 'Phthalocyanine-Grafted Titania Nanoparticles for Photodegradation of Ibuprofen', *Catalysts*, vol. 10, no. 11, p. 1328, Nov. 2020, doi: 10.3390/catal10111328.

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* Correspondence: rafal.krakowiak@student.ump.edu.pl

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[illegible] (1)*, [illegible] (2), [illegible] (3), [illegible] (2), [illegible] (1), [illegible] (1), [illegible] (1)

(1) Chair and Department of Chemical Technology of Drugs, Poznan University of Medical Sciences, [illegible], Poznan, Poland
(2) Chair and Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, [illegible], Poznan, Poland
(3) Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, [illegible], Poznan, Poland
* Corresponding author: [illegible]

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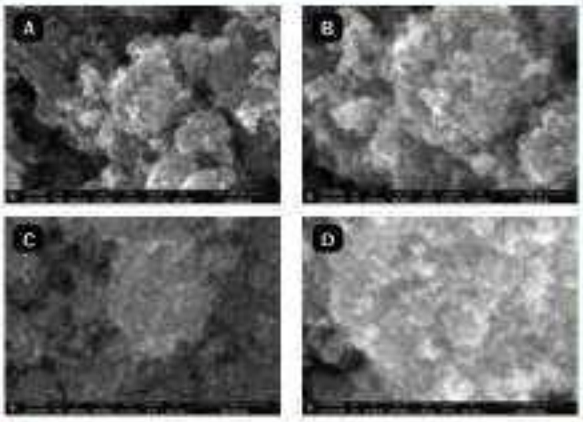
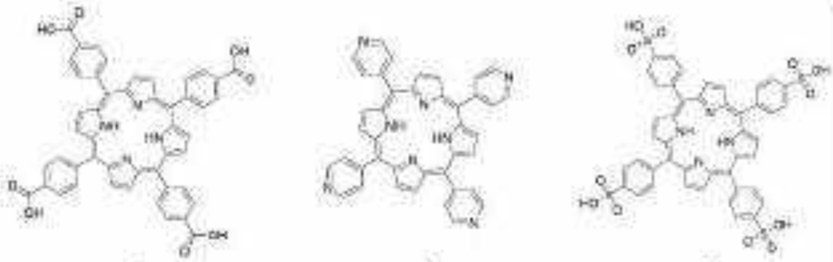


Fig. 1. SEM images of (A) bare 40 nm anatase TiO_2 and (B–D) functionalized TiO_2 with porphyrins. The images show visible aggregation of the material. Although porphyrin particles are not visible in the images, the change in image's resolution suggests that the material's composition has changed.

HYBRID MATERIALS BASED ON PORPHYRINOIDS AND NANOMETRIC TiO_2



1 2 3

Fig. 2. Porphyrins used for functionalization of TiO_2: 1) meso-tetraphenylporphine-4,4',4'',4'''-tetracarboxylic acid, 2) 5,10,15,20-tetra(4-pyridyl)porphyrin and 3) meso-tetraphenylporphine-4,4',4'',4'''-tetrasulfonic acid.

Porphyrins presented in Fig. 2 were deposited onto 40 nm anatase TiO_2's surface by suspending both materials in organic solvents and stirring for 24 h. Methanol was used for 1 and 3 as the solvent, whereas for 2 methanol acidified with HCl was used. Interestingly, after deposition the material changed color. Initially, 1 was a bluish powder, 2 was dark purple and 3 blue-green crystallites. The obtained powders were pale orange for 1 and 3 and dark green for 2.

After the process, the HMs were thoroughly characterized with techniques such as ATR-FTIR, BET assay, electron microscopy (SEM, supplemented with TEM where necessary), TGA, XRD and XPS to confirm successful deposition of the porphyrins on TiO_2's surface.

PHOTOCATALYTIC STUDIES

For photocatalytic experiments four sets of three diode lamps were used for separate irradiations. Used wavelengths were 365 nm (UV), 425 nm (UV2), 525 nm (G) and 660 nm (R). Ibuprofen was dissolved in water at concentration of 10 mg/L to serve as the model emerging contaminant. The irradiations were performed over the period of 6 hours. The results are presented on Fig. 3.

Only materials functionalized with 1 and 2 showed photocatalytic activity in the visible range. No photodegradation was observed in red light. However, 1 achieved 29% reduction of initial ibuprofen concentration after 6 hours of irradiation with green light. For 2 the reduction was 38%. Although these values are not high, one needs to have in mind relatively small absorption peaks for porphyrins in the Q band range.

Out of all the prepared hybrid materials 2 showed the highest overall photodegradation efficiency.

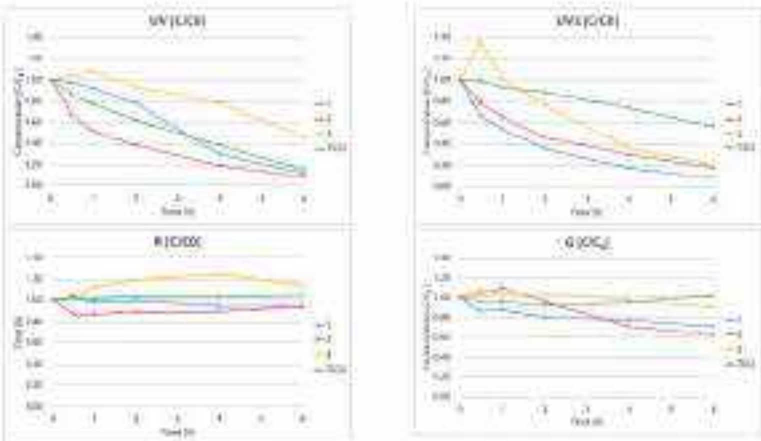


Fig. 3. Photocatalytic degradation of ibuprofen water solution with prepared hybrid materials under irradiation with different wavelengths.

References:
[1] [illegible]
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[3] [illegible]

This study was supported by the National Science Centre, Poland, under grant No 2016/21/B/NZ9/00783.

Inhibiting LIM Kinase: A possible treatment for neurofibromatosis type 1?

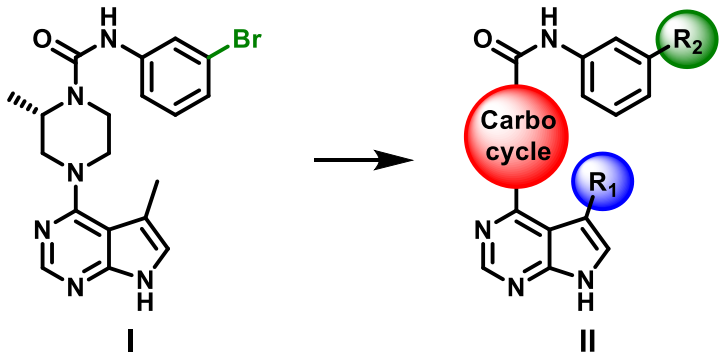
Rayan Berabez [1]*, Anthony Champiré [1], Béatrice Vallée [2], Hélène Bénédetti [2], Karen Plé [1], Sylvain Routier [1].

(1) Institut de Chimie Organique et Analytique (ICOA), UMR CNRS 7311, Orléans, France.
(2) Centre de Biophysique Moléculaire, UPR4301, CNRS, Orléans, France.

Restricted to organizers

Neurofibromatosis is a disease which touches 1 in 3500 people worldwide. LIM-Kinase has been shown to be involved in the signalling pathway of this pathology,[(a)] and its inhibition is a promising therapeutic target.[(b)] As part of a project to develop new molecules for LIMK inhibition, we wished to use our synthetic experience for the preparation of new biologically active compounds.

Starting from the structure of compound I, one of the most efficient inhibitors of LIMK[(c)], we have designed several new molecular series. Structural modifications were made in three positions, and have lead to a library of over 130 compounds. To date, these original pyrrolopyrimidines have been tested for their activity against LIMK1 and LIMK2 as well as their inhibition of cofilin phosphorylation *in cellulo* with very promising results. New chemical modifications are in progress to improve pharmacokinetic properties as well as to target ROCK, another kinase deregulated in patients affected by neurofibromatosis.



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[(b)] Petrilli, A.; Copik, A.; Posadas, M.; Chang, LS.; Welling, DB.; Giovannini, M.; Fernández-Valle C. *Oncogene*, **2014**, 33(27), 3571-3582.

[(c)] Harrison, B. A. et al. *J.Med.Chem*. **2009**, 52, 6515–6518.

* Correspondence: rayan.berabez@univ-orleans.fr

Inhibiting LIM Kinase: A possible treatment for neurofibromatosis type 1?

Rayan Berabez[1], Anthony Champiré [1], Béatrice Vallée [2], Hélène Bénédetti [2], Karen Plé [1], Sylvain Routier [1]

[1] ICOA, Université d'Orléans, UMR CNRS 7311, Orléans, France, [2] Centre de Biophysique Moléculaire, UPR4301, CNRS, Orléans, France

Introduction

Neurofibromatosis type I (NF1) is a common genetic disease which affects 1 in 3500 people worldwide. Clinical expression of NF1 varies widely from simple "Café au lait" spots on the skin to the development of potentially cancerous tumors in the central and peripheral nervous system. Cognitive disorders are also associated with the disease in 50-70% of cases. Currently, there is no treatment for NF1.

Neurofibromin Nf1

Neurofibromatosis type I is due to mutations on the *NF1* gene, a tumor suppressor gene. *NF1* encodes neurofibromin, Nf1, involved in many cell signaling pathways regulating vital processes in the body. Recently, our team of biologists has demonstrated that Nf1 inhibits the Rho/ROCK/LIMK2/cofilin pathway by interacting with LIMK2 kinase[1]. Other studies have shown that Nf1 also inhibits the Rac/PAK1/LIMK1/cofilin pathway[2].

LIM kinases 1 and 2

LIM kinases (LIMK1 and LIMK2) thus appear as new therapeutic targets of choice. These kinases are involved in the remodeling of the actin cytoskeleton and in microtubule organization. No known LIMK inhibitor has ever been tested for the treatment of NF1. Targeting the LIM kinase pathway is thus a totally new approach toward a potential therapeutic solution in the treatment of this disease

As part of this collaborative project, we wished to develop new LIM kinase inhibitors using the expertise of our molecular modeling (ICOA), medicinal chemistry (ICOA) and biology (CBM) teams. The clinicians, present in this translational project, will subsequently validate these molecules for preclinical entry.

Molecular modeling

1. Unknown 3D structures :
   - LIMK 2 active conformation (DFG-in)
   - LIMK 1 inactive conformation (DFG-out)

2. Structure building Homology modeling

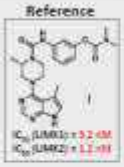
3. Predicting interactions Docking reference inhibitors

4. Rational proposition of new ligands

Synthesis

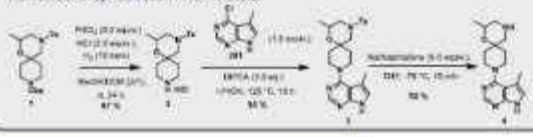
In 2009, Lexicon Pharmaceuticals described molecule I [3], an excellent inhibitor of LIMKs developed to treat glaucoma and ocular hypertension.
Our work was inspired by this skeleton to design new derivatives with improved physicochemical and pharmacological properties to ensure development for neurofibromatosis.

Reference

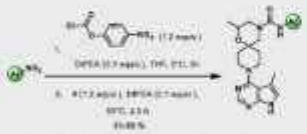


Modification of the central ring

A strategy to replace the central piperazine ring was developed to modify pharmacokinetic properties as well as the orientation of the molecule in the active site. Among the changes made, a novel piperazine-like spirocycle was synthesized, increasing the number of sp^3 carbons in the molecule.



The selective deprotection of the Cbz group gave the key spirocyclic amine 2 which was further transformed into amine 4. Formation of the final urea compounds was performed *via* a mixed carbamate in moderate yield.



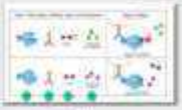
These new promising series of LIMK inhibitors showed not only good selectivity between LIMK1 and LIMK2 (in the nM range) but also an excellent selectivity against the upstream ROCK kinase.

Based on these results, efforts are being made to design new spirocyclic compounds from the reference I and 4. The interesting 3D conformation of this series could bring very good selectivity for LIMK among the kinase domain.

Biological tests

In vitro tests

The LanthaScreen Eu kinase binding assay was used to test inhibition of LIMK1 and LIMK2 (Invitrogen™), and the Z'Lyte assay used for ROCK1 and ROCK2.



	compounds with Ki <10 nM	compounds with 10<Ki<50 nM
LIMK1	17	47
LIMK2	14	45

- ~70 compounds are specific for LIMK1/2
- ~13 compounds are LIMK1 vs LIMK2 selective (5 times Ki ratio)

In cellulo tests

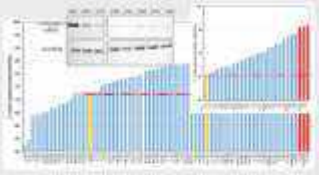
Cytotoxicity

Cell viability in presence of the different inhibitors
- CellTiter-Glo® Luminescent Cell Viability assay (Promega™) based on quantification of present ATP

- Most of our compounds exhibit low cytotoxicity

Cofilin phosphorylation inhibition

HeLa cells +/- 25 µM inhibitor for 2 hours, lysis.
- Western Blot α-Phospho-cofilin/α-cofilin



75 compounds have been tested

- 25 compounds are more efficient on HeLa cells than LX7101
- 3 compounds are extremely efficient

References

1. Vallée *et al.* Nf1 RasGAP Inhibition of LIMK2 Mediates a New Cross-Talk Between Ras and Rho Pathways. *PLoS One.* 2012; 7(10):e47203.
2. Stanvitsky-Elbaz *et al.* The pre-GAP-related domain of neurofibromin regulates cell migration through the LIM kinase/cofilin pathway. *Mol. Cell. Neuroscience.* 2009, 45, 278-287.
3. Harrison, B. A. *et al.* Novel Class of LIM-kinase 1 Inhibitors for the Treatment of Ocular Hypertension and Associated Glaucoma. *J.Med.Chem.* 2009, 52, 6515–6518.

Acknowledgments

This project is funded by the APR IR program of the Region center val de Loire and by the Ligue contre le cancer.

Conclusion

- ✓ To date, a library of more than 190 compounds have been prepared and tested for LIMK and ROCK inhibition.
- ✓ 53 molecules exhibit a Ki for LIMK < 50 nM arid ten of them a Ki <10 nM
- ✓ Compounds exhibit low cytotoxicity.
- ✓ 25 compounds are more efficient for cofilin phosphorylation inhibition in HeLa cells than the reference molecule LX7101. 3 compounds are extremely active.

Work is in progress toward the preclinical validation of these promising inhibitors

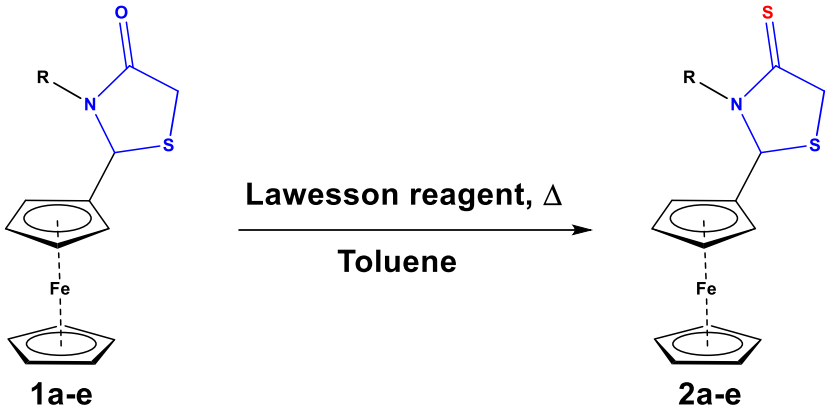
The synthesis and spectral characterization of novel 2-ferrocenyl-1,3-thiazolidine-4-thiones

Anka Todosijević[(1)]*, Aleksandra Minić[(2)], Danijela Ilić Komatina [(2)].

(1) Faculty of Agriculture, University of Niš, Serbia.
(2) Faculty of Technical Sciences, University of Priština, Serbia.

Restricted to organizers

Thiazolidinone ring is of substantial interest as it represents is a core structure in numerous synthetic medicaments displaying a wide variety of biological activities.[a] Besides, the thiazolidinone scaffold is found in various natural products, especially thiamines, molecules possessing cardiac and glycemic benefits such as troglitazone[b] and many metabolic products of fungi and primitive marine animals, including 2-(aminoalkyl)-4-carboxylic acids.[c] Therefore, the synthesis of these heterocycles are of considerable interest to organic and medicinal chemists. In this work we will report the synthesis of novel 2-ferrocenyl-1,3-thiazolidine-4-thiones which include quite simple procedure according to which ferrocenyl thiazolidinones (**1a-e**) were treated by Lawesson's reagent in toluene as solvent. In addition, a detailed characterization of the obtained 2-ferrocenyl-1,3-thiazolidine-4-thiones (**2a-e**) has been completed by IR and NMR spectroscopy.



1a-e **2a-e**

R = butyl; hexyl; octyl; *m*-tolyl; *p*-tolyl;

Acknowledgement: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. 451-03-68/2020-14/200122).

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[(a)] M. Baumann at al., *Beilstein J. Org. Chem*. **9** (2013) 2265–2319.
[(b)] M. N. Ghazzi at al., *Diabetes* **46** (1997) 433–439.
[(c)] U. Scmidt at al., Synthesis (1987) 233–236.

* Correspondence: anka.pejovic@gmail.com

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(1) Faculty of Agriculture, University of Niš, Serbia.
(2) Faculty of Technical Sciences, University of Pristina, Serbia.

Correspondence: anka.pejovic@gmail.com

INTRODUCTION

Thiazolidinone ring is of substantial interest as it represents as a core structure in numerous synthetic medicaments displaying a wide variety of biological activities[a]. Several years ago, our team reported easily and efficient method for the synthesis of the novel thiazolidinone-derivatives 2-ferrocenyl-1,3-thiazolidine-4-ones. These heterocycles which showed strong acetylcholinesterase activity also demonstrated great potential as ligands in the synthesis of another ferrocene containing thiazolidinones[b,c]. In this paper we will present the synthesis of the 2-ferrocenyl-1,3-thiazolidine-4-thiones using 2-ferrocenyl-1,3-thiazolidine-4-ones as starting materials.

Spectral characterization: The structure of all five thiazolidine-4-thiones **2a-e** were utterly confirmed by standard spectroscopic techniques (IR, ^{1}H- and ^{13}C NMR). The IR spectra of compounds contained a strong band in range 1203-1165 cm^{-1} which is attributed to the C=S stretching vibrations. Three groups of signals have been observed in the ^{1}HNMR spectra. The first belongs to aliphatic protons, the second to protons of the ferrocene moiety and the third to the aromatic protons. The ^{1}H- and ^{13}C NMR spectra of **2a** are presented on Figure 1 and Figure 2.

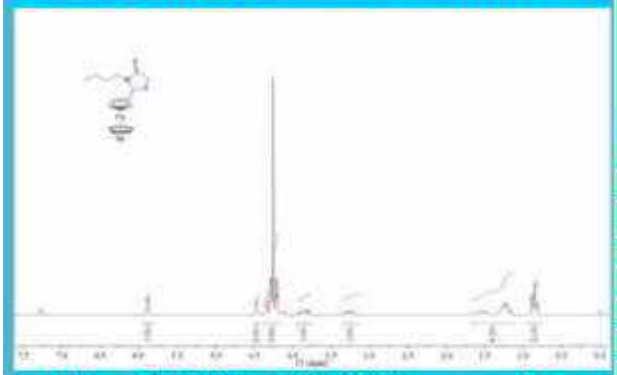


Figure 1. ^{1}HNMR (200 MHz, $CDCl_3$) spectrum of **2a**

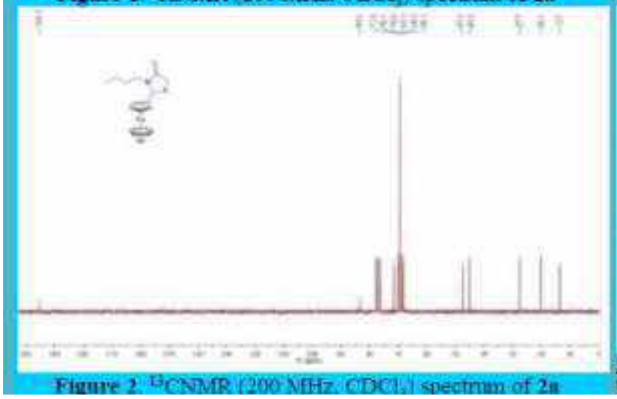
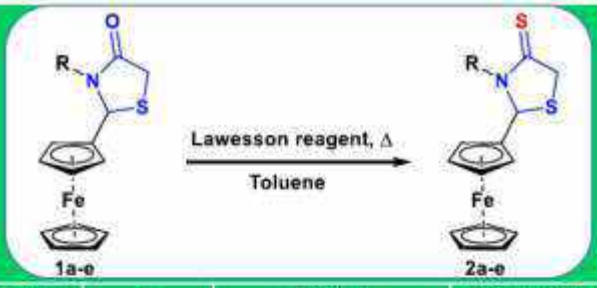


Figure 2. ^{13}CNMR (200 MHz, $CDCl_3$) spectrum of **2a**

RESULTS AND DISCUSSION

Synthesis: The obtaining of target compounds was conceived as the functionalization of the 2-ferrocenyl-1,3-thiazolidine-4-ones (Table 1, **1a-e**). It should comprise their thionation to afford the series of appropriate 2-ferrocenyl-1,3-thiazolidine-4-thiones. The preliminary examinations showed that the thionation of the 2-ferrocenyl-1,3-thiazolidine-4-ones (**1a-e**) is possible under mild reaction conditions. Namely, in the opening experiments in the reaction between **1a** and Lawesson's reagent in toluene as solvent in our debuts, the desired thiazolidine-4-thione **2a** was obtained in very good yield (88%, Table 2). We accepted these conditions as the optimal ones so we applied them on another fourth known ferrocene-containing thiazolidinones. All reactions were performed and the 2-ferrocenyl-4-thiazolidinethiones (**2b-e**) were isolated pure in good to high yields (Table 1).

Table 1. Substrate scope of synthesis of 2-ferrocenyl-1,3-thiazolidine-4-thiones **2a-e**



1a-e **2a-e**

Entry	R	Product	Yields* (%)
1	*n*-Butyl	2a	88
2	*n*-Hexyl	2b	90
3	*n*-Octyl	2c	88
4	*m*-Tolyl	2d	65
5	*p*-Tolyl	2e	60

*Isolated yield after column chromatography.

Conclusion

The five novel 2-ferrocenyl-1,3-thiazolidine-4-thiones were synthesized.

Structures of all obtained products were utterly confirmed by standard spectroscopic techniques.

The synthesized thaizolidinethiones are interesting material for biological examination.

References:

[a]M. Baumann at al., *Beilstein J. Org. Chem.* **9** (2013) 2265–2319.
[b]A. Pejović at al., *J. Organomet. Chem.* **873** (2018) 78-85.
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Acknowledgement: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No: 451-03-68/2020-14/200133)

Synthèse des 2- (benzooxazolyl)imidazo[1,2-x]azinesvia O-Arylation catalysée par le cuivre

A. El AKKAOUI [a], S. BERTEINA-RABOIN [b], A. MOUADDIB [c] & G. GUILLAUMET[b]

[a]Laboratoire de Chimie Analytique et Biomoléculaire. Université cadi ayyad - Faculté polydisciplinaire de Safi. Sidi Bouzid. B.P. 4162, 46000 SAFI - Maroc
[b]Institut de Chimie Organique et Analytique, UMR CNRS 6005, Université d'Orléans, BP 6759, 45067 ORLEANS Cedex 2, France
[c]Laboratoire de Chimie Bio-Organique et Analytique. Université Moulay Slimane. BP 523, 23000 BENI-MELLAL, Maroc

Introduction

Les imidazo [1,2-x]azines sont des bioisosters de l'indole et ont un très vaste spectre d'activité biologique et thérapeutique [1]. Récemment, divers travaux ont mis en évidence l'intérêt biologique de certains composés présentant, dans leur squelette de base, un noyau imidazole en tant qu'agents anticancéreux, anxiolytiques ou antiviraux [2]. En liaison avec notre programme consacré à la synthèse de dérivés structurellement diversifiés à base d'imidazoazines [3]. Nous présentons dans cette communication voie rapide et efficace d'accès aux composés de type 2-(benzooxazolyl)imidazo[1,2-x]azine. (Schéma 1).

Schéma 1

Synthèse de dérivés 2-(benzooxazolyl)imidazo[1,2-x]aziniques via des réactions multicomposant

ylation intramoléculaire catalysée par le cuivre de dérivés d' imidazo[1,2-x]azines-2-carbamide. Deux séquences sont à prendre en considération, la préparation des l'intermédiaires de type imidazo[1,2-x]azines-2-carbamide par condensation entre l'ester imidazo[1,2-b]azinique et une aniline ortho-bromée (Schéma 2), puis la cyclisation intramoléculaire assistée par le cuivre(Schéma 3).

Synthèse des l'intermédiaires de type imidazo[1,2-x]azines-2-carbamide

Schéma

Imidazo[1,2-a]pyridines polyfonctionnalisées

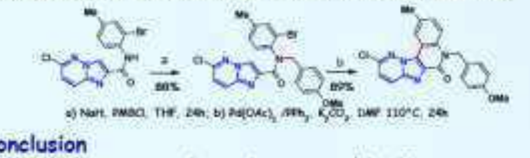
Nous avons appliqué cette méthodologie à la synthèse d'imidazo[1,2-a]pyridine diversement substituées (Schéma 4).

Schéma 4

Cyclisation intramoléculaire des imidazo[1,2-x]azines-2-carbamide

Schéma

Synthèse de 10-chloro-5-(4-méthoxybenzyl)-2-alkyl-pyridazo [2',1':2,3]imidazo[5,4-c]quinolin-6(5H)-one



Conclusion

En conclusion, nous avons mis au point une stratégie efficace permettant la synthèse de composés polycycliques, la réaction intramoléculaire catalysée par le cuivre au départ de imidazo[1,2-x]azines-2-carbamide non protégé, conduit à des bicycles de structure 2-benzoxazolylimidazo[1,2-x]aziniques par formation d'une liaison C-O. Cette méthodologie a été mise à profit pour engendrer une petite bibliothèque de 2-benzooxazolylimidazo[1,2-x]azines avec de très bons rendements. Alors que pour l'imidazo[1,2-b]pyridazine-2-carbamide protégé, la réaction d'arylation intramoléculaire conduit au 10-chloro-5-(4-méthoxybenzyl)-2-alkyl-pyridazo[2',1':2,3]imidazo[5,4-c]quinolin-6(5H)-one par formation d'une liaison C-C. La combinaison des réactions d'arylation inter et intramoléculaire donne accès aux imidazo[1,2-a]pyridine diversement substituées .

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IN SILICO ANALYSIS OF IL-3 AND IL-3RA ACROSS STRUCTURE AND EVOLUTION

Jade Fogha[1,2,3], Julien Diharce[1,2,3,*], Alexandre G. de Brevern[1,2,3,4,*]

[1] Université de Paris, Inserm, Biologie Intégrée du Globule Rouge, UMR_S 1134, DSIMB, F-75739 Paris, France.
[2] Institut National De La Transfusion Sanguine (INTS), F-75739 Paris, France.
[3] Laboratoire D'excellence GR-ex, F-75739 Paris, France.
[4] Université De La Réunion, Inserm, Biologie Intégrée du Globule Rouge, UMR_S 1134, DSIMB, F-97744 Saint-Denis, La Réunion, France.
*julien.diharce@inserm.fr, alexandre.debrevern@inserm.fr

INTRODUCTION

Interleukin-3 (IL-3) is a cytokine belonging to the cytokine family of common β (βc) and is involved in various biological systems. Its activity is mediated by the interaction with its receptor (IL-3Ra), a glycoprotein member of the βc receptor family.[1] IL-3 and IL-3Ra, play a crucial role in various pathologies such as hematological malignancies.[2] Thus, IL-3 and IL-3Ra are interesting therapeutic targets and we proposed a thorough investigation of those proteins and their interaction based on structural and evolutionary information.

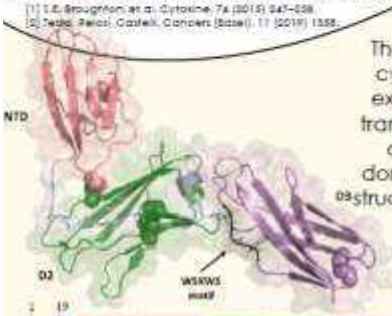
[1] J.D. Broughton et al., Cytokine, 74 (2015) 247–258.
[2] Tefferi, Pardol, Cadrell, Cancers (Basel), 11 (2019) 1358.

IL-3 protein

A cytokine constituted by 4 principal α-helices (A, B, C and D). PDB ID 5UV8

Evolutionary analyses were performed on IL-3 and IL-3Ra in 3 steps: (1) non-human sequences similarity search using PSI-BLAST, NCBI (2) MSA and (3) conservation analysis with ConSurf, resulting in conservation scores determination.

IL-3Ra protein

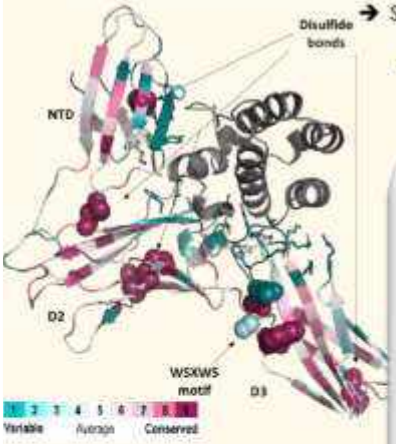


The α subunit of the IL-3 cytokine receptor. The extracellular part of this transmembrane protein is constituted by three domains with β sandwich structures: NTD, D2 and D3. PDB ID 5UV8

Multiple sequence alignment (MSA) from IL-3 species

→ Structural architecture conservation
→ Disulfide bond conservation only in primates and rodents

IL-3Ra evolutionary conservation during evolution



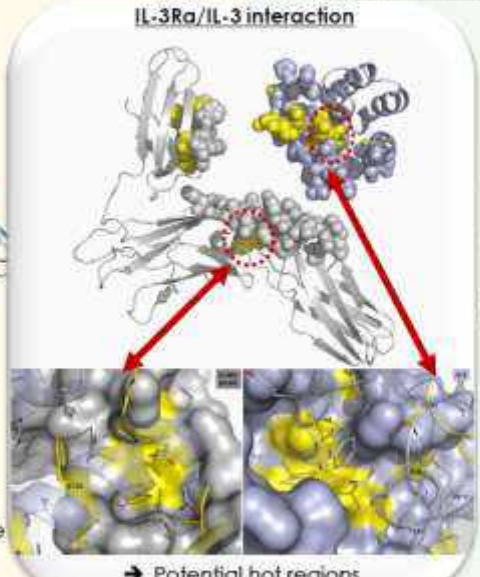
→ Structural architecture conservation
→ Disulfide bond conservation

→ Non-conservation of IL-3Ra interfacing residues.
→ Few IL-3Ra interfacing residues conserved across evolution:
❖ **Lys 54**, **Ala 62** and **Tyr 67** in NTD region.
❖ **Ser 203**, **Phe 232** and **Asn 233** in D2 and D3 regions, are spatially close and form a subpocket (203 Å³).

Evolutionary conservation of IL-3 interfacing residues during evolution

	A					A-α1					α1					
Position	36	**39**	40	43	47	53	54	55	56	**60**	61	**62**	**63**	64	65	66	68
Sequence	S	T	D	I	R	L	L	D	F	**N**	G	**E**	**D**	Q	D	I	M
Max AA %	S 53.7	T **83.4**	D 29.3	I 34.1	N 45.9	L 56.1	L 38.9	D 50	F 42.1	**N 70.7**	S 29.3	**E 46.3**	**D 53.7**	Q 36.6	R 26.8	I 53.6	M 36.6
Grade	3	**7**	1	3	4	4	2*	2*	4	**6**	1	**7**	**7**	4	1	4	3

IL-3Ra/IL-3 interaction



→ Potential hot regions

R	C-D					D							
75	112	113	114	117	**128**	**131**	**132**	135	136	138	139	140
R	T	R	H	H	**R**	**T**	**F**	K	T	E	N	A
R **56.1**	T **65.8**	R 29.3	H 31.7	R 34.1	**R 41.4**	**T 56.1**	**F 46.3**	K 39.1	T 41.4	E 42.5	N 45	S 20
7	5	4	3	3	**5**	**5**	**6**	3	3	4	4	2

→ Non-conservation of IL-3 interfacing residues.
→ Few IL-3 interfacing residues conserved across evolution: **Arg 128**, **Thr 131** and **Phe 132** are spatially close and form a subpocket (414 Å³).

A FAMILY AFFAIR

IL-5Rα/IL-5 and GMRα/GM-CSF complexes are also constituted by proteins involved in the βc family. Cytokines (IL-5 and GM-CSF) and their receptors have **the same structural architecture** than IL-3 and IL-3Ra respectively. However, using sequence and structure alignment, we observed **a different binding mode** between the 3 complexes.

CONCLUSION

This study highlighted potential hot regions and potential key residues for IL-3 and IL-3Ra interaction. Further analysis should be carried out to determined the energetical contribution of these residues. These results could be an interesting stating point in drug discovery process

Indolesulfonamides as spindle poisons: design, synthesis and study of the antitumor mechanism of action.	***Restricted to organizers***

A. Vicente-Blázquez [1,2]*, M. González [2], R. Álvarez [2], M. Medarde [2], R. Peláez [2], F. Mollinedo [1].

(1) Laboratory of Cell Death and Cancer Therapy, Biological Research Center Margarita Salas CSIC, E-28040 Madrid, Spain.
(2) Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca, E-37007 Salamanca, Spain.

Microtubules participate in cell division, cell morphology, and vesicle transport, to name a few functions. Given that, it is not surprising that microtubules constitute a validated target in cancer chemotherapy. α,β-Tubulin dimers are the building blocks of microtubules, with seven druggable sites characterized to date.

The FDA has approved a few tubulin-binding drugs, such as paclitaxel or vinblastine. Despite their indisputable clinical success, there are some handicaps associated with these therapies, such as non-optimal pharmacokinetics, resistance mechanisms, or toxicity. In this work, we have selected the colchicine binding domain in tubulin as an antitumor target with the primary goal of obtaining novel compounds with ameliorated properties.

Herein, we present the design and synthesis of novel colchicine-site ligands and the study of their mechanism of action. Our work has yielded a series of compounds that harbor two aromatic rings restricted into a relative folded conformation by a sulfonamide bridging the rings. These new ligands exert anti-proliferative activity *in vitro* against several tumor cell lines with IC_{50} values in the submicromolar or nanomolar range. Two mechanisms may contribute to their antitumor activity. On the one hand, these compounds induce apoptotic cell death in the human tumor cell line HeLa (cervix adenocarcinoma) upon a sustained mitotic arrest. On the other hand, we have observed angiogenesis inhibition *in vitro* using the murine endothelial cell line 3B-11.

Aside from the design and synthesis, the detailed mechanism of action and the characterization of the molecular target will be presented.

Acknowledgements:
This work was funded by Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA262P18 and SA116P20) and Ministerio de Ciencia, Innovación y Universidades (SAF2017-89672-R and RTI2018-099474-BI00).

* Correspondence: avicenteblazquez@usal.es

Indolesulfonamides as spindle poisons: design, synthesis and study of the antitumor mechanism of action

A. Vicente-Blázquez [1,2], M. González [2], R. Álvarez [2], M. Medarde [2], R. Peláez [2], F. Mollinedo [1]

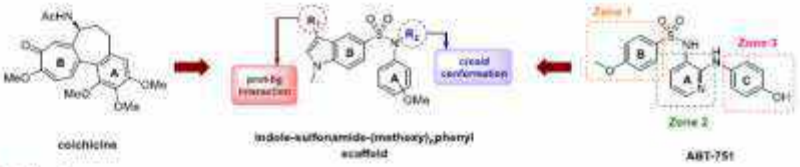
[1] Laboratory of Cell Death and Cancer Therapy, Biological Research Center CSIC (E-28040 Madrid, Spain)

[2] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca (E-37007 Salamanca, Spain)

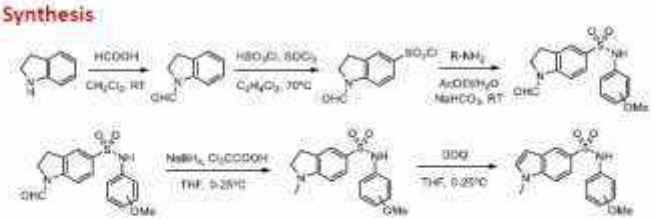
Introduction

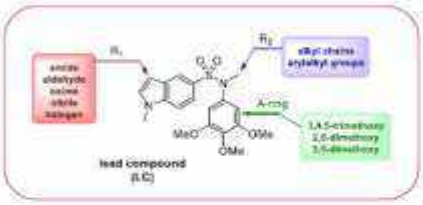
Tubulin has emerged as a successful target for cancer treatment given its key role in cell division and morphology. Despite the huge investment in the development of antitubulin therapies, none of the discovered colchicine site ligands has overcome clinical trials yet. In this work, we have accomplished the design and synthesis of a novel family of colchicine site tubulin inhibitors with the aim of improving the potency and pharmacokinetic properties of the lead compounds that bind at the same pocket (Fig. 1), and overcome resistance mechanisms. Structure-activity relationship studies after biological evaluation unraveled the *N*-methyl-trimethoxyphenyl-indole-sulfonamide (LC) as the most potent ligand. Beyond cytotoxicity evaluation, we have focused on the study of the molecular pathways beneath treatment controlling the chemotherapy response.

Fig. 1. ABT-751 binding mode at the colchicine site of tubulin. The three different binding pockets at the colchicine domain are represented in colors: the *p*-methoxyphenyl B ring of ABT-751 binds at the zone 1 (orange), the *m*-phenylenediamine moiety (A ring) is located at the zone 2 (green) and the phenolic C ring is embraced by the aminoacids at the zone 3 (pink).



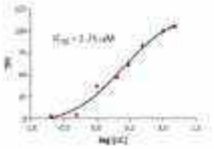
Synthesis



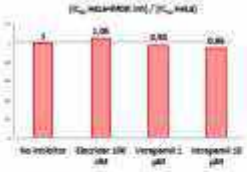


LC targets tubulin polymerization

LC was tested against isolated tubulin from calf brain, showing a dose-dependent tubulin polymerization inhibition (TPI) with an IC_{50} value of 2.75 μM, thus, confirming the data suggesting an optimal colchicine site binding and microtubule disruption (Fig. 2). A potent antiproliferative profile with nanomolar IC_{50}'s was manifested against three different tumor cell lines (HeLa, HT-29 and HL-60). The LC proved not to be a MDR family efflux pumps substrate, retaining the same *in vitro* potency when treated with MDR inhibitors.



Cell line	LC IC_{50} (nM)	CA-4 IC_{50} (nM)	ABT-751 IC_{50} (nM)
HeLa	2	3	1000
HT-29	4	4160	-
HL-60	1	2	-



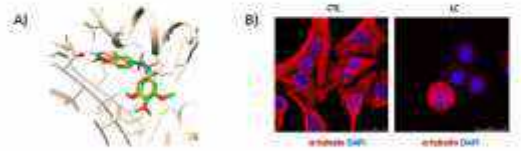
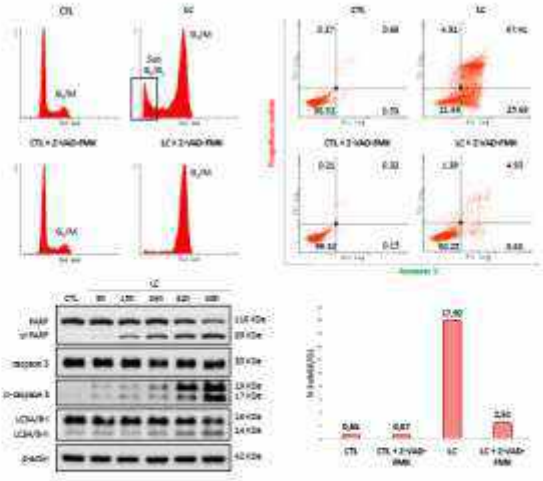


Fig. 2. A) Docking of LC bound at the colchicine site of the SLW x-ray structure. SLW corresponds to the co-crystalized CA-4 (a natural product that binds at the colchicine site) in complex with tubulin. This screenshot shows the docked pose of the LC (orange) that overlaps very well with CA-4 (green). B) Immunofluorescence images obtained by confocal microscopy after staining the nucleus in blue (DAPI) and the cytoskeleton in red. Cells completely lose the morphology after treatment, becoming round. Microtubule network is totally disrupted which is in agreement with the lead compound altering tubulin polymerization.

Apoptotic cell death following treatment

A prolonged G_2/M arrest was observed after LC treatment, followed by cell death that was exacerbated 48h post-treatment. Cell death was triggered through the caspase-mediated apoptotic pathway, as assessed by the presence of an annexin V-positive cell population which did not occur when cells were pre-treated with a pan-caspase inhibitor (Z-VAD-FMK). These results were confirmed by Western blot.



Acknowledgements

Financial support came from Consejería de Educación de la Junta de Castilla y León and FEDER funds (SA263P18 and SA118P20) and the Spanish Ministerio de Ciencia, Innovación y Universidades (RTI2018-098474-B-I00).

New nitric oxide-releasing indomethacin derivatives with 1,3-thiazolidin-4-one scaffold: synthesis, in silico and in vitro studies

Alexandru Sava[1,2*], Frederic Buron[2], Sylvain Routier[2], Ioana Vasincu[3], Maria Apotrosoaei[3], Luminita Confederat[3], Oana Dragan[3], Andreea Iacob[3], Florentina Geanina Lupascu[3] Lenuța Profire[3]

"Grigore. T. Popa" University of Medicine and Pharmacy from Iasi, Faculty of Pharmacy [1]Department of Analytical Chemistry, [3]Department of Pharmaceutical Chemistry

[2]University of Orleans, CNRS, Institut de Chimie Organique et Analytique, UMR 7311, Rue de Chartres BP 6759, F-45067 Orléans, France

Restricted to organizers

Non-steroidal anti-inflammatory drugs (NSAIDs) are principal drugs which are used to treat different conditions where inflammation is involved (1-3). They inhibit cyclo-oxygenase (COX) in a wide variety of systems. Therefore, COX inhibition has become definitively the main mechanism that is responsible for both the therapeutic and side effects of these drugs (4). Briefly, despite the clinical benefits of NSAIDs through nonselective COX inhibition or to the relative COX-1 and COX-2 inhibition, long term use of them increase the incidence of side effects (gastrointestinal, renal, allergic skin reactions, increased risk of acute coronary syndromes, bleeding) (4; 5). To improve clinical efficacy of NSAIDs, two platform strategies are currently emerging. The first one is drug combination in order to reduce the side effects of NSAIDs. The second strategy assumes that a single compound can hit multiple targets (single drug-multiple targets).

Nitric oxide releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) are a new class of anti-inflammatory drugs developed with the aim to reduce gastric-ulcerations through releasing of NO. The 1,3-thiazolidin-4-one scaffold serve as a core of many synthetic compound from a wide spectrum of biological activity such as antioxidant, antitubercular, antibacterial, anti HIV, anti-inflammatory and analgesic properties [6]. Also it was identified in natural compound like actithiazic acid (acidomycin), from *Strepomyces* strains and have act on Mycobacterium tuberculosis.

Literature gives a lot of proofs that sustain that a drug which operates on several therapeutic targets it can subtly influence the activity of many enzymes. Therefore, the multi-target pathways and increase of NO level on the inflammatory site may be a new therapeutic approach in the case of inflammatory diseases.

In the present study we present the synthesize of new improved pharmacological and safety profile drugs to find out new indomethacin nitric oxide donors (NO-IND) with significant anti-inflammatory properties. They were developed by our research group as new therapeutic multi-targets strategy, able to inhibit COX and to release NO in the gastric medium.

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* Correspondence: E-mail alexandru_sava1@yahoo.com

GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

New nitric oxide-releasing indomethacin derivatives with 1,3-thiazolidin-4-one scaffold: synthesis, in silico and in vitro studies

Alexandra Sava[1,2], Frederic Buron[2], Sylvain Routier[2], Ioana Vasincu[1], Maria Apotrosoaei[1], Luminita Confederat[1], Oana Dragan[1], Andreea Iacob[1], Florentina Geanina Lupascu[1] Lenuta Profire[1]

[1]"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Department of Analytical Chemistry, [2]Department of Pharmaceutical Chemistry
[2]University of Orléans, CNRS, Institut de Chimie Organique et Analytique, UMR 7311, Rue de Chartres BP 6759, F-45067 Orléans, France

The ancient NO-sauge depicts a significant molecule that can freely cross cell borders and allow cell death, transfer messages between cells (e.g. between neurons or endothelial and vascular smooth muscle cells) and can protect the body from pathogens as a weapon of cellular immunity. Artwork by Peter Dirnhofer

The current state of knowledge

➢Inflammation is a defense reaction (non-specific and local) of advanced living organisms to any injury that is caused by trauma, microbial toxins or harmful chemicals. In essence, the inflammation remains a crucial physiological response for the human body to any kind of aggression. This can become a chronically pathological process when acute inflammatory process fails to resolve the cause of inflammation and to re-establish the body homeostasis.[1]

➢The inhibition of the arachidonic acid cascade is one of the most used strategies for the treatment of the inflammatory diseases.

➢Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common medications in the treatment of various inflammatory through inhibition of prostaglandin H synthase (COX).

➢However, the downside of widespread and long-term usage NSAIDs is the manifestation of adverse reactions like gastrointestinal, cardiovascular, renal and hepatic toxicity[2].

➢Therefore, the modulation of the NOSs (Nitric Oxide Synthases) with the release of NO and inhibition of dual COX/LOX may be a new therapeutic approach in the case of inflammatory diseases.

➢The rationale for NO-NSAIDs development was based on the observation that NO possesses some of the same properties as PGs within the gastric mucosa.

➢NO increases mucosal blood flow, mucus release, and repair of the mucosa, whereas it inhibits neutrophil activation and adherence.

➢These effects can theoretically compensate for gastric PG reduction.

➢Coupling a NO-releasing moiety to a NSAID might deliver NO to the site of NSAID-induced damage and thus decrease gastric toxicity.

The synthesis was preceded by *molecular docking study* [3] in order to support the design of suitable chemical structures able to release NO and to have dual inhibition of COX-1 and COX-2.

The objectives

-synthesis of new heterocyclic compounds that combine indomethacin, 1,3-thiazolidin-4-one heterocycle and a NO donor group

-In vitro antioxidant and antiinflammatory evaluation

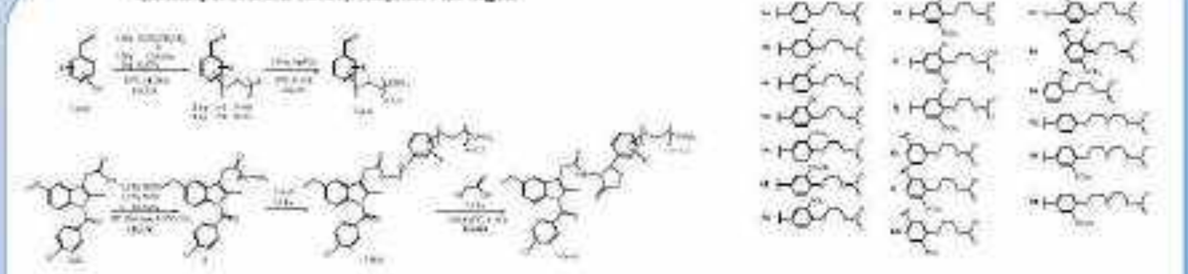
Docking of SC-558 (Cox2 selective inhibitor) and *Indomethacin* (non selective inhibitor)

Docking of new hybrid molecules

➢The 1,3-thiazolidin-4-one moiety interacts with polar side-pocket of COX2 through hydrogen binding with Arg513 and Gln364.

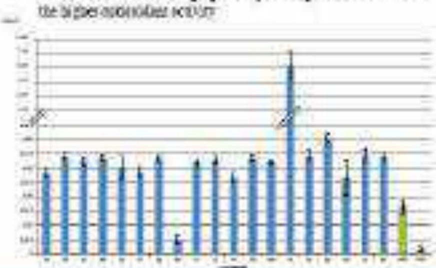
➢The substitution on phenyl moiety can increase the Cox selectivity through interaction of ligands with specific COX2 aminoacids: Val518, Ala516, Phe518, Gln192, His90.

Synthesis of new NO-IND with a 1,3-thiazolidin-4-one scaffold



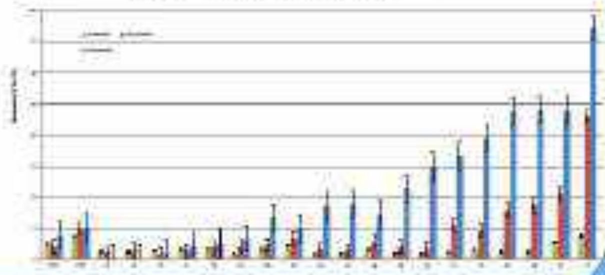
In vitro radical scavenging assay

The DPPH radical scavenging activity, the higher CEAC value means the higher antioxidant activity



Anti-inflammatory effect of the tested compounds

Effects of NO-IND on protein denaturation



Conclusions. We described herein the synthesis, molecular docking study and in vitro antioxidant/antiinflammatory effects of a novel nitric-oxide-indomethacin drug with 1,3-thiazolidin-4-one moiety.

➢The most efficient method for synthesis of final products is through use of aldehydes containing NO donor.

➢The molecular docking studies further help in understanding the various interactions between the ligands and enzyme active site and thereby help to design the novel potent inhibitors.

➢It was observed that the presence of nitro group in the ortho position of the phenyl ring (6m) exhibited the strongest antioxidant activity.

➢The presence of halide (Cl, Br) on the phenyl ring and elongation of nitrate ester spacer (6b, 6d, 6j, 6n, 6q and 6r) increase anti-inflammatory properties.

Perspectives. The research will be continued by the pharmacological studies of the compounds synthesized and checking a possible correlation between studies in vivo and in silico.

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Synthesis and biological activity of some new hydrazone metal-complexes of diclofenac

Maria Apotrosoaei[(1)*], Alin Focșa[(1)], Drăgan Maria[(2)], Ioana Vasincu[(1)], Sandra Constantin[(1)], Alexandru Sava[(1)], Andreea Iacob[(1)], Florentina Lupașcu[(1)], Cătălina Stan[(2)], Lenuța Profire[(1)]

(1) University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Iasi 700115, Romania . (2) University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Department of Drug Industry and Pharmaceutical Biotechnology, Iasi 700115, Romania

Restricted to organizers

Introduction: Diclofenac, a nonsteroidal anti-inflammatory drug of the phenylacetic acid class, is on top of the prescription. On the other hand, it is known that metals play a crucial role in life processes, being involved in cellular and subcellular functions. In addition, the studies on NSAIDs complexation with metals have established that these drugs exhibit improved anti-inflammatory/antioxidant properties in comparison to free ligands or exhibit different biological activities than the parent NSAIDs. **Material and methods:** The aim is to obtain new metal-complexes based copper, zinc, nickel and as ligand-scaffold, diclofenac derivatives. The optimization of the synthesis reaction of the new metal-complexes was performed. The complexes formed were characterized by determination of physicochemical parameters (yields, melting point), and their structure were proved, using IR and 1H-NMR analysis. The stability of the complexes were studied using UV-VIS method and differential thermal analysis. The evaluation of the antioxidant potential was also performed using in vitro methods: total antioxidant capacity and reducing power. **Results:** It was observed that the best yield of the compound bis-(2-[(E)-(2-(2,6-dichlorophenyl) acetyl-hydrazino-methyl-phenoxy)-zinc (68%) was obtained following the reaction between 2-[2-(2,6-dichloroanilino)phenyl]-N-[(E)-(2-hydroxyphenyl)-methylidenamino] acetamide (1 eq.) and $Zn(CH_3COO)_2 \cdot 2H_2O$ (1.25 eq.), using ethanol as the reaction medium, at reflux, for 4 h. Using the optimization method, another 11 complexes with hydrazone structure were synthetized. According to a comparative analysis of the results obtained for the 2-hydroxy-substituted hydrazones and the corresponding complexes, it is highlighted that 10 of the 12 synthesized complexes showed a better reducing activity than the corresponding hydrazones. **Conclusions:** The reaction of 2-hydroxy-substituted hydrazones with organic salts of some bivalent metals: $Cu(CH_3COO)_2 \cdot H_2O$, $Zn(CH_3COO)_2 \cdot 2H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, in ethanol medium, led to the obtaining of 12 complexes. The yields for obtaining the complexes were between 45% and 72%. Moreover, it was noticed that the antioxidant effect increases with concentration and it is influenced by the metal used for complexation of 2-hydroxy-substituted hydrazones.

Scientific research funded by the UMF "Grigore T Popa" Iasi, based on contract no. 10308/29.06.2020 and d by the grant of UEFISCDI, PN III Program, AUF-RO,AUF-IFA 2019-2020, contract no. 28/2019.

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(a) Spencer J, Walden B. Special focus: Metals in medicine. *Future Med Chem* 2018; 10(6):607–9.

(b) Leung CH, Lin S, Zhong HJ, Ma DL. Metal complexes as potential modulators of inflammatory and autoimmune responses. *Chem Sci* 2015; 6(2):871–84.

*** Correspondence:** apotrosoaei.maria@umfiasi.ro

Young Research Fellow Meeting 2021
(11-12 February, 2021, Paris)

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Maria Apotrosoaei(1)*, Alin Focşa(1), Drăgan Maria(2), Ioana Vasincu(1), Sandra Constantin(1), Alexandru Sava(1), Andreea Iacob(1), Florentina Lupaşcu(1), Cătălina Stan(2), Lenuţa Profire(1)

(1) University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Iasi 700115, Romania

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Introduction

It is known that metals play a crucial role in life processes, being involved in cellular and subcellular functions. In addition, the studies on NSAIDs complexation with metals have established that these drugs exhibit improved anti-inflammatory/antioxidant properties in comparison to free ligands or exhibit different biological activities than the parent NSAIDs.

Aim → To obtain new metal-complexes based copper, zinc, nickel and as ligand-scaffold diclofenac derivatives and the evaluation of the antioxidant potential of diclofenac derivatives with hydrazone structure have been performed.

Materials and methods

The complexes formed were characterized by determination of physicochemical parameters (yields, melting point), and their structure were proved, using IR analysis. The evaluation of the antioxidant potential was also performed using in vitro methods: total antioxidant capacity and reducing power.

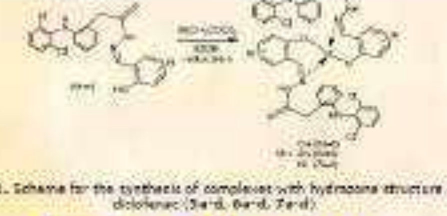

Fig 1. Scheme for the synthesis of complexes with hydrazone structure of diclofenac (5a-d, 6a-d, 7a-d)

Results and discussions

Table 1. Physico-chemical characteristics of complexes with hydrazone structure of diclofenac (5a-d, 6a-d, 7a-d)

Compound	M	R	η (%)	IR	Mp (°C)
5a	Cu^{2+}	-H	45	[illegible]	252
5b		3-OH	53	[illegible]	254
5c		4-OH	65	[illegible]	255
5d		5-OH	60	[illegible]	258
6a	Zn^{2+}	-H	56	[illegible]	285
6b		3-OH	58	[illegible]	281
6c		4-OH	72	[illegible]	279
6d		5-OH	69	[illegible]	273
7a	Ni^{2+}	-H	59	[illegible]	235
7b		3-OH	71	[illegible]	305
7c		4-OH	53	[illegible]	306
7d		5-OH	50	[illegible]	302

It was observed that the best yield of the compound bis-[2-[(E)-[2-[2-(2,6-dichlorophenyl) acetyl-hydrazono-methyl-phenoxy]-zinc (68%) was obtained following the reaction between 2-[2-(2,6-dichloroanilino)phenyl]-N-[(E)-(2-hydroxyphenyl) methylideneamino] acetamide (1 eq.) and $Zn(CH_3COO)_2 \cdot 2H_2O$ (1.25 eq.), using ethanol as the reaction medium, at reflux, for 4 h. Using the optimization method, another 11 complexes with hydrazone structure were synthetized.

Table 2. EC_{50} values (mg / mL) of antioxidant activity (reducing power) for 2-hydroxy-substituted hydrazones (4t-w) and corresponding complexes (5a-d, 6a-d, 7a-d)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
Diclofenac	0.4351 ± 0.0363	5b	0.0566 ± 0.0089	6d	0.0867 ± 0.0091
4t	0.2315 ± 0.0196	5c	0.0678 ± 0.0073	7a	0.0802 ± 0.0049
4u	0.2924 ± 0.0245	5d	0.1132 ± 0.0108	7b	0.0527 ± 0.0022
4v	0.0885 ± 0.0113	6a	0.2089 ± 0.0189	7c	0.1123 ± 0.0138
4w	0.2327 ± 0.0155	6b	0.0968 ± 0.0097	7d	0.1562 ± 0.0177
5a	0.3216 ± 0.0240	6c	0.0684 ± 0.0068	Ascorbic acid	0.0162 ± 0.0031

The most active compounds were 7b (M = Ni, R = 2,3-diOH, EC_{50} = 0.0527 ± 0.0022), 5c (M = Cu, R = 2,4-diOH, EC_{50} = 0.0678 ± 0.0073) and 6c (M = Zn, R = 2,4-diOH, EC_{50} = 0.0684 ± 0.0068) which were found to be 8.1 times (7b), 6.4 times (5c), respectively 6.3 times (6c) more active than diclofenac.

Table 3. EC_{50} values (mg / mL) of antioxidant activity (antioxidant capacity) for 2-hydroxy-substituted hydrazones (4t-w) and corresponding complexes (5a-d, 6a-d, 7a-d)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
Diclofenac	0.4999 ± 0.0182	5b	0.1156 ± 0.0188	6d	0.2279 ± 0.0288
4t	0.7212 ± 0.0188	5c	0.0893 ± 0.0086	7a	0.6843 ± 0.0236
4u	0.4157 ± 0.0157	5d	0.3461 ± 0.0197	7b	0.2099 ± 0.0112
4v	0.1989 ± 0.0134	6a	0.2845 ± 0.0182	7c	0.1566 ± 0.0185
4w	0.3865 ± 0.0195	6b	0.1352 ± 0.0185	7d	0.1513 ± 0.0199
5a	0.3397 ± 0.0131	6c	0.1885 ± 0.0173	Ascorbic acid	0.0362 ± 0.0051

From the analysis of the EC_{50} values (Table 3) we noticed the compounds were 5c (M = Cu, R = 2,4-diOH, EC_{50} = 0.0893 ± 0.0086), 5b (M = Cu, R = 2,3-diOH, EC_{50} = 0.1156 ± 0.0188) and 6b (M = Zn, R = 2,3-diOH, EC_{50} = 0.1352 ± 0.0185) which were shown to be 4.9 times (5c), 3.8 times (5b), respectively 3.2 times (6b) more active than diclofenac.

Conclusions

The reaction of 2-hydroxy-substituted hydrazones with organic salts of some bivalent metals: $Cu(CH_3COO)_2 \cdot H_2O$, $Zn(CH_3COO)_2 \cdot 2H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, in ethanol medium, led to the obtaining of 12 complexes. The yields for obtaining the complexes were between 45% and 72%. Moreover, it was noticed that the antioxidant effect increases with concentration and it is influenced by the metal used for complexation of 2-hydroxy-substituted hydrazones.

Acknowledgements
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• Leung CH, Lin S, Zhong HJ, Ma DL. Metal complexes as potential modulators of inflammatory and autoimmune responses. Chem Sci 2015; 6(2):871–84.

Silafluorene as a promising core for cell-permeant, highly bright and two-photon excitable fluorescent probes for live-cell imaging

M. Auvray [1,2], F. Bolze[3], G. Clavier[4] and F. Mahuteau-Betzer [1,2]

(1) Institut Curie, Université PSL, CNRS UMR9187, Inserm U1196, Chemistry and Modeling for the Biology of Cancer, 91400 Orsay, France
(2) Université Paris-Saclay, CNRS UMR9187, Inserm U1196, Chemistry and Modeling for the Biology of Cancer, 91400 Orsay, France
(3) Faculté de pharmacie, UMR7199, 67401 Illkirch-Graffenstaden Cedex, France
(4) PPSM, CNRS, ENS Paris-Saclay, 91190 Gif-sur-Yvette, France

Restricted to organizers

Over the past decades, fluorescent probes had become a powerful tool to study cell function from mitosis to intracellular transport as small fluorescent molecules were designed to label selectively most of organelles.[1] However, their application is limited because of unmet needs like high emission and excitation wavelengths in order to avoid degradation and allow deep penetration in tissues. As a consequence, one of the most challenging issue is to develop probes that have high emission and excitation wavelengths.

From the triphenylamine scaffold, our team developed biocompatible OFF/ON fluorescent conjugated systems with high charge transfer possessing good two-photon absorption cross-sections. [2] Here we describe the design and the synthesis a new OFF/ON silicon-based fluorescent probe. Its ability to label organelles in live cells is also studied thanks to confocal microscopy.[3]

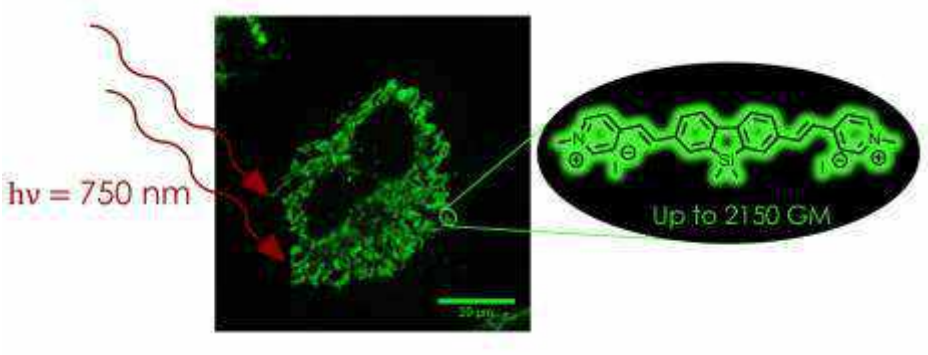

Figure 2 : Confocal microscopy imaging of live A549 cells incubated with **SiFluo-L** at 2 µM during 2h under two-photon excitation (750 nm)

Bibliographic references:

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[2] (a) B. Dumat *et al*., *J. Am. Chem. Soc*., 2013, **135** (4), 12697-12706 (b) B. Dumat *et al*., *Org. Biomol. Chem*., 2016, **14** (1), 358-370
[3] M. Auvray *et al*., *Dyes and Pigments* , **2021**, accepted

* Correspondence: marie.auvray@curie.fr

SILAFLUORENE AS A PROMISING CORE FOR CELL-PERMEANT, HIGHLY BRIGHT AND TWO-PHOTON EXCITABLE FLUORESCENT PROBES FOR LIVE-CELL IMAGING

Marie Auvray [a,b], Frédéric Bolze [c], Gilles Clavier [d] and Florence Mahuteau-Betzer [a,b]

[a] Université Paris Sud, Université Paris-Saclay, CNRS, INSERM, UMR 9187-U 1196, 91405 Orsay Cedex, France
[b] Institut Curie, PSL University, CNRS, INSERM, UMR 9187-U 1196, 91405 Orsay Cedex, France
[c] Faculté de pharmacie, UMR7199, 67401 Illkirch-Graffenstaden Cedex, France
[d] PPSM, CNRS, ENS Paris-Saclay, 91 190 Gif-sur-Yvette, France
marie.auvray@curie.fr

Introduction

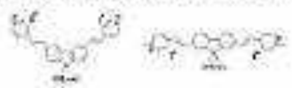
Over the past decades, fluorescent probes had become a powerful tool to study cell function from mitosis to intracellular transport as small fluorescent molecules were designed to label selectively most of organelles.[1]

However their application is limited because of some needs like high emission and excitation wavelengths in order to avoid degradation and allow deep penetration in tissues.

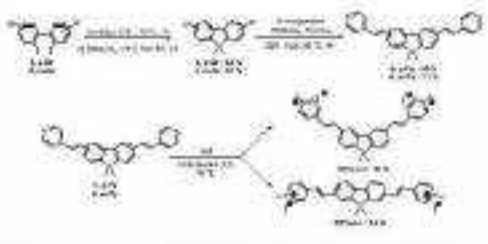
As a consequence one of the most challenging issue is to develop probes that have high emission and excitation wavelengths.

To overcome this problem, one of the solution is to use two-photon excitable fluorescent probes. Unfortunately, most of the commonly used dyes are not suitable for two photon absorption. One of the way to design such probe is to synthesize quadrupolar or octupolar structure.[2]

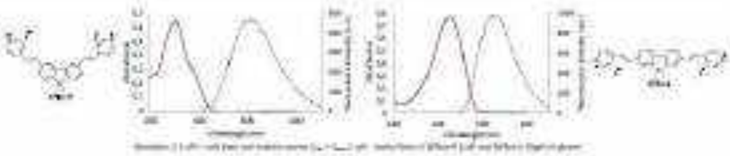
Most of the time such probes possess a high molecular weight and a poor solubility. That is why our goal was to design a water-soluble probe with a high cross-section to molecular weight ratio to make it biocompatible. We focused our work on two probes from the silafluorene family:



Synthesis



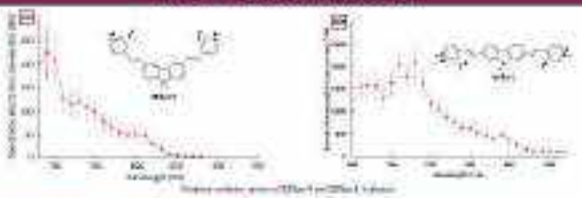
Photophysical properties



Compound	ε (L.mol⁻¹.cm⁻¹)	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (cm⁻¹)	Φ	Brightness εΦ (L.mol⁻¹.cm⁻¹)
SiFluo-V	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SiFluo-L	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Similar linear photophysical properties:
- Absorbance in the blue region
- Strong large emission centered in the green region

Non-linear absorption properties



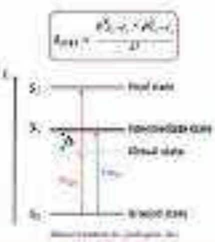
Compound	λ_{max}^{2PA} (nm)	δ (GM)	Φ	δ.Φ (GM)
SiFluo-V	[illegible]	[illegible]	[illegible]	[illegible]
SiFluo-L	[illegible]	[illegible]	[illegible]	[illegible]

SiFluo-L (δ/M.W. ↑) displays far better two-photon absorption properties than SiFluo-V

→ Could molecular modeling help us to understand?

Molecular Modeling

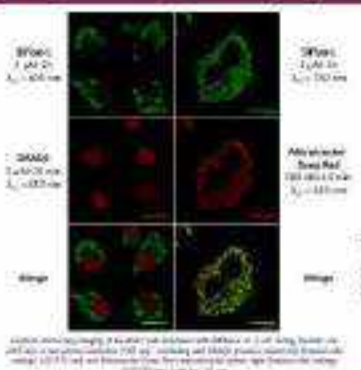
➢ For quadrupolar structure, δ_{max} can be linked to transition dipole moment through the formula:



Compound	μ_{0}-μ_{1}	μ_{1}-μ_{2}	Δ (eV)	[illegible]
SiFluo-V	3.41	[illegible]	[illegible]	[illegible]
SiFluo-L	4.03	3.34	[illegible]	[illegible]

=> Consistent with experimental observations

Confocal microscopy of SiFluo-L



High contrast with one and two-photon excitation at low power of laser

Imaging possible with [probe] = 100 nM

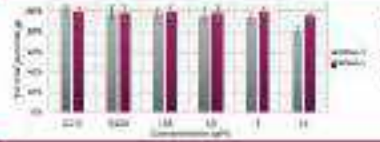
- Highly bright probe under one (405 nm) or two-photon excitation (750 nm)
- Imaging in the red window
- Mitochondrial labeling confirmed

Photostability of SiFluo-L under two-photon excitation

More than 80 % of the signal remains after [illegible] excitation

- Possibility of a long-term imaging

Cytotoxicity – MTT Assay



IC_{50} = 10 µM for both probes after 24h of incubation

- These two probes are not cytotoxic for usual concentration for live-cell imaging

Conclusion

- Highly bright probe under one (405 nm) or two-photon excitation (750 nm)
- Imaging in the red window
- Photostable: possibility of long-term imaging
- Non-cytotoxic
- Suitable for two-photon microscopy

PERSPECTIVES:
- Add other group to target organelles

- For further information:

[1] The Molecular Probes Handbook ... [illegible]
[2] [illegible]
[3] [illegible]
[4] [illegible]
[5] Auvray et al., [illegible]

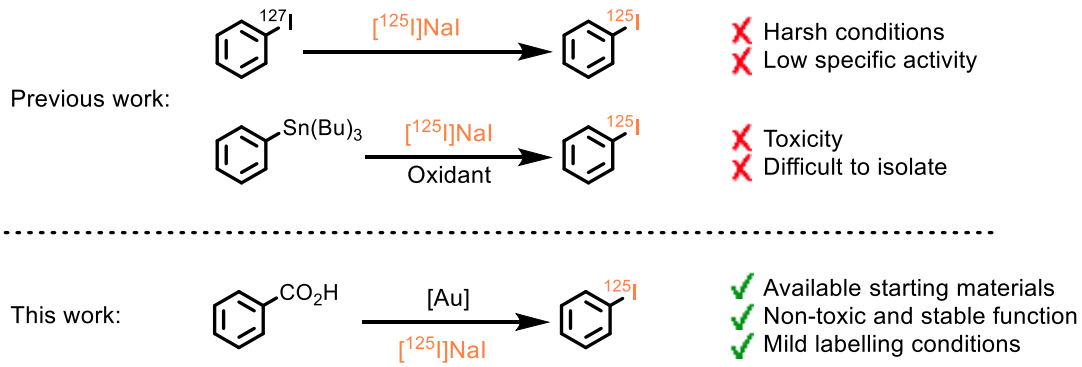
Gold(I) mediated radio-iododecarboxylation of arenes

Hugo BLOUX[(1)*], Emmanuelle DUBOST[(1)], Frédéric FABIS[(1)], Thomas CAILLY[(1)(2)(3)*]

(1) Normandie Univ, UNICAEN, Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN), 14000 CAEN.
(2) Normandie Univ, UNICAEN, IMOGERE, 14000 CAEN
(3) Department of Nuclear Medicine, CHU Côte de Nacre, 14000 CAEN

Restricted to organizers

Radiolabelling of pharmaceutical compounds has a direct impact on the discoveries in the field of life sciences and nuclear medicine. Indeed, radiolabelled compounds are, nowadays, usually used for non-invasive studies, such as medical imaging, drug development and radiotherapy. Radio-iodinated tracers allow to work in several fields of application. Four radioactive-isotopes can be used, each one with a specific application: ^{123}I and ^{124}I for SPECT and PET imaging respectively, ^{125}I for binding studies, ^{131}I for radiotherapy. Different methods are already described for radio-iodination[1] and are usually based either on isotopic exchange or on the replacement of a functional group by iodine.[2–5] Isotopic exchange requires harsh conditions and leads to a low specific activity. Among the many functional groups that can be used to perform radio-iodination, organotin derivatives are the most popular due to the obtained high specific activities and the possibility to perform iodo-destannylation in mild conditions. However, organostannane are notoriously fragile, toxic and difficult to isolate. The use of function more stable and less toxic would make the synthesis of radio-iodinated compound more attractive. The carboxylic acid function fulfils these requirements. The easy access to carboxylic acid derivatives and their low prices due to their wide presence in natural products, and common chemicals make the development of this method very attractive. We wish to describe here a gold(I) mediated radio-iododecarboxylation based on the work of *Cornella et al.,*[6] this approach could find other applications in radiolabelling with other radio-isotopes.



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(6) Cornella, J.; Rosillo-Lopez, M.; Larrosa, I. *Adv. Synth. Catal.* **2011**, *353* (8), 1359–1366.

* Correspondence: hugo.bloux@unicaen.fr ; thomas.cailly@unicaen.fr

Gold(I) mediated radio-iododecarboxylation of arenes

Hugo BLOUX[1*], Emmanuelle DUBOST[1], Frédéric FABIS[1], and Thomas CAILLY[1,2,3]

(1) Normandie Univ, UNICAEN, Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN), 14000 Caen, France
(2) Normandie Univ, UNICAEN, IMOGERE, 14000 CAEN, France
(3) Department of Nuclear Medicine, CHU Côte de Nacre, 14000 CAEN, France

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Radiolabelling of pharmaceutical compounds has a direct impact on the discoveries in the field of life sciences and nuclear medicine. Radiolabelled compounds are, nowadays, usually used for non-invasive studies, such as medical imaging, drug development and radiotherapy.

Radioactive iodine : multiples application

- ^{123}I — SPECT imaging
- ^{124}I — PET imaging
- ^{125}I — Binding studies Biology
- ^{131}I — Radiotherapy

Different methods are already described for radio-iodination[1] (Figure 1) from radiolabeling precursors. S_NAr and S_EAr show major drawbacks but metal mediated/catalyzed approaches are to date the more innovative pathways to promote radio-iodination.

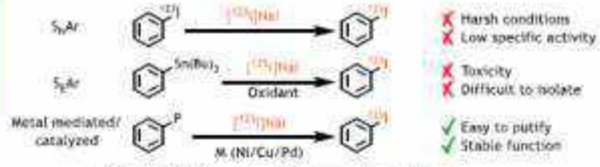

Figure 1: Previous work on radio-iodination

The use of more stable and less toxic radiolabeling precursors would make the synthesis of radio-iodinated compound more attractive. The carboxylic acid function fulfils these requirements and can be introduced in the early stage of a multistep synthesis. The easy access to carboxylic acid derivatives and their low prices due to their wide presence in natural products, and common chemicals make the development of this method very attractive.

Based on the work of Cornella and *al.*,[2] the decarboxylation of arene was mediated with gold (I) complex to give the corresponding aryl-gold complex. Radio-iodination was performed with [^{125}I]NIS, generated *in situ* from [^{125}I]NaI and NCS, in presence of TFA (Figure 2).

Scope of the reaction was realized directly from crude solutions of aryl gold(I) complexes. Overall, the obtained RadioChemical Conversions (RCC) are very good in most of the studied cases and the amount of PPh_3AuCl can be reduced to 0.05 equiv. for ortho-substitued substrates while maintaining very good RCC (see Figure 3).

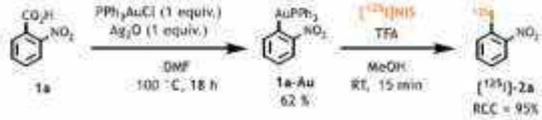

Figure 2: Radio-iodination from pure aryl-gold (I) complex

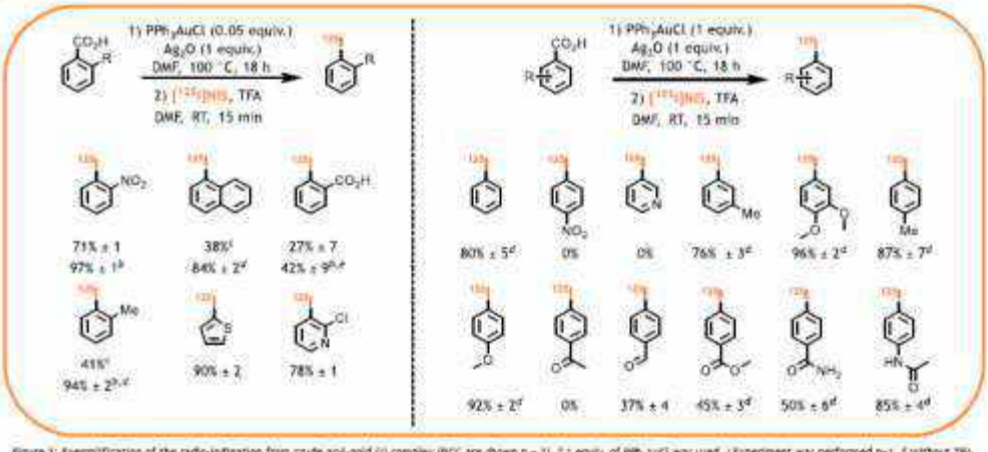

Figure 3: Exemplification of the radio-iodination from crude aryl-gold (I) complex (RCC are shown n = 2). [a] 1 equiv. of PPh_3AuCl was used, [c] Experiment was performed n=1, [d] without TFA, [e] [^{125}I]-1,2-diiodobenzene was generated with a RRC of 13 % ± 3.

According to these results, we are now turning our attention toward the labelling of molecule of interest (Figure 4). Currently, synthesis of carboxylic acid precursors of MIBG and Iniparib are achieved and the radio-iodination will be performed soon.

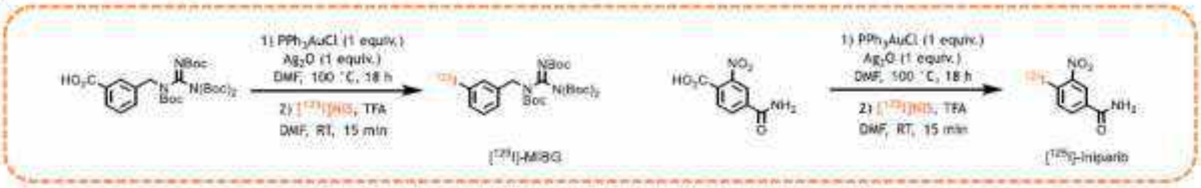

Figure 4: Perspectives, synthesis of [^{125}I]MIBG and [^{125}I]Iniparib via gold(I) radio-iododecarboxylation method.

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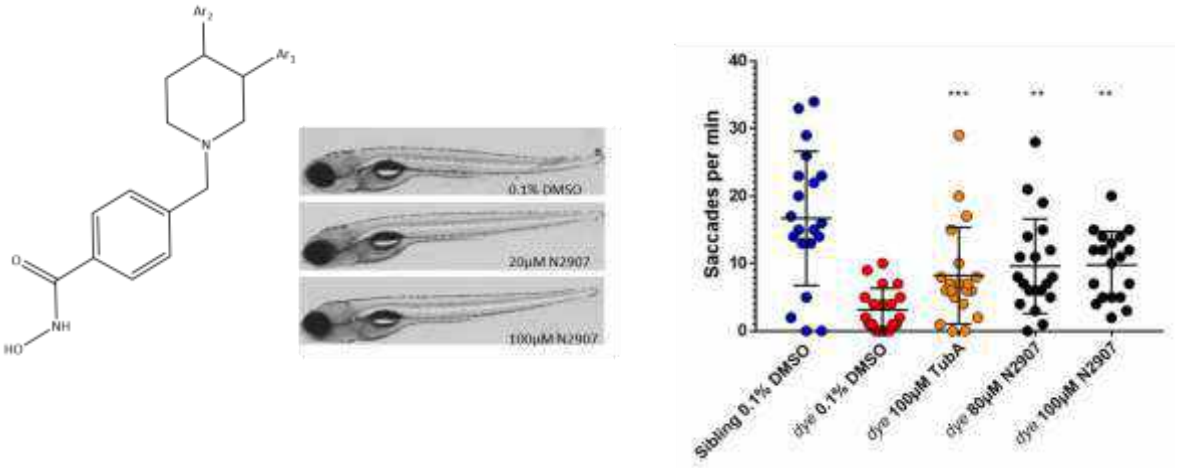
Selective HDAC6 Inhibitors for Restoring Cone Photoreceptor Vision in Zebrafish Model of Retinal Blindness	***Restricted to organizers***

Gabriele Carullo* **and Giuseppe Campiani.**

Department of Biotechnology, Chemistry and Pharmacy, Department of Excellence 2018-2022, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy.

Retinitis pigmentosa (RP) is one of the inherited retinal disorders leading to intense vision loss or blindness resulting from the degeneration of photoreceptor cells. Histone deacetylase enzymes resulted highly expressed in photoreceptors, where their inhibiton delays cone death and supports long-term cone survival. The selective HDAC6 inhibitor Tubastatin A significantly restored visual function in *atp6v0e1*$^{-/-}$, a zebrafish model of retinal blindness compared to other HDAC6 inhibitors such as Tubacin and NF2373. Starting from these observations, a small set of Tubastatin A analogues was prepared to evaluate if these molecules were able to selectively inhibit HDAC6 and restore vision in the same RP model. The best derivatives NF2899 and NF2907 showed *h*HDAC6 IC_{50} values of 97 and 20 nM respectively (SI 33 and 61 respect to HDAC1) and good physico-chemical properties in terms of LogP and solubility. The molecules occupied the same binding pocket of Tubastatin A, with the phenyl linker moiety interacting with His651, Phe680 and Phe620. NF2907 at 100µM and 80µM showed a statistically significant improvement in dye vision (80µM and 100µM p≤0.01), with a maximum tolerate dose deemed at 100 µM.



Acknowldgements: *Prof. Manfred Jung* (Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg; *Prof Breandán Kennedy* (UCD Conway Institute, UCD School of Biomolecular and Biomedical Science University College Dublin)

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* Correspondence: gabriele.carullo@unisi.it

28th YOUNG RESEARCH FELLOWS

Selective HDAC6 Inhibitors for Restoring Cone Photoreceptor Vision in Zebrafish Model of Retinal Blindness

Gabriele Carullo,[a] Luca Pozzetti,[a] Sandra Gemma,[a] Stefania Butini,[a] Justine O'Brien,[b] Alicia Gómez Sánchez,[b] Manfred Jung,[c] Breandan Kennedy,[b] Giuseppe Campiani[a]

[a]Department of Biotechnology, Chemistry and Pharmacy (Department of Excellence 2018-2022), University of Siena, Via Aldo Moro 2, Siena, Italy [b]UCD Conway Institute, University College Dublin, Dublin, Ireland, UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland. [c]Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg, Albertstraße 25, 79104, Freiburg, Germany
gabriele.carullo@unisi.it

Inherited retinal disorders

Stargardt disease
Xelinked retinoschisis
achromatopsia
Choroideremia
Leber congenital amaurosis

Retinitis pigmentosa

Human retina : a plethora of biological targets

CAP — LINKER — ZBG

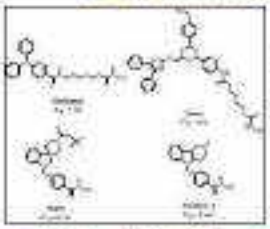
HDAC6 inhibitor: typical structure

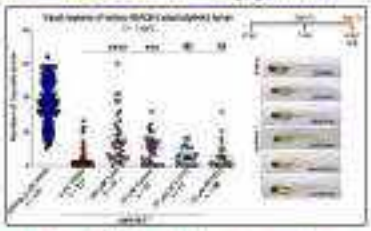
HDAC6 inhibition:

- promotes cell survival after oxidative stress by upregulating HSP70 and HSP25 proteins
- upregulates regulatory protein peroxiredoxin 1, which exerts protective effects in photoreceptors
- recovers visual function and retinal morphology
- reduce oxidative stress

Design of new HDAC6 inhibitors

HDAC6 inhibitors in zebrafish model of retinitis pigmentosa

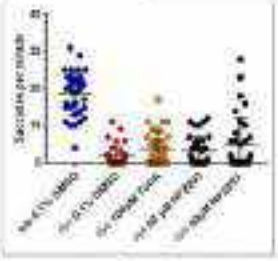


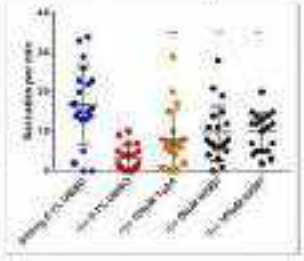


Efficacy of NF2893 and NF2907 in zebrafish model of retinitis pigmentosa

Efficacy test on 5 dpf *dye* larvae

Cpd	IC_{50} hHDAC3 (μM)	IC_{50} hHDAC6 (nM)	Selectivity index
NF2893	1.36 ± 0.6	47 ± 7	31
NF2898	1.70 ± 0.2	228 ± 28	8
NF2908	5.21 ± 0.7	186 ± 40	28
NF2907	3.22 ± 0.39	20 ± 1	61





NF2893: in the efficacy test on dye model, vision was slightly restored at 80μM ($p^*=0.0463$). There was no significative differences at 60 μM.

NF2907: at 100μM and 80μM a statistically significant improvement in dye vision was observed (80μM and 100μM $p≤0.01$)

HIGHLIGHTS

HDAC6 is an epigenetic target useful to treat retinitis pigmentosa
The synthesized compounds are promising, water-soluble HDAC6 inhibitors with good efficacy in retinitis pigmentosa model
Further studies are necessary to investigate new and more potent chemical entities

Chitosan-based polymeric systems with applications in diabetes mellitus treatment

Luminita Confederat [1]*, Cristina Tuchiluș [1], Iustina Condurache [2], Maria Drăgan [3], Andreea Iacob [3], Ioana Vasincu [3], Florentina Lupașcu [3], Maria Apotrosoaei [3], Alexandru Sava [3], Lenuța Profire [3].

(1) Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi 700115, Romania.
(2) Faculty of Medical Bioengineering, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi 700115, Romania.
(3) Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi 700115, Romania,

Restricted to organizers

Objectives: Recent research activities are focused on the use of different drug delivery systems based on natural polymers in order to improve the pharmacokinetic and pharmacological profile of some drugs. Chitosan is a wide-spread natural polymer, being accessible, biocompatible, without toxicity and low immunogenicity. In addition to this, chitosan presents some important pharmacological effects beneficial for the management of diabetes mellitus including hypoglycemic, cholesterol-lowering, antioxidant and favorable effects in reducing obesity. The aim of this study was the development and biological evaluation of some polymeric systems containing chitosan, gliclazide and lipoic acid. **Material and Method:** The chitosan-based systems were prepared as microparticles, being obtained by inotropic gelation, using pentasodium tripolyphosphate (TPP) as cross-linking agent. Medium molecular weight chitosan 1.5% was used, the concentration of cross-linking agent was 2% and the chitosan: gliclazide: lipoic acid ratio was 1: 0.5: 0.5. The microparticles obtained were characterized in terms of size, structure and morphology using IR Spectroscopy and Scanning Electronic Microscopy (SEM). The percentage of drug loading and the release of the drugs from the polymeric matrix was determined using a validated HPLC method. The systems obtained were evaluated *in* vivo on diabetic Wistar rats. After the induction of diabetes, the microparticles were administered orally for 30 days. During the experiment and at the end of it, it was evaluated the influence of the systems developed on the blood glucose and glycosylated hemoglobin values, body weight, food and water consumption. **Results:** The size of the microparticles obtained ranged between 732.1 and 790.42 µm. The IR analyses proved the drug encapsulation and the SEM analyses showed the surface morphology. The drug loading percentage was 33.91% for gliclazide and 42.53% for lipoic acid. The polymeric systems developed proved a favourable influence on the glycaemic profile, reducing significantly the blood glucose and glycosylated hemoglobin values. Also, the developed microparticles showed the benefit on body weight and food consumption. **Conclusions:** The developed chitosan-gliclazid-lipoic acid systems present all the theoretical premises to act as a multi-target treatment of diabetes mellitus.

Bibliographic references:

[(a)] Ahsan SM, Thomas M, Reddy KK et al. Chitosan as biomaterial in drug delivery and tissue engineering. Int J Biol Macromolec 2018 ; 110 :97-109.

[(b)] Guan G, Azad AK, Lin Y et al. Biological effects and applications of chitosan and chito-oligosaccharides. Front Physiol 2019 ; 10 : 1-10.

* Correspondence: luminita.confederat@yahoo.com

Chitosan-based polymeric systems with applications in diabetes mellitus treatment

Luminita Confederat (1)*, Cristina Tuchilus (1), Maria Drăgan (2), Kristina Condurache (3), Andreea Iacob (2), Ioana Vasincu (2), Florentina Lupascu (2), Maria Apotrosoaei (2), Alexandru Sava (2), Lenuta Profire (2)

Introduction

- Recent research activities are focused on the use of different drug delivery systems based on natural polymers in order to improve the pharmacokinetic and pharmacological profile of some drugs.
- Chitosan is a wide-spread natural polymer, being accessible, biocompatible, without toxicity and low immunogenicity.
- In addition to this, chitosan presents some important pharmacological effects beneficial for the management of diabetes mellitus including hypoglycemic, cholesterol-lowering, antioxidant and favorable effects in reducing obesity.

Aim — The aim of this study was the development and biological evaluation of some polymeric systems containing chitosan, gliclazide and lipoic acid.

Materials and methods

- The chitosan-based systems were prepared as microparticles, being obtained by inotropic gelation, using pentasodium tripolyphosphate (TPP) as cross-linking agent.
- Medium molecular weight chitosan 1.5% was used, the concentration of cross-linking agent was 2% and the chitosan: gliclazide: lipoic acid rabo was 1: 0.5: 0.5.
- The microparticles obtained were characterized in terms of size, structure and morphology using IR Spectroscopy and Scanning Electronic Microscopy (SEM).
- The percentage of drug loading and the release of the drugs from the polymeric matrix was determined using a validated HPLC method.
- The systems obtained were evaluated in vivo on diabetic Wistar rats. After the induction of diabetes, the microparticles were administered orally for 30 days. During the experiment and at the end of it, it was evaluated the influence of the systems developed on the blood glucose values, glycosylated hemoglobin and body weight.

Conclusions

- The polymeric system developed proved a favourable influence on the glycaemic profile, reducing significantly the blood glucose and glycosylated hemoglobin values.
- The developed chitosan-gliclazid-lipoic acid systems present all the theoretical premises to act as a multi-target treatment of diabetes mellitus.

Results and discussions

- The size of the microparticles obtained ranged between 732.1 and 790.42 µm.
- The drug loading percentage was 33.91% for gliclazide and 42.53% for lipoic acid.

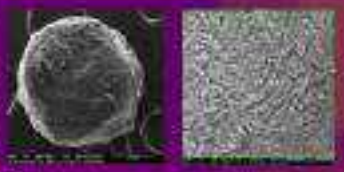


Figure 1. SEM images of the developed microparticles

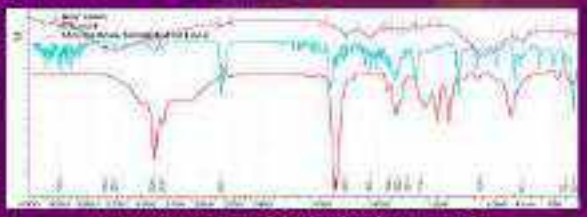


Figure 2. IR spectra of the developed microparticles

Figure 3. Glycosylated hemoglobin in diabetic rats: Lot 9: Gliclazide - Lipoic acid microparticles; Lot 11: Diabetes control; Lot 12: Healthy control

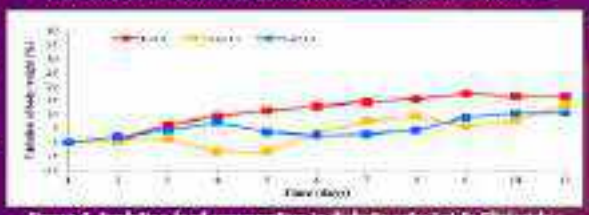


Figure 4. Evolution food consumption in diabetic rats: Lot 9: Gliclazide - Lipoic acid microparticles; Lot 11: Diabetes control; Lot 12: Healthy control

Acknowledgments

This research was financially supported by UNESCO - L'Oréal through the fellowship „For Women in Science".

References:

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[2] Ozkan G, Asad AK, Lin Y et al. Biological effects and applications of chitosan and chito-oligosaccharides. Front Physiol 2019 ; 10 : 1-10.

Heteroaromatic analogues of combretastatins as anticancer agents

C. Sanz[1, 2], S. del Mazo [1, 2, 3], P. Puebla[1, 2, 3], M. González[1, 2, 3], S. Ramos[1, 2], M. Marín[1, 2], R. Fuentes[1, 2] R. Álvarez[1, 2, 3], M. Medarde[1, 2, 3], L. Gallego-Yerga[1, 2, 3], R. Peláez[1, 2, 3]

[1]Laboratorio de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, España
[2]Instituto de Investigaciones Biomédicas de Salamanca (IBSAL), Hospital Universitario de Salamanca, Hospital Virgen de la Vega, 10ª Planta, Paseo de San Vicente, 58-182, 37007 Salamanca, España

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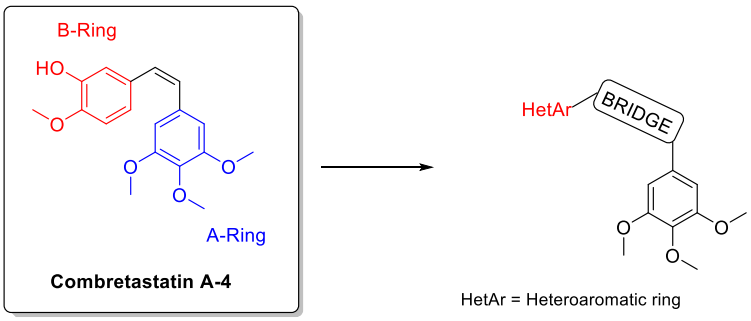
Cell division process is regulated by a series of control mechanisms that, when they are altered, produce an uncontrolled proliferation of cells, leading to cancer. To avoid this, attempts are made to develop drugs capable of preventing mitosis, a process in which microtubules are essential (protein polymers formed by 13 linear protofilaments, composed in turn by heterodimers of α/β tubulin). As dynamic structures, microtubules continually polymerize and depolymerize. Drugs that use tubulin as a target alter these dynamics and are effective therapies for the treatment of tumours.
Combretastatin A-4 (CA-4) is a natural product that affects microtubule dynamics by binding to the colchicine site of tubulin and shows potent cytotoxicity. However, its main disadvantages are the low solubility in aqueous medium.[1]
In this work B-ring of CA-4 has been replaced by heteroatomic rings, which has proven to lead to potent derivatives[2, 3,4] and the olefinic bridge has been replaced to improve aqueous solubility.



Synthesis of compounds and its biological evaluation will be presented in this work.

Acknowledgements:

Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA262P18 and SA0116P20).
Ministerio de Ciencia, Innovación y Universidades, Proyectos I+D+i «Retos Investigación» del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad RTI2018-099474-B-I00.
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* Correspondence: cristina.scuesta@usal.es

HETEROAROMATIC ANALOGUES OF COMBRETASTATINS AS ANTICANCER AGENTS

C. Sanz[1,2], S. del Mazo[1,2], P. Puebla[1,2], M. González[1,2], S. Ramos[1,2], M. Marín[1,2], R. Fuentes[1,2] R. Álvarez[1,2], M. Medarde[1,2], L. Gallego-Yerga[1,2], R. Peláez[1,2]
[1]Laboratorio de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, España
[2]Instituto de Investigación Biomédica de Salamanca (IBSAL), Hospital Universitario de Salamanca, Hospital Virgen de la Vega, 10ª Planta, Paseo de San Vicente, 58-182, 37007 Salamanca, España
Email: [illegible]

INTRODUCTION AND OBJETIVES

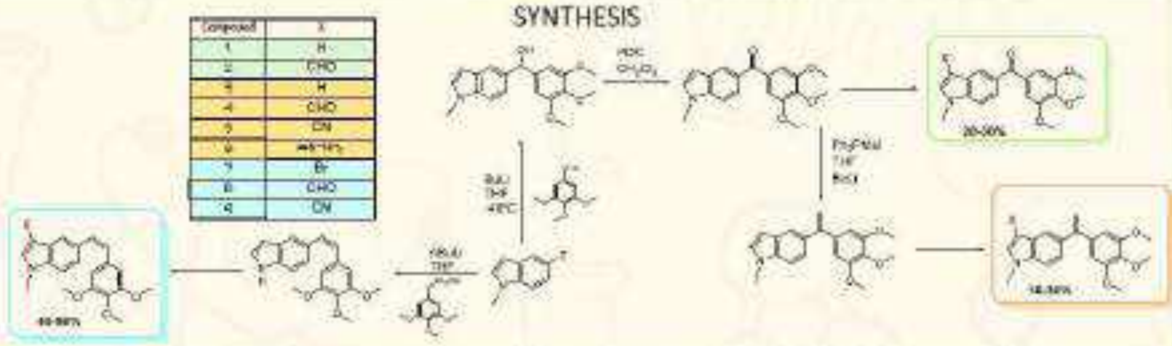
The process of cell division is regulated by a series of control mechanisms that could be altered, producing an uncontrolled proliferation of cells, leading to cancer. To avoid this, attempts are made to develop drugs capable of preventing mitosis, a process in which microtubules are essential. They are protein polymers formed by 13 linear protofilaments, composed in turn by heterodimers of α/β-tubulin. As a dynamic structures, they are constantly polymerizing and depolymerizing. Considering that, drugs that use tubulin as a target are effective therapies for the treatment of tumours.

Combretastatin A-4 (CA-4) is a natural product that affects microtubule dynamics by binding to the colchicine site of tubulin and shows potent cytotoxicity. However, its main disadvantages are the low solubility in aqueous media.[1]

In this work B-ring of CA-4 has been replaced by heteroatomic rings, which has proven to lead to potent derivatives[2,3,4] and the olefinic bridge has been replaced to improve aqueous solubility.

SYNTHESIS

Compound	X
1	H
2	CHO
3	H
4	CHO
5	CN
6	[illegible]
7	Br
8	CHO
9	CN



BIOLOGICAL EVALUATION

Cytotoxicity against HeLa cancer cell line of the new synthesized compounds was determined and compared to CA-4

As we can see in the graph, compound 9 is the most cytotoxic, even more than CA-4. This compound has a cis-ethylene bridge, and a nitrile group at indole 3-position. In addition, compounds 3, 5 and 8 display similar potency to CA-4. It is observed that olefins have the best activity profile and, within the substituents at indole 3 position, the best option seems to be a nitrile group. While for combretastatin family introduction of substituents at indole 3-position lead to a cytotoxicity increase, for the other three families (phenstatins, isocombretastatins and amides) the most active compound is that with the unsbstitued indole at 3 position.

Compound	[illegible] (µM)
CA-4	3
1	1.6
2	[illegible]
3	0.7
4	0.9
5	1.5
7	2.5
8	0.9
9	0.5

Tubulin polymerization inhibition (TPI) assays have confirmed that cytotoxic effect is due to interaction of the analogues with the protein avoiding microtubule formation.

CONCLUSIONS

The most active compounds belong to families with an olefin in the bridge: compounds 3, 5 and 8 are as potent as CA-4, while compound 9 is even more potent. Regarding indole substituents, introduction of a nitrile group has proven to be the best option.

It is observed that bridge changes modify the spatial disposition of the aromatic rings, which implies different orientation of ring substituents.

The comparation of the TPI and cytotoxic IC50s show a correspondence, which implies that the mechanism of action involves interaction with tubulin and, therefore, inhibition of cell division.

Acknowledgements:
Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA030U16 and SA262P18).
Ministerio de Ciencia, Innovación y Universidades. Proyectos I+D+i Retos Investigación del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad RTI2018-[illegible]
Fundación Memoria D. Samuel Solórzano Barruso FS/[illegible]-2018

UNIVERSIDAD DE SALAMANCA

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(4) Álvarez, R.; Puebla, P.; [illegible] Peláez, R. Endowing Indole-Based Tubulin Inhibitors with an Anchor for Derivatization: Highly Potent 3-Substituted Indolephenstatins and Indoleisocombretastatins. J. Med. Chem. 2018, [illegible]

Anti-angiogenic, anti-inflammatory, and anti-edematous effects of mineralocorticoid receptor antagonism in a rat model of corneal neovascularization

Daniela Rodrigues-Braz (1)*, Clemence Bonnet (2), Min Zhao (1), Francine Behar-Cohen (1,3).

(1) Centre de Recherches des Cordeliers, Sorbonne Université, Inserm, Université de Paris, UMRS1138, Team 17, Paris, France
(2) Stein Eye Institute, Los Angeles, California, United States
(3) Ophtalmopole, AP-HP, Cochin Hospital, Paris, France

Restricted to organizers

Purpose: Corneal neovascularization (CNV) is a major cause of blindness worldwide [1]. Topical steroids are widely used to treat CNV, but the results are highly variable. Glucocorticoids act through binding to both glucocorticoid (GR) and mineralocorticoid receptors (MR). MR overactivation contributes to retinal and choroidal neovascularization [2,3], and endothelial MR invalidation reduces CNV in mice. We thus aimed to evaluate the pharmacological effects of MR antagonism in CNV.
Methods: CNV was induced in one eye of Lewis rats by a 360° circumstance total corneal de-epithelialization and limbal scratching. Rats were injected subcutaneously with MR antagonist (MRA) spironolactone 25mg/kg/day or vehicle for 14 days. Corneal morphology and thickness were assessed *in vivo* at day 3, 7 and 14 using Micron III optical coherence tomography (OCT). Corneal re-epithelialization was evaluated by fluorescein staining under slit lamp at day 3 and 7. Fluorescein (FA) and indocyanine green angiographies (ICG) were performed at day 14 to evaluate the surface of CNV. Rats were sacrificed at day 16 and eyes removed for immunostaining of ED1, IBA1 and GSI-B4. Peripheral cornea and tissues adjacent to limbus were also dissected at day 3 and day 7 for quantitative PCR and transcriptomic analysis. A more specific MRA eplerenone (200mg/kg/day in chew for 14 days) was also used to confirm the effect of MR antagonism.
Results: Spironolactone significantly reduced the CNV and corneal thickness compared to vehicle. There is no difference in corneal re-epithelialization between spironolactone and vehicle. Spironolactone reduced infiltration of ED1 and IBA1 positive inflammatory cells and decreased the isolectin-positive neovascular surface in the cornea. The transcriptomic signatures showed 212 differentially expressed genes (26 up-regulated and 186 down-regulated) involved in corneal wound healing and differentiation, infectious responses, inflammatory and immune responses, myogenesis and hypoxia. The qPCR showed an up-regulation of GR in spironolactone treated group tilting the GR/MR balance in favor of GR pathway. Eplerenone confirmed the anti-angiogenic effect of MRA.
Conclusions: MRA is anti-angiogenic, anti-inflammatory, and anti-edematous in rat CNV. The potential additive effect of MRA and glucocorticoids on CNV will be further tested.

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* Correspondence: daniela.rodrigues.braz@gmail.com

Anti-angiogenic, anti-inflammatory, and anti-edematous effects of mineralocorticoid receptor antagonism in a rat model of corneal neovascularization

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[1] *Centre de Recherches des Cordeliers, Sorbonne Université, Inserm, Université de Paris, UMR51138, Team 17, Paris, France* ; [2] *Stein Eye Institute, Los Angeles, California, United States* ; [3] *Ophtalmopole, AP-HP, Cochin Hospital, Paris, France.*

Introduction

Corneal neovascularization (CNV) is a leading cause of blindness worldwide (1). The incidence of CNV in the United States is estimated at 1.4 million patients per year (2). Intraocular corticosteroids, a family of potent anti-inflammatory and vasoconstrictor drugs, are the most widely used pharmacological treatment for CNV but results are highly variable. Glucocorticoids act through binding to both glucocorticoid (GR) and mineralocorticoid (MR) receptors. MR overactivation contributes to retinal and choroidal neovascularization (3,4), and disabling endothelial MR reduces CNV in mice. Our hypothesis is that MR pathway over-expression contributes to the pathogenesis of CNV. We therefore assessed the pharmacological effects of MR antagonism in CNV to open novel targeted therapeutic options.

Materials and Methods

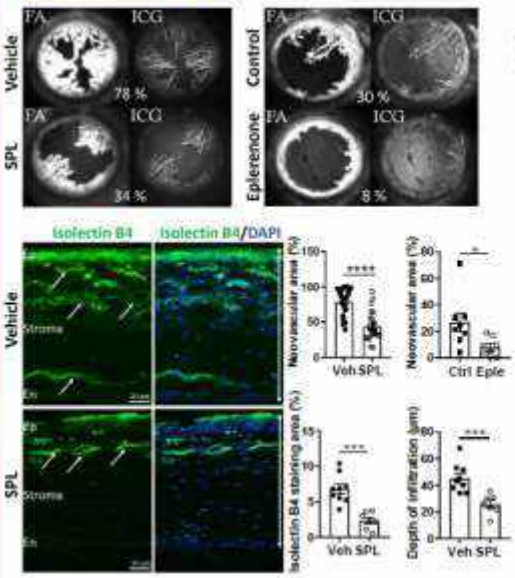
Model of corneal neovascularization: Removal of the corneal and limbal epithelium

Treatments:
- Subcutaneous **Spironolactone** (25mg/kg/day), **Vehicle** (90% olive oil and 10% DMSO)
- **Eplerenone** (per os 200mg/kg/day),
Control (normal chow)

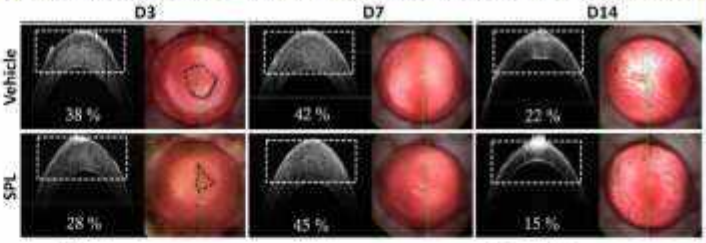
Analysis:
- Corneal thickness
- Corneal ulcer
- Quantitative PCR
- Corneal neovessels
- Immunohistochemistry

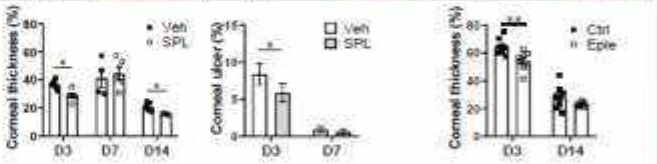
Results

1. Mineralocorticoid receptor antagonism has an anti-angiogenic effect

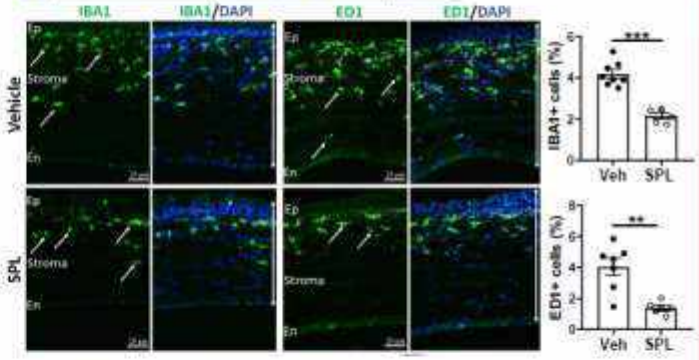


2. Spironolactone reduces corneal thickness and promotes re-epithelialization

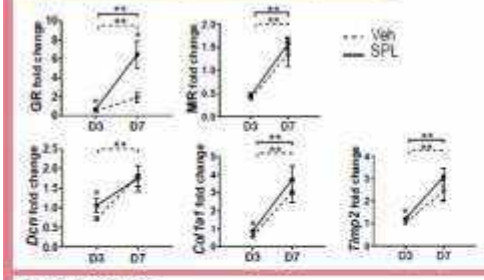




3. Spironolactone has an anti-inflammatory effect



4. Spironolactone promotes GR pathway



FA: fluorescein angiography, ICG: indocyanine green, SPL: spironolactone, Veh : vehicle, Eple: eplerenone, Ctrl: control, Ep: epithelium, En: endothelium, D: day, *Dcn*: decorin, *Col1a1*: collagen type 1 alpha 1, *Timp2*: tissue inhibitor of metalloproteinases 2

Wilcoxon-Mann-Whitney test : * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$

Conclusions

The mineralocorticoid receptor antagonism (MRA) significantly reduced the infiltration of neovessels and inflammatory cells into the cornea, decreased corneal thickness and improved re-epithelialization. Spironolactone upregulated the expression of *Dcn*, *Col1a1*, *Timp2* and particularly GR. The potential additive effect of MRA and glucocorticoids on CNV will be further tested. Treatment with eplerenone, a more specific mineralocorticoid receptor antagonist, confirmed the anti-angiogenic, anti-inflammatory, and anti-edematous effects of MR antagonism.

References

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(4) Zhao, M. et al. Nat. Commun. 10, 569 (2019).

Contact information

BIOLOGICAL EVALUATION OF AZETIDINE-2-ONE DERIVATIVES 2 OF FERULIC ACID AS POTENT ANTI-INFLAMMATORY AGENTS

Maria Drăgan[(1)]*, Andreea Iacob[(1)], Oana Dragostin[(2)], Luminiţa Confederat[(1)], Ioana Vasincu[(1)], Oana Ionescu[(1)], Maria Apotrosoaei[(1)], Florentina Lupascu[(1)], Alin Focşa[(1)], Alexandru Sava[(1)], Lenuţa Profire[(1)], Cătălina Stan[(1)]

(1) University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Iasi 700115, Romania
(2) University of Medicine and Pharmacy "Dunărea de Jos", Faculty of Pharmacy, Galaţi, 800008, Romania

Restricted to organizers

The study objective. The purpose of this study was to evaluate new azetidine-2-one derivatives of ferulic acid; the in vivo acute toxicity of azetidine-2-one derivatives of ferulic acid on Swiss white mice was investigated. Based on the results obtained, it can be stated that the studied derivatives fall into the category of compounds with moderate toxicity. **Materials and methods:** The in vivo anti-inflammatory potential of these derivatives was evaluated on a model of acute inflammation induced by carrageenan in rats and on chronic inflammation model induced on rats using the granuloma test. **Results**: In the acute inflammation model, all the studied compounds had a maximum anti-inflammatory effect, 24 hours after administration, which suggests that these compounds may be classified, from a pharmacokinetic point of view, in the category of long-acting compounds. The most active compound in the series was found to be compound **6b**. In the case of the chronic inflammation model it was observed that both the studied compounds (6a-f) and the reference substances (diclofenac sodium, indomethacin) had a more intense effect of inhibiting the proliferative component, respectively of granulation tissue formation, than on the transudative component of chronic inflammation. The most important inhibitory effect of the transudative process was recorded for compound **6b**. Also, investigation of liver function was performed by determining serum levels of liver enzymes (AST, ALT, LDH) and bilirubin (total and direct). The results showed that in the series of azetidin-2-one derivatives, the liver enzyme concentration values were close to those recorded for the reference anti-inflammatories (diclofenac sodium and indomethacin) and slightly higher compared to the values for the healthy control group. At the end of the experiment, the animals were euthanized, taking fragments of liver, lung and kidney tissue from all groups in the study. These were processed in order to perform the histopathological examination, not noticing major changes in the groups treated with azetidine 2-one derivatives compared to the healthy groups. **Keywords:** azetidine-2-one derivatives, ferulic acid, acute inflammation, chronic inflammation, biochemical parameters, histopatological study.

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* Correspondence: mwolszleger @yahoo.com

XXVIIIth Young Research Fellow Meeting, Paris France, 2021

BIOLOGICAL EVALUATION OF AZETIDINE-2-ONE DERIVATIVES 2 OF FERULIC ACID AS POTENT ANTI-INFLAMMATORY AGENTS

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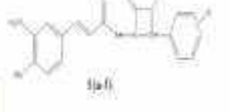
(1) University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Iasi 700115,Romania
(2)University of Medicine and Pharmacy "Dunărea de Jos", Faculty of Pharmacy, , Galați, Romania
*Corespondence: mwdulzeger@yahoo.com

INTRODUCTION

The purpose of this study was to evaluate the in vivo biological potential of new azetidine-2-one derivatives of ferulic acid (6a-f). First, the in vivo acute toxicity of azetidine-2-one derivatives of ferulic acid on Swiss white mice was investigated and, based on the obtained results, it can be stated that the studied derivatives belong to compounds with moderate toxicity. The in vivo anti-inflammatory potential of these derivatives was determined in a model of acute inflammation induced by carrageenan in rats and in a chronic inflammation model induced in rats using the granuloma test. In the acute inflammation model, all the studied compounds had a maximum anti-inflammatory effect 24 h after administration, which suggests that these compounds may be classified, from a pharmacokinetic point of view, in the category of long-acting compounds. The most active compound in the series was found to be compound 6b. In the case of the chronic inflammation model, it was observed that the studied compounds (6a-f) reduced the formation of granulation tissue compared to the control group, having an intense effect of inhibiting the proliferative component. The most important inhibitory effect of inhibiting the proliferative component was recorded for compound 6b. Additionally, the investigation of liver function was performed by determining the serum levels of liver enzymes aspartate transaminase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and bilirubin (total and direct). The results showed that, in the series of azetidin-2-one derivatives, the liver enzymes concentration values were close to those recorded for the reference anti-inflammatories (diclofenac sodium and indomethacin) and slightly higher compared to the values for the healthy control group. At the end of the experiment, the animals were euthanized and fragments of liver, lung, and kidney tissue were taken from all groups in the study. These were processed for histopathological examination, and we noticed no major changes in the groups treated with the azetidine 2-one derivatives of ferulic acid compared to the healthy groups.

MATERIALS AND METHODS



5(a-f)

Table 1
Azetidin-2-one derivatives of ferulic acid (5a-f)

Compound	R	Compound	R
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

Determination of Acute Toxicity

Ferulic acid derivatives were evaluated in vivo, determining the lethal dose 50 (LD50) on Swiss white mice [9,10,11]. The study was carried out in accordance with the current guidelines on the ethics and ethics of the study on laboratory animals (Law no. 206/27 May 2004, Eu/2010/63-CEE/609/ EEC) and with the opinion of the commission of 07.01.2014 of the Research Ethics of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi for acute oral toxicity tests.

Table 2. Lethal dose 50 (LD50 in mg/kg body) for azetidine-2-one derivatives.

Compound	R	LD50 (mg/kg body)	Compound	R	LD50 (mg/kg body)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ferulic acid	[illegible]				

From the analysis of the obtained results, presented in Table 2, it can be appreciated that all the studied derivatives can be classified in the category of substances with moderate toxicity, having values of LD50 in the range of 500-5000 mg/kg body.

Acute inflammation model induced with carrageenan in rats

The study of the anti-inflammatory effect on a model of acute inflammation was performed on white, Wistar breed, adult, male rats weighing between 140 and 220 g, which were raised under identical laboratory conditions and acclimated for 7 d before the start of the experiment under conditions of a relatively constant environment: temperature 23 ± 2 °C, humidity 40-60%, cycle of 12 h light/dark. Then, 18 h before the start of the experiment, the animals were deprived of food, receiving only water ad libitum. All the chemicals, Tween 80, ferulic acid, diclofenac sodium, indomethacin, carrageenan were purchased from Sigma-Aldrich Company, Taufkirchen, Germany. Acute inflammatory edema was induced by carrageenan, according to the working protocols described in the literature with some modifications [12,13]. After weighing, the rats were divided into groups of 6 rats by body weight. The test compounds, azetidine 2-one derivatives of ferulic acid (6a-f) were administered by gavage in 1/10 doses of LD50 by dispersion in Tween 80. The study also included a group considered positive control, treated with ferulic acid, two reference groups, treated with diclofenac sodium (5 mg/kg body) and indomethacin (1.5 mg/kg body), and a control group in which the animals received only the vehicle used for the administration of the substances to be studied, Tween 80 (0.5 mL/100 g body) [14,15]. According to the working protocol described in the literature, before the administration of the compounds, the volume of the left hind paw of the rat was determined using the LE 7500 digital plethysmometer (Panlab, Barcelona, Spain) (Figure 1). Edema was induced by the intra-implantation, in the left hind paw of 0.2 mL of 1% λ-carrageenan suspension in physiological serum (sodium chloride 0.9 mg/mL). Immediately after the carrageenan injection, the compounds to be studied were gavaged, after which the volume of the left hind paw was measured at 2, 4, 6, and 24 h after administration.

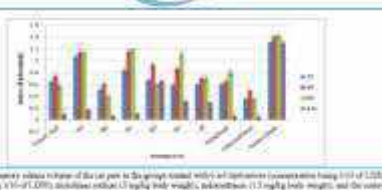


Figure 1. [illegible]

Model of chronic inflammation induced in rats—granuloma test

The study of the anti-inflammatory effect of the azetidin-2-one derivatives of ferulic acid in a chronic inflammation model was performed using the granuloma test [16,17,18,19]. Work was performed on lots of 6 rats, Wistar breed white, weighing between 140 and 220 g, raised under identical laboratory conditions, and acclimated for 7 d before the start of the experiment under relatively constant environmental conditions: temperature 21 ± 2°C, 40-60% humidity, 12 h light/dark cycle. Eighteen hours before the beginning of the experiment, the animals were deprived of food, with the water kept ad libitum. All the chemicals, ketamine, Tween 80, ferulic acid, diclofenac sodium, indomethacin, were purchased from Sigma-Aldrich Company, Taufkirchen, Germany. The rats were anesthetized with ketamine (80-100 mg/kg body) and then incised in the interscapulohumeral area; in the incision were introduced two sterile 60 mg cotton fiber pellets. The pellets were kept in the respective area for 7 d, during which time the experimental animals received by groups, during the 24 h interval, the tested substances, azetidin-2-one derivatives, in a dose of 1/10 of LD50 by dispersion in Tween 80. The study also included a group considered a positive control, treated with ferulic acid, two reference groups, treated with diclofenac sodium (5 mg/kg body) and indomethacin (1.5 mg/kg body), and a control group in which the animals received only the vehicle used for the administration of the substances to be studied—namely, Tween 80 (0.5 mL/100 g body). After 7 d, the pellets were extracted, weighed in a wet state, dried for 18 h at 57°C, and weighed again. The lower the mass of the pellet, the more intense the anti-inflammatory action of the studied compound is considered to be.

Table 4. Effect of the azetidine-2-one derivatives (6a-f) on the proliferative process (granulation tissue formation) of chronic inflammatory edema induced in rats.

Compound	R	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ferulic acid		[illegible]	[illegible]	[illegible]
Diclofenac sodium		[illegible]	[illegible]	[illegible]
Indomethacin		[illegible]	[illegible]	[illegible]
Control		[illegible]	[illegible]	

The most important inhibitory effect of granulation tissue formation and consequently the most important anti-inflammatory effect was recorded for compound 6b (R = 4 - F). This compound inhibited granulation tissue formation in 78.02%, the effect being comparable to that of indomethacin (81.25%). Under similar experimental conditions, ferulic acid showed a significant anti-inflammatory effect, inhibiting the process of granulation tissue formation by 77.94%, the effect being comparable to that of indomethacin.

Histopathological study

In order to evaluate the effect of the administration of the selected compounds in the form of suspension on the chronic inflammation, a histopathological study was carried out on liver, pulmonary, and renal tissue fragments to identify any morphological changes. At the end of the experiment, the animals were euthanized and fragments of liver, lung, and kidney tissues were taken from certain groups in the study [24,25,26]. The 1 cm liver, kidney, and lung samples were put in vials with 10% neutral formalin solution (Sigma Aldrich, Taufkirchen, Germany) and fixed at room temperature for 48 h. These were processed for inclusion in paraffin, followed by microtome sectioning and H&E staining. To examine the histological preparations thus obtained, a Nikon Eclipse 50i microscope was used.

Figure 4. Morphology of the investigated organs for the control group: (a) liver normal morphology, col.HE, x20; (b) lung normal morphology, col.HE, x20; (c) kidney normal morphology, col.HE, x20.

Figure 5. Morphology of the investigated organs for the ferulic acid-treated group: (a) liver normal morphology, col.HE, x20; (b) lung normal morphology, col.HE, x20; (c) kidney normal morphology, col.HE, x20.

Figure 6. Morphology of the investigated organs for the diclofenac-treated group: (a) liver dilated sinusoids, col.HE, x20; (b) lung massive alveolar destruction, col.HE, x20; (c) kidney dilated tubular lumens, distortion of the tubular epithelium, col.HE, x20.

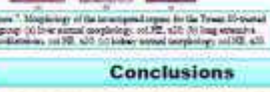


Figure 7. Morphology of the investigated organs for the Tween 80-treated group: (a) liver normal morphology, col.HE, x20; (b) lung extensive vasodilatation, col.HE, x20; (c) kidney normal morphology, col.HE, x20.

Figure 8. Morphology of the investigated organs for the group treated with 6b (R=4-F) derivative: (a) liver normal morphology, col.HE, x20; (b) lung bronchial dilatation, inflammatory reactions, col.HE, x20; (c) kidney normal morphology, col.HE, x20.

Conclusions

- The in vivo biological potential of six novel azetidine-2-one derivatives of ferulic acid was evaluated in the present study. The acute toxicity study revealed that the studied derivatives belong to compounds with moderate toxicity. The anti-inflammatory potential was determined in a model of acute and chronic inflammation, the obtained results suggesting that these compounds may be classified in the category of long-acting compounds with an intense effect of inhibiting the proliferative component. The most active compound in the series was found to be compound 6b (R=4-F). A liver function evaluation showed that the AST, ALT, LDH, and bilirubin (total and direct) concentration values were close to those recorded for the reference drugs. Additionally, no major changes were found in the histopathological investigation. All the obtained data designate a lower toxicological profile than diclofenac sodium.
- We can record that all six new azetidine-2-one derivatives of ferulic acid, and mainly compound 6b, have promising anti-inflammatory activity.

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(b) Pulo-Peláez, X.H.D.; McEwen, N.B.; Gómez-Soto, J.G.; Alvarez-Martínez, R.C.; Olvera-Ramírez, A.M. Metatranscriptomic and Histopathological Study of Rabbit Epizootic Enteropathy in Mexico. *Animals* **2020**, *10*, 936. doi:10.3390/ani10060936

Conception of 3D models of Tau protein aggregates in the service of conception and synthesis of potential Tau aggregation disruptors

Johanna GIOVANNINI[(1)(2)]*, Marie JOUANNE[(1)], Jana SOPKOVA-DE OLIVEIRA SANTOS[(1)], Marco CATTO[(2)] and Anne Sophie VOISIN-CHIRET[(1)].

[(1)] Centre d'Etudes et de Recherche sur le Médicament de Normandie, Normandie Université, Unicaen, CERMN, 14000 Caen, FRANCE.

[(2)] Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari 'Aldo Moro', 70125 Bari, ITALIA.

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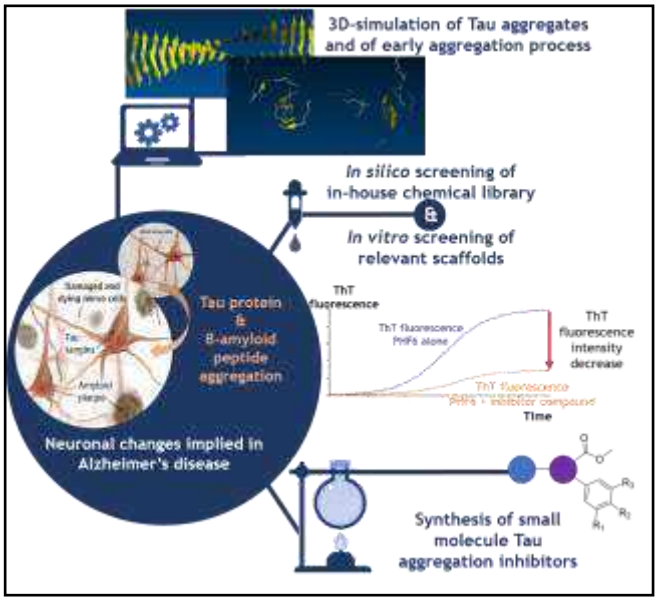
Alzheimer's disease is a neurodegenerative illness characterized by short term memory confusion, executive performance disturbance and space and time orientation function disruption among other troubles. Brain studies on Alzheimer's disease patients constantly revealed two types of damages: amyloid plaques and neurofibrillary tangles. Both of these damages are related to abnormal protein aggregation: beta-amyloid peptide (Aβ) for amyloid plaques and tau protein for neurofibrillary tangles. For both of these proteins, key-peptide sequences were identified as responsible for early oligomerization, initiating the whole amyloidogenic process.[(a, b)] In fact, those peptides adopt a beta-sheet structuration and pile themselves up, guiding the protein aggregation initiation.

We are aiming to synthesize small molecules as protein-protein interaction disruptors in order to prevent early stage aggregation.

The present work was initiated by a conformational analysis of the Tau key hexapeptide implied in Tau aggregation, called PHF6 (Paired Helical Filament hexapeptide). We built preformed PHF6 aggregates and assessed their stabilities through Molecular Dynamic (MD) simulations and analyses of intra- and intermolecular interactions. Then, MD simulations of PHF6 aggregates with palmatine, a Tau aggregation disruptor,[(c)] were launched and mechanisms of aggregation disruption will be proposed.



Then, similarity screening of our in-house chemical library[(d)] based on palmatine and *in vitro* tests provided 40 scaffolds as starting points for the rational design and synthesis of small molecules that could disturb the amyloid fibril interactions. Finally, we set up a new kind of 3D-simulation to comprehend early Tau protein aggregation process that bring up together disordered PHF6 gradually aggregating. This simulation was also carried out with palmatine in order to assess its aggregation inhibition capacity in very early stages.

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[(c)] Haj, E., Losev, Y. et al. BBA – General Subjects 2018, *1862*, 1565-1575.

[(d)] cermn.unicaen.fr/plateformes/chimiotheque/

* Correspondence: johanna.giovannini@unicaen.fr

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[1] Centre d'Etudes et de Recherche sur le Médicament de Normandie, Normandie Université, Unicaen, CERMN, 14000 Caen, FRANCE. [2] Dipartimento di Farmacia – Scienze del Farmaco, Università degli Studi di Bari 'Aldo Moro', 70125 Bari, ITALIA.

For any enquiries please contact johanna.giovannini@unicaen.fr

YRFM 2021 Paris

Pictures taken from : Jin J., JAMA, 2015[1]

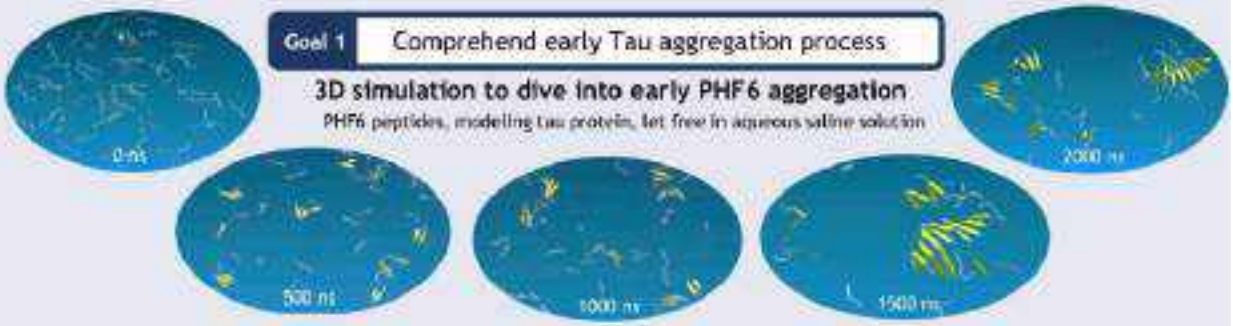
Tau aggregation: a critical issue in Alzheimer's disease

NEUROTOXICITY & DEMENTIA

Goal 1 — Comprehend early Tau aggregation process

3D simulation to dive into early PHF6 aggregation

PHF6 peptides, modeling tau protein, let free in aqueous saline solution

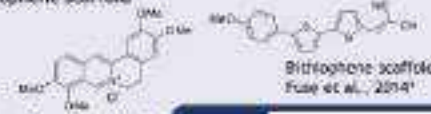


Goal 2 — Design potential Tau protein aggregation disruptors

***In silico* & *In vitro* screening-guided rational drug design**

Constitution of a 200 compound chemical library

- Structural similarity-built on our in-house chemical library[2]
- Based on 2 described tau protein aggregation disruptors: Palmatine chloride & a bithiophene scaffold



Palmatine chloride[3] — Bithiophene scaffold Fuse et al., 2014[4]

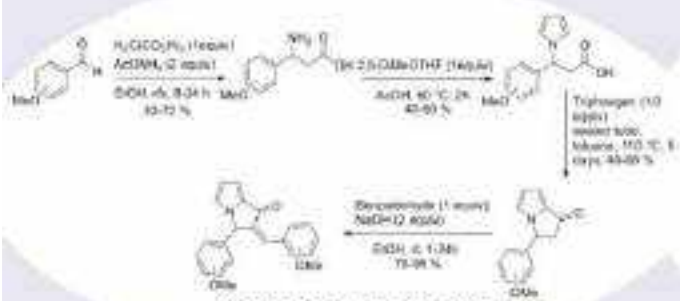
In vitro assessment of aggregation inhibition

- Inhibition of PHF6 aggregation experiment: Thioflavin T fluorescence assay

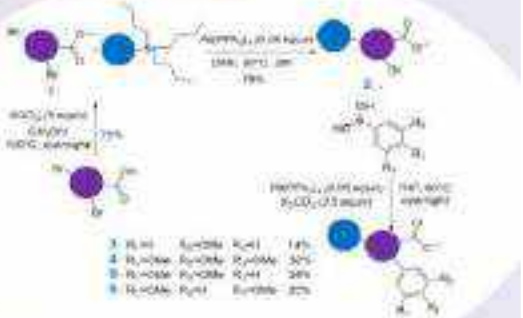
⇒ From 200 compounds: 40 scaffolds were able to inhibit PHF6 aggregation (Compound 10 µM - PHF6 50 µM)

⇒ 40 starting points for rational design of compounds that could disturb the interactions between amyloid fibrils.

Goal 3 — Synthesize Tau protein aggregation disruptors



Synthesis of pyrrolizinone derivatives.



Synthesis of bicyclic compounds

References [1] Jin, JAMA Alzheimer Disease, 2015;313 [2] cermn.unicaen.fr/plateformes/chimiotherapie/ [3] Hsu et al., RSC, 2018, 1862 [4] Fuse et al., Eur. J. Med. Chem., 2014, 85

Fragment-based library generation for the development of XIAP/caspases interaction disruptors	***Restricted to organizers***

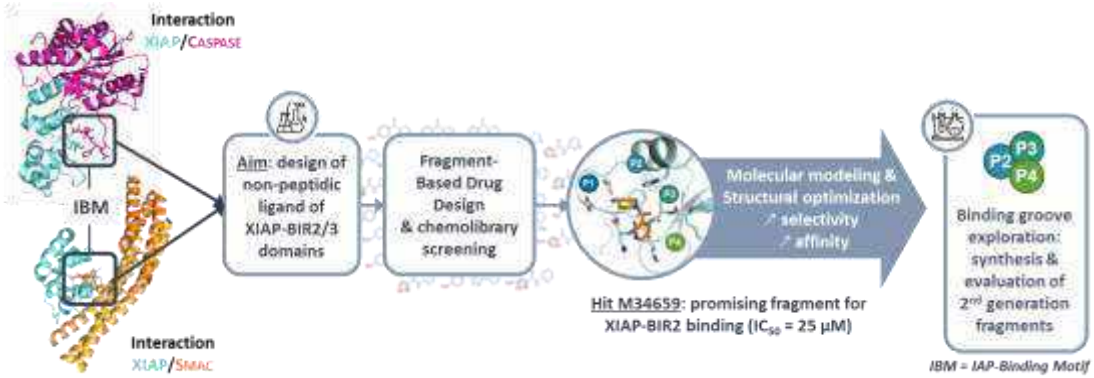
<u>Guedeney, N.</u>(1)*, Lo Caputo C.(1), Jouanne, M.(1), Ragui, M.(1), Sopková de Oliveira Santos, J.(1), Voisin-Chiret, A. S.(1), Kieffer, C.(1)

(1) Centre d'Etude et de la Recherche sur le Médicament de Normandie (CERMN), EA 4258 FR CNRS 3038 INC3M, SF 4206 ICORE, Université de Caen Normandie, 14000 Caen, France.

Apoptosis, an essential form of programmed cell death, is a tightly regulated cellular process to eliminate unwanted or damaged cells. Resistance of apoptosis is a hallmark of cancer cells.[a] X-linked inhibitor of apoptosis proteins (XIAP) is one of key apoptosis regulators that promote cancer cell resistance to apoptosis when overexpressed, like in ovarian cancer.[b] Disrupting the binding of XIAP with their functional partners (caspase 3/7/9) therefore is a promising strategy to restore the apoptotic response to proapoptotic stimuli and overcome chimioresistance.[c]

In medicinal chemistry approaches, the most successful example is the use of small molecules to mimic interactions between BIR2 or BIR3 XIAP domains and the binding motif of SMAC, an endogenous peptide inhibitor. If several peptidomimetic compounds have been developed last years, recurrent drawbacks are: the difficulty to achieve selectivity between members of the IAP proteins family, or between XIAP-BIR2 or XIAP-BIR3 domains, and unfavorable pharmacokinetic parameters.[d]

These past years, we have been conducted a rational approach to develop original non-peptidic inhibitors of XIAP-BIR2 domain. A step of fragment-based drug design and optimization of *in vitro* evaluation allowed us to obtain a first library. As the fragment MR34659 showed a promising selectivity for XIAP-BIR2, we kept it as the starting point for our pharmacomodulation study, based on difference between BIR2 and BIR3 domains[e] in order to improve both its affinity and its selectivity. All our work is actually supported by molecular modelling and in vitro assays (Alphascreen, Fluorescence Polarization Assay).



Bibliographic references:

(a) Hanahan D., *et al. Cell* **2011**, *144*, 646-674.

(b) Momenimovahed Z., *et al. Int. J. W. Health* **2019**, *11*, 287–299; Chessari G., *et al. J. Med. Chem.* **2015**, *58*, 6574-6588.

(c) Rathore R., *et al. Apoptosis* **2017**, *22*, 898-919.

(d) Cong H., *et al. J. Med. Chem*. **2019**, *62*, 5722-5750.

(e) Lukacs C., *et al. Acta Cryst.* **2013**, *D69*, 1717-1725; Donnell A. F., *et al. J. Med. Chem*. **2013**, *56*, 7772-7787.

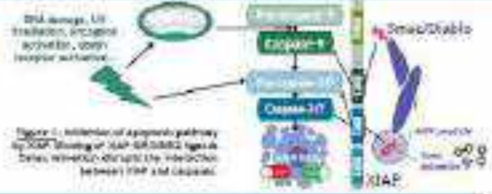
* Correspondence: nicolas.guedeney@unicaen.fr

Fragment-based library generation for the development of XIAP/caspases interaction disruptors

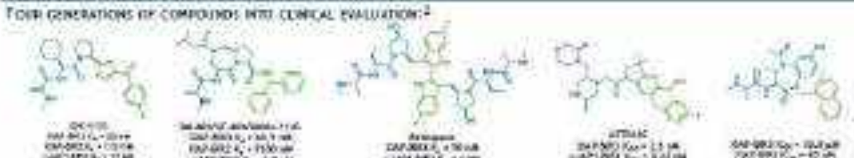
Guedeney, N.*, Lo Capoto, C., Jouanne, M., Ragol, M., Sopková-de Oliveira Santos, J., Voisin-Chiret A. S., and Kieffer, C.
Centre d'Etudes et de Recherche sur le Médicament de Normandie, EA 4258 FR CNRS 3038 INC3M, SF 4206 ICORE, Université de Caen Normandie, 14000 Caen, France
* Corresponding author : nicolas.guedeney@unicaen.fr

RESISTING APOPTOSIS & CHEMORESISTANCE, HALLMARKS OF CANCER

- Key apoptosis regulator: X-linked Inhibition Apoptosis Protein (XIAP)[1]:
  - Inhibition of caspase-3/7 (via BIR2 domain) and caspase 9 (via BIR3 domain),
  - Downregulated by Smac/Diablo via AVPI N-terminal peptide.
- Disrupting protein-protein interactions (PPI) between IAP family and caspase with Smac mimetic is a validated therapeutic approach.[2]



FOUR GENERATIONS OF COMPOUNDS INTO CLINICAL EVALUATION:[2]

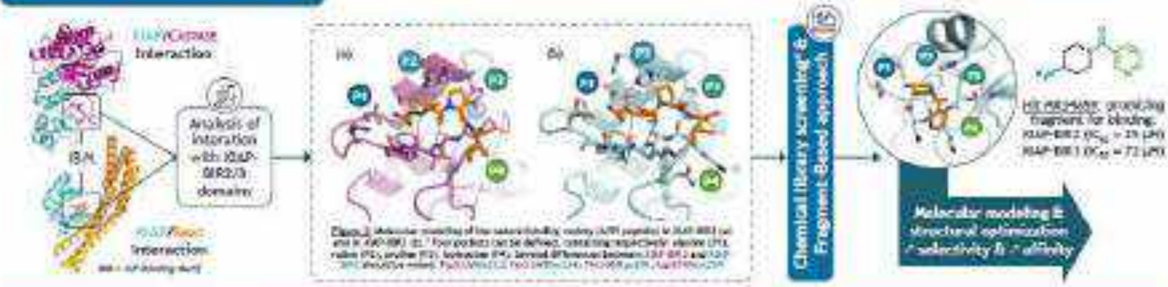


Peptidomimetics drawbacks:

- Low stability (peptidase),
- Low bioavailability,
- Cytokine release syndrome (c-IAP),
- Difficulties to achieve selectivity between c-IAP and XIAP; between XIAP-BIR2 and XIAP-BIR3.

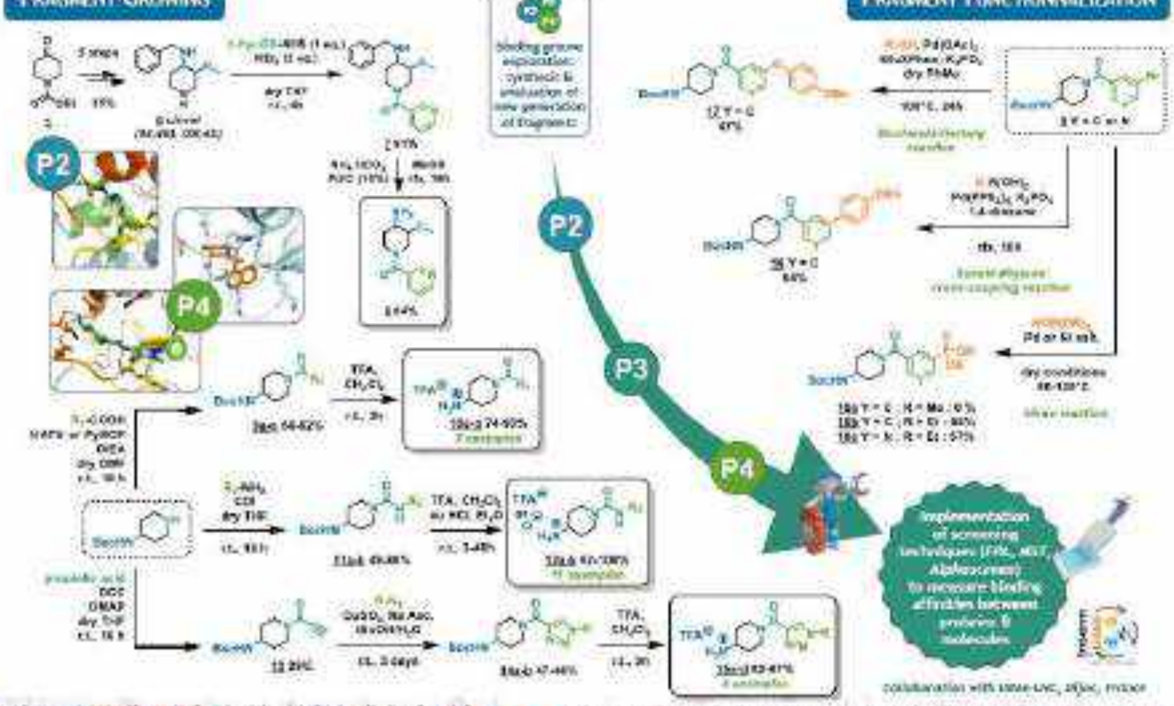
FRAGMENT-BASED DRUG DESIGN

DEVELOPMENT OF A NEW SMALL SCAFFOLD WITH BETTER SELECTIVITY FOR XIAP-BIR DOMAINS:



FRAGMENT GROWING

FRAGMENT FUNCTIONNALIZATION



collaboration with ...

This project was selected and financed by Fondation ... and La Ligue Contre le Cancer

References: [1] Hanahan, D., Weinberg, R.A., Cell, 2011, 144, 646-674. [2] Cong, H. et al., J. Med. Chem. 2019, 62, 5750-5772. [3] (a) Lozato, C. et al., ... 2013, ... [4] ...

Design and synthesis of new analogues of donecopride with potential interest for Alzheimer's disease treatment.

Jouanne, M.,[1] Lecoutey, C.,[1] Davis, A.,[1] Freret, T.,[2] Boulouard, M.,[2] Claeysen, S.,[3] Rochais, C.,[1] Dallemagne, P.[1]

(1) UNICAEN, CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie), Caen, France
(2) UNICAEN, INSERM U1075 COMETE, Caen, France
(3) IGF, Univ Montpellier, CNRS, INSERM, Montpellier, France

- In view of the multifatorial origin of Alzheimer's disease (AD), our lab is specialized in the design and synthesis of new molecules as Multi Target Directed Ligands (MTDLs), compounds that are effective in treating complex diseases because of their ability to interact with the multiple targets thought to be responsible for the disease pathogenesis.
- Our group recently succeeded in combining 5-HT_4 receptor partial agonism and acetylcholinesterase inhibitory activity into a single pleiotropic compound, **donecopride**, which is potentially able to display both symptomatic and disease-modifying therapeutic effects in the treatment of AD.

In vitro studies[1]

- ✓ 5-HT_4 receptor partial agonist
- ✓ Acetylcholinesterase inhibitor

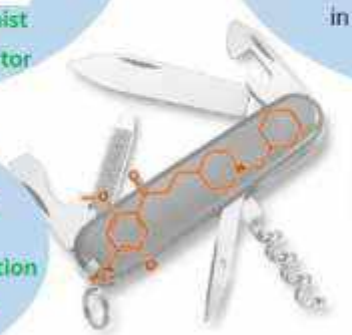
In vivo studies [2]

→ Chronic administration of donecopride :

- ✓ Decrease in amyloid load and in astrogliosis in some brain regions.
- ✓ Potent anti-amnesic properties in two animal models of AD

In cellulo studies[2]

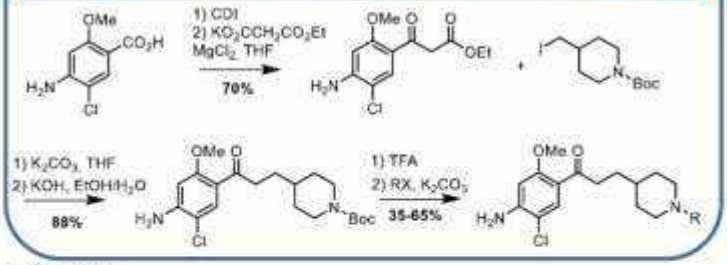
- ✓ Exerts a neuroprotective effect against amyloid toxicity
- ✓ Reduces Tau hyperphosphorylation
- ✓ Promotes the formation of new synapses

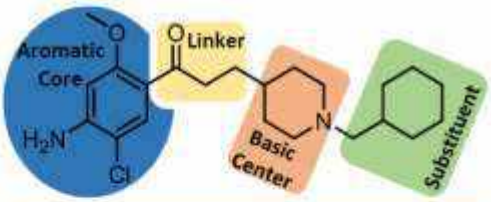


Donecopride, pleiotropic compound, acts like a Swiss army knife against AD

- ✓ Regulatory preclinical study for AD treatment *In progress...*

Scheme 1 : General synthesis of donecopride analogues[3]





Many pharmacomodulations have been realized according to this scaffold and following the synthesis presented in scheme 1 . Currently, new analogues are still synthesized in order to further improve activities.

References:

[1] Lecoutey, C. *PNAS*, **2014**, 111, E3825–E3830. https://doi.org/10.1073/pnas.1410315111 ;

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[3] Rochais, C. *et al. J. Med .Chem.*, **2015**, *58*, 3172-3187.

marie.jouanne@unicaen.fr

@CermnUnicaen

Biological evaluation of chitosan electrospun nanofibers as wound dressing materials.

Andreea-Teodora Iacob [(1)]*, Oana-Maria Ionescu [(1)], Florentina Lupașcu[(1)], Maria Apotrosoaei[(1)], Ioana Vasincu[(1)], Luminița Confederat [(2)], Maria Drăgan[(1)], Alexandru Sava[(1)], Lenuța Profire[(1)].

[(1)]University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Iasi 700115, Romania

Restricted to organizers

Aim. In recent years, the use of nanomaterials for biomedical and pharmaceutical applications had gained significant attraction. Thus, a considerable percentage of nanomaterials are used in various biomedical applications for wound dressings, drug delivery and other medical purposes. The goal of this work is the development of innovative electrospun nanofibers based on chitosan and polyethylene oxide (CS/PEO) with arginine and propolis incorporated as active substances and their biological evaluation as new dressing materials in terms of antibacterial activity and anti-hemolytic potential by erythrocyte membrane stabilization. **Materials and method.** The choice of bio-polymers and bioactive agents was not arbitrary, but was based on their beneficial properties in tissue healing. The *in vitro* assay used for proving the anti-inflammatory potential allows the determination of the ability of the compounds to protect the erythrocyte membrane from hypotonicity-induced lysis, expressed as the percentage of erythrocyte membrane stability. The antimicrobial activity of the samples was studied using Gram positive bacterial strains (*Staphylococcus aureus* ATCC 25923), Gram negative bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeasts (*Candida albicans* ATCC 10231) by disc-diffusion method, where the diameters of the inhibition area (mm) including disc size, were measured. **Results and discussions.** By varying the essential parameters in the formation of electropsun nanofibers, it was concluded that the best results were obtained at a flow rate of 5ml/h applying a voltage of 20 kV and a distance from the syringe needle to the collecting plate of 25 cm. Analysis of the literature and SEM micrographs of the obtained nanofibers showed that chitosan has an outward orientation of the nanofibers, while PEO has an inward orientation. After analyzing the results obtained, it was concluded that the best results were obtained for the formulations containing dry propolis and arginine in 7.5% conc. **Conclusions.** New chitosan-based electrospun nanofiber dressings were formulated that incorporated arginine and propolis into the polymer matrix and were biologically evaluated by determining the spectrophotometric anti-hemolytic potential and the antimicrobial potential. The results obtained justify the use of these formulations as potential dressings for a faster healing and with minimal risk of infection.

Acknowledgment: Scientific research funded by the University of Medicine and Pharmacy "Grigore T Popa Iasi", based on contract no. 27496/20.12.2018.

Bibliographic references:

(a) Daemi H, Mashayekhi M, Modaress MP. Carbohydr. Polym 2018; 198: 481-485.

(b) Thakur K, Sharma G, Singh B, Chhibber S, Patil AB, Katare OP. Int J Biol Macromol. 2018; 115: 1012-1025.

* Correspondence: panzariu.andreea.teodora@gmail.com

28th Young Research Fellows Meeting
11-12January 2021, Caen

Biological evaluation of chitosan electrospun nanofibers as wound dressing materials

Andreea-Teodora Iacob (1)*, Oana-Maria Ionescu (1), Florentina Lupaşcu(1), Maria Apotrosoaei(1), Ioana Vasincu(1), Luminița Confederat (2), Maria Drăgan(1), Alexandru Sava(1)), Lenuța Profire(1).

(1)University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Iasi 700115, Romania

Introduction

- In recent years, the use of nanomaterials for biomedical and pharmaceutical applications had gained significant interest. Thus, a considerable percentage of nanomaterials are used in various biomedical applications for wound dressings, drug delivery and other medical purposes [1, 2].
- **The goal of this present work** is the development of innovative electrospun nanofibers based on chitosan and polyethylene oxide (CS/PEO) with arginine and propolis incorporated as active substances and their biological evaluation as new dressing materials in terms of antibacterial activity and anti-hemolytic potential by erythrocyte membrane stabilization.

Fig 1. The advantages of nanotechnology in wound healing

Material and methods

1. The nanofiber formulation was carried out in 3 steps:

- the first step consisted in the preparation of chitosan LMW (1% w/v) and PEO 1.000.000 Da (3% w/v) polymer solutions (molar ratio 1: 1 chitosan: PEO) using as solvent: acetic acid (70% v/v, 30% v/v), followed by:
- the second step: the inclusion of arginine and propolis in various concentrations in the colloidal dispersion obtained;
- For the third step, the *electrospinning process* (nanospinner, INOVENSO), a syringe with different ranges of gauge needle was filled with the colloidal and then applied different flow rates, different values of applied voltage and also different tip-to-collector distance [3].

2. Determination of the stabilizing capacity of the erythrocyte membrane

- The effect of the studied compounds on the stability of erythrocyte membranes was determined spectrophotometrically, on blood collected on citrate from clinically healthy patients.
- Sadigue method, a method that allows the determination of the ability of compounds to protect the erythrocyte membrane from hypotonicity-induced lysis.
- The absorbance of the supernatant was read at 560 nm from the sample control.
- All determinations were performed in triplicate, and diclofenac sodium was used as a reference substance (positive control) and processed similarly to the tested samples [4].

$$\% \text{ stability} = 100 - [(A_{probe}/A_{allogo})x100]$$

3. Determination of antimicrobial activity

- **Microorganisms.** The antimicrobial activity of the samples 1-10 was studied using Gram positive bacterial strains (Staphylococcus aureus ATCC 25923), Gram negative bacterial strains (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and pathogenic yeasts (Candida albicans ATCC 10231). The bacterial and fungal suspensions were obtained by dispersing a small amount of each microbial culture in sterile NaCl 0.9% until a turbidity equivalent to McFarland standard no. 0.5 (106 CFU/mL).
- **Culture medium** used for antimicrobial susceptibility tests was Mueller Hinton agar for antibacterial tests and Sabouraud agar for antifungal tests. The culture medium was spread on sterile Petri plates, in volume of 25 mL/Petri plate.
- **Disc-diffusion method.** Antimicrobial tests were performed according to CLSI specifications. The bacterial and fungal suspensions, prepared as described above, were inoculated on the surface of the culture medium spread in Petri plates. Sterile stainless steel cylinders) were applied on the agar surface in Petri plates and 200 µL of each sample tested was added into cylinders. Commercial discs containing Ciprofloxacin (5 µg/disc) and Voriconazole (1 µg/disc) were used as positive control. The plates were incubated at 37°C for 24 h (antibacterial activity) and at 24°C for 48 h (antifungal activity). After incubation, the diameters of the inhibition area (mm) including disc size, were measured [5].

Results and discussions

1. Obtaining CS/PEO electrospun nanofibers with arginine/propolis

- The dried samples were coated with gold before observation in a scanning electron microscope. SEM will provide informations about the composition of the sample studied by X-ray detection, backscatter electrons and Auger electrons and allow us to examine the details with a high magnitude and resolution and provides 3D- images. Analysing the images provided datas about the nanofibers diameter, porosity and distribution.

Table 1 Parameters of CS/PEO electrospun nanofibers which incorporated arginine

Polymeric Solutions	Parameters	Average diameter± DS (nm)
PEO 3% CS 1%	5mL/h; 20kV; 25 cm	251.79 ± 84.08
PEO 3% CS 1%-Arg		275± 101.85
PEO 3% CS 1%Arg-dry propolis		227.4±70.51
PEO 3% CS 1%-Arg-alcoholic tincture of propolis		274.52 ± 66.95

The morphology of chitosan membranes with arginine is examined by using scanning electron microscope (SEM) SEMscope INOVENSO IEM 10 (Fig. 2).

Fig. 2. SEM images for CS 1%/PEO3% and arginine 1% (5a), insulin (5b) and propolis (5c).

2. Representation of stabilizing capacity of the erythrocyte membrane

After analyzing the data obtained, it was concluded that the nanofibers with propolis obtained the best results, as shown in Fig. 3.

Fig. 3. Determination of the stabilizing capacity of the erythrocyte membrane for eNFs PEO/CS/Arg (A); PEO/CS/Arg- dry propils (B); PEO/CS Arg-alcoholic tincture of propolis (C)

3. Determination of antimicrobial activity

Compound	Diameter of inhibition zone (mm)			
	S. aureus ATCC 25923	E. coli ATCC 25922	P. aeruginosa ATCC 27853	C. albicans ATCC 10231
PEO/CS	23	17	22	24
PEO/CS/Arg	24	25	25	25
PEO/CS/Arg-propils dry	25	25	25	27
PEO/CS-Arg-propolis alc.	26	25	26	27
CIP 5µg/ disc	26	28	30	NT
VRC	NT	NT	NT	27

Conclusions

- New chitosan and PEO electrospun nanofibers were formulated, as potential wound dressing materials comprising arginine and propolis dry and alcoholic solution.
- The studies and results obtained justify the evaluation of the biological, antibacterial and pro-healing potential in the treatment of various wounds, starting from the antibacterial effects of chitosan and the beneficial role of applied topical propolis and arginine in the treatment of wounds.
- All correlated data of this study show that the developed novel chitosan-PEO arginine electrospun nanofibers are potential materials for wound dressing, in order to further evaluation for *in vivo* testing.

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Acknowledgments

Scientific research funded by the University of Medicine and Pharmacy "Grigore T Popa Iasi", based on contract no. 27496/20.12.2018.

Antioxidant potential of some new derivatives of isobutylphenylpropionic acid.

Ioana-Mirela Vasincu[(1)]*, Alexandru Vasincu[(1)], Maria Apotrosoaei[(1)], Sandra Constantin[(1)], Florentina Lupașcu[(1)], Alexandru Sava[(1)], Andreea Iacob[(1)], Maria Drăgan[(1)], Luminița Confederat[(2)], Frederic Buron[(3)], Sylvain Routier[(3)], Lenuța Profire[(1)].

(1)"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Iasi, Romania.
(2)"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Faculty of Medicine, Iasi, Romania.
(3) University of Orléans, Institute of Organic and Analytical Chemistry, Orléans, France.

Restricted to organizers

Propionic acid derivatives are a class of nonsteroidal anti-inflammatory drugs (NSAIDs) used for treating pain or inflammation. The first member of this class introduced in therapy was 4-(2-methyl propyl) phenyl] propionic acid (ibuprofen) as a better alternative of acetylsalicylic acid (aspirin). Ibuprofen has important analgesic, antipyretic and anti-inflammatory properties, but also some studies evidenced antioxidant activity. It seems that inflammation and oxidative stress have been implicated as pathogenic mechanisms in some neurodegenerative disorders such as Parkinson or Alzheimer diseases. Some evidences confirmed that the use of NSAIDs could be useful in the prophylaxis of these types of diseases. Ibuprofen can scavenge the reactive oxygen species and reduce the oxidative damage.

Some hydrazones and thiazolidin-4-ones of ibuprofen were synthesized and the antioxidant potential of them was evaluated using four *in vitro* assays: DPPH and ABTS radical scavenging, total antioxidant capacity and reducing power tests.

Structural modulations of the free carboxyl group of ibuprofen aimed to obtain derivatives that showed an antioxidant effect comparable or more intense than the basic structure. Most compounds with thiazolidin-4-one structure have been shown to be more active than the corresponding hydrazones, which supports the importance of the thiazolidin-4-one scaffold for the antioxidant effect. Some derivatives presented an important antioxidant potential, which recommends them as potential therapeutic agents in diseases where inflammation and oxidative stress have an important role (inflammatory, neurodegenerative, neoplastic diseases).

Bibliographic references:

[(a)] Vasincu IM, Apotrosoaei M, Panzariu A, Buron F, Routier S, Profire L. Molecules 2014; 19:15005-15025.

[(b)] Saravanakumar K, Sarikurkcu C, Sarikurkcu RT, Wang MH. Industrial Crops and Products 2019; 142:111878.

* Correspondence: ioana-mirela.vasincu@umfiasi.ro

ANTIOXIDANT POTENTIAL OF SOME NEW DERIVATIVES OF ISOBUTYLPHENYLPROPIONIC ACID

Ioana-Mirela Vasincu(1)*, Alexandru Vasincu(1), Maria Apotrosoaei(1), Sandra Constantin(1), Florentina Lupaşcu(1), Alexandru Sava(1), Andreea Iacob(1), Maria Drăgan(1), Luminița Confederat(2), Frederic Buron(3), Sylvain Routier(3), Lenuța Profire(1)

(1) GRIGORE T. POPA University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, Iasi, Romania
(2) GRIGORE T. POPA University of Medicine and Pharmacy Iasi, Faculty of Medicine, Iasi, Romania
(3) University of Orléans, Institute of Organic and Analytical Chemistry, Orléans, France
*Correspondence: ioana-mirela.vasincu@umfiasi.ro

Introduction

- ✓ the discovery of new bioactive compounds with improved pharmacotoxicological and pharmacokinetic profile towards traditional drugs is the major concern of pharmaceutical researchers
- ✓ aryl-propionic acid derivatives (ibuprofen, fenoprofen, ketoprofen, naproxen, etc.) are some of the most commonly used agents within nonsteroidal anti-inflammatory drugs (NSAIDs)

- ✓ it seems that inflammation and oxidative stress have been implicated as pathogenic mechanisms in some neurodegenerative disorders such as Parkinson or Alzheimer diseases
- ✓ some evidences confirmed that the use of NSAIDs could be useful in the prophylaxis of these types of diseases

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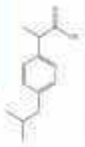
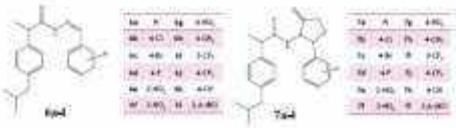


- ✓ the first member of this class introduced in therapy was 4-(2-methyl propyl) phenyl] propionic acid (ibuprofen) as a better alternative of acetylsalicylic acid (aspirin)
- ✓ has important analgesic, antipyretic and anti-inflammatory properties, but also some studies evidenced that ibuprofen can scavenge the reactive oxygen species and reduce the oxidative damage

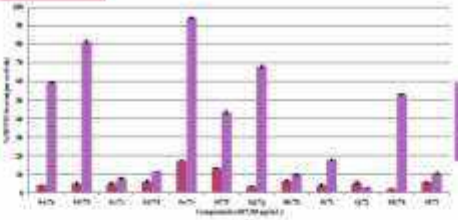
Material and methods

- ✓ some hydrazones and thiazolidin-4-ones of ibuprofen were synthesized and the antioxidant potential of them was evaluated using four *in vitro* assays: DPPH and ABTS radical scavenging, total antioxidant capacity and reducing power tests

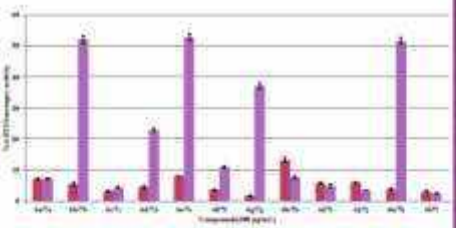


Results

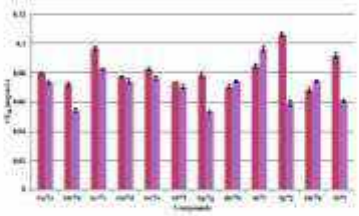
DPPH radical scavenging assay



ABTS radical scavenging assay



Total antioxidant capacity



Reducing power

Compounds	CE_{50} (mg/mL)	Compounds	CE_{50} (mg/mL)
7a	0,2934±0,0048	7b	0,4747±0,0064
7h	0,0219±0,0006	7m	2,4726±0,0294
7c	4,4876±0,0513	7i	3,5935±0,0377
7d	2,5112±0,0460	7j	3,3202±0,0659
7e	0,8208±0,0170	7k	0,4914±0,0056
7f	1,3803±0,0147	7l	4,8647±0,0701
Ascorbyl	0,0214±0,0008	Ibuprofen	13,9467±0,4407

Conclusions

- ➢ structural modulations of the free carboxyl group of ibuprofen aimed to obtain derivatives that showed an antioxidant effect comparable or more intense than the basic structure
- ➢ most compounds with thiazolidin-4-one structure have been shown to be more active than the corresponding hydrazones, which supports the importance of the thiazolidin-4-one scaffold for the antioxidant effect
- ➢ some derivatives presented an important antioxidant potential, which recommends them as potential therapeutic agents in diseases where inflammation and oxidative stress have an important role (inflammatory, neurodegenerative, neoplastic diseases)

Selected refereences
- Vasincu IM, Apotrosoaei M, Panzariu A, Buron F, Routier S, Profire L. Molecules 2014; 19:15006-15025.
- Saravanakumar K, Sarikurkcu C, Sarikurkcu RT, Wang MH. Industrial Crops and Products 2019; 142:111878.

28th Young Research Fellows Meeting, Paris, France, 2021

Comparison of the fungi *Trichoderma harzianum* and *Trametes trogii* for the bioremediation of antibiotics in a fungal microbial fuel cell (FMFC)

Marwa JAIDI[(1,2)*], Mehri Shabani [(1,3)], Tahar Mechichi [(2)], and Maxime Pontié [(1)*]

(1) University of Angers, Faculty of Sciences Group Analysis and Processes, 2 Bd. Lavoisier, F-49045 Angers Cedex 01, FRANCE.
(2) University of Sfax, National Engineering School of Sfax, Laboratory of biochemistry, enzymes engineering and lipases, TUNISIA.
(3) Tarbiat Modares University, Department of Environmental Science, Faculty of Natural Resources, Tehran, IRAN.

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Abstract

Antibiotics presence contribute to the development of the anti-bioresistance diseases. Tetracycline (TCL) presently studied is the most commonly used antibiotic despite its high toxicity and persistence in the environment and especially in wastewaters [(a)]. Two fungal strains, one basidiomycota *Trametes trogii* and one ascomycota *Trichoderma harzianum* were previously selected for their high ability to grow on different culture media (YNB, YPDA, PDA) with and without TCL. In addition, these strains were selected for their non-pathogenic properties for human. Then a comparative study was performed between both strains to determine their potential for the bioremediation of TCL in a two compartments fungal microbial fuel cell (FMFC)[(b,c)]. A solution of 100mg/l of TCL was used in the bioanode compartment as the sole source of carbon and was exposed successively to the studied bioanodes. HPLC and UV-VIS analysis allowed to follow TCL concentrations and determine the kinetical parameters of degradation. The results revealed that the two strains were able to biodegradate TCL in a first order kinetic model. Furthermore, *T. harzianum* was faster with a kinetic constant value of $k_{20°C}$=0.011 h^{-1} vs 0.005 h^{-1} for *T. trogii*, also a percentage of biodegradation in 24h hours at 20% and only 7%, respectively. Besides, the bioanode with *T. harzianum* was able to provide the higher amount of power density with 0.2 mW/m² compared to only 0.05 mW/m for T. trogii. Moreover, we have tested a previously developed[(b)] cathode recovered of a chemical catalyzor poly-NiTSPc with a new biocathode with a biofilm of T. trogii. The result showed an enormous increase of the power density from 0.2 to 6 mW/m^2 with the biocathode C/T. trogii vs chemical cathode. This can be explained by the ability of T. trogii to produce high laccase activity up to 10000 mU/m [(d)]l, a well-known enzyme for catalyzing the reduction of O_2. Consequently, T. trogii seems to be more efficient as biocathode and T. harzianum as a bioanode in our FMFC. Future work will confirm the first results reported here particularly the interest in replacing a chemical Ni cathode by a biocathode. Others antibiotics will be also tested soon.

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* Correspondence E-mail: marwajaidi.enis@gmail.com / *maxime.pontie@univ-angers.fr*

XXVIII the Young Research Fellow Meeting - SCT 2021

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M. Jaidi[1,2], M.Shabani[1,3], T. Mechichi[2] and M.Pontié[1]

[1]Angers University, Dpt. of Chemistry, GA&P, FRANCE; [2] National Engineering School of Sfax, LGBEL, TUNISIA; [3] Tarbiat Modares University, Tehran, IRAN.

Keywords: Fungal microbial fuel cell, Trametes trogii, Trichoderma harzianum, Tetracycline, Biocathode.

Introduction

Antibiotics presence in wastewaters contribute to the development of the anti-bio-resistance diseases. Tetracycline (TCL) presently studied is the most commonly used antibiotic despite its high toxicity and persistence in the environment and especially in wastewaters (1). Current study investigates the removal of TCL in a novel microbial fuel cell. Two fungal strains non pathogenic, one basidiomycota *Trametes trogii* and one ascomycota *Trichoderma harzianum* were selected for their high ability to grow on different culture media (YNB, YPDA, PDA) with and without TCL. Therefore, this comparative study was performed between both strains to determine their potential for the bioremediation of TCL in a two compartments fungal microbial fuel cell (FMFC) recently developed (2) and to hence its performances.

Fig1. Growth comparison in Agar media with and without TCL (100 mg/L)

Fig 1 shows the ability of *T.harzianum* and *T. trogii* to growth in various media agar in presence of TCL. Results revealed that growth rate of *T.harzianum* is more perturbed than *T trogii* in presence of TCL. Also, we have observed that *T.harzianum* presented the higher growth rate vs T trogii which is confirmed by another study reported recently by Shabani et al. (2)

Tetracycline biodegradation in the FMFC : T.trogii Vs *T. harzianum*

In order to evaluate the TCL's biodegradation in the FMFC, samples were taken periodically from the anodic compartment with *T.harzianum* Vs. *T.trogii*

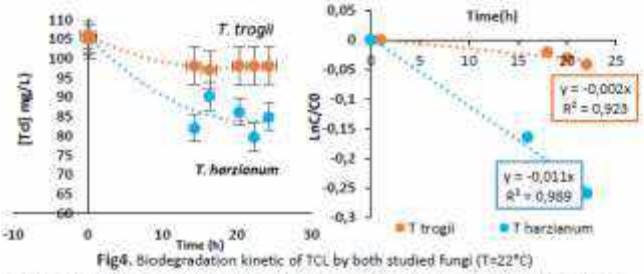

Fig4. Biodegradation kinetic of TCL by both studied Fungi (T=22°C)

TCL biodegradation occurred at 100 mg/L and measured by HPLC at 272 nm. After 24h, a decrease in TCL concentration with 20% of tetracycline biodegradation by *T. harzianum* as only 5% by *T.trogii* were observed.

A first kinetic order of biodegradation was observed with *T. harzianum* a constant rate of k= 0.011 h-1 and a half life time t1/2= 62 hours (3 days) was calculated as for *T. trogii* the constant rate was 5 time lower k= 0.002 h-1 and t1/2= 150 hours (6 days).

Furthermore, the maximum power density obtain was 0.7 mW/m² with *T. harzianum* biofilm Vs 0.05 mW/m² for *T. trogii* in correlation with the rate of biodegradation of TCL.

FMFC T. trogii / T. harzianum performances (power density, optimal resistance)

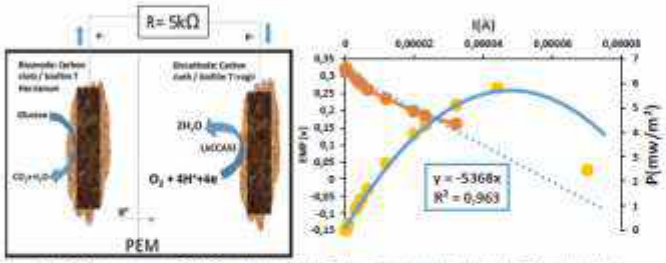

Fig6. Schema view of FMFC with bioanode *T.harzianum* and biocathode *T. trogii* and its polarization curve (with 5g/l Glucose as carbon source to facilitate Cell growth) 24h, phosphate buffer pH=7.2)

Conclusion and Perspectives

Considering the first results reported presently it seems that *T. harzianum* was more efficient than *T.* trogii in biodegradation of TCL with biodegradation's percentage (24h hours) was 20% for *T. harzianum* and only 5% for *T. trogii*, also the kinetic rate (ordre1) was k (20°C)= 0.011 h-1 >> 0.002 h-1 respectively.

The new biocathode *C/T. trogii* with *T. harzianum* as bioanode seems to be useful for TCL biodegradation (Pmax=6 mW/m² in presence of glucose)

Future work will confirm the first results reported here particularly the interest in replacing the CC modified poly NiTsPc cathode used presently (3) by a biocathode Modified with a biofilm of *T. trogii*. Others antibiotics will be also tested i.e. sulfanilamide, Ampicillin...

T.harzianum and *T.trogii* growth in petri's dishes, on carbon cloth electrodes and SEM microscopies images

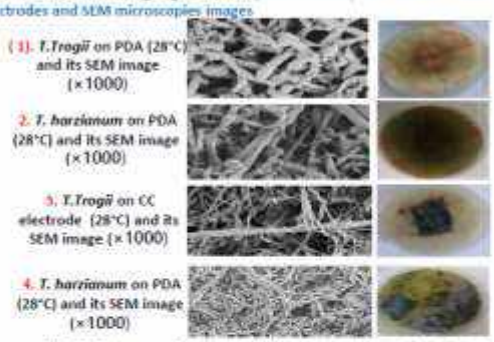

Fig2. SEM images of *T.trogii* and *T.harzianum* developed on PDA agar (1,2) and on carbon cloths CC(3,4)

The two fungi strains were developed abundantly on carbon cloths formed fungi biofilms which will be used as catalysts in the bioanodic compartments.

FMFC Elaboration with T harzianum bioanode (T trogii in second time) and NiTsPc cathode

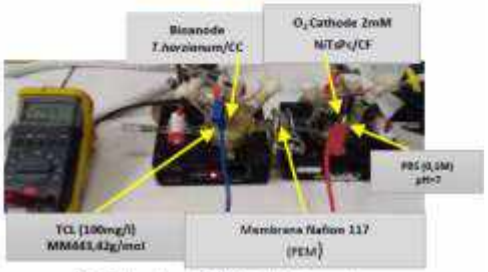

Fig3. Elaboration of FMFC with TCL as carbon source

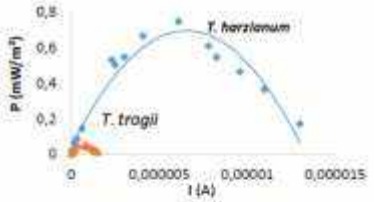

Fig5. Power density generated in FMFC with *T. harzianum* Vs *T. trogii* bioanode, respectively

T. trogii was able to produce laccases with high activity of 10,000 mU/ml (4). This multicopper oxidases enzyme is well known to catalysis O2 reduction and generating water as by-products with the reaction bellow (5) :

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (5)$$

Our results showed that *T. trogii* can preferably be used as biocathode with *T. harzianum* as bioanode in order to try to improve the FMFC performances. With a faster carbon source as glucose, this FMFC showed improvement of the energy performances with Pmax = 6 mW /m² and EMF = 500 mV (Figure 6)

In principle, this energy derive from the complete oxidation of glucose to carbon dioxide and water and provide 24 electrons per molecule through the reaction bellow :

$$C6H12O6 + 6O2 \rightarrow 6CO2 + 6H2O \quad (6)$$

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(1) J. Hou, C. Wang, D. Mao, Y. Luo, Environmental Science and Pollution Research, 2015, 23, 1722-1731; (2) M. Shabani, M. Pontié et al., J. Applied Electrochemistry ,2021; (3) S. Mbokou, M. Pontié et al., J Applied Electrochem, 2017, 47 (2) 273-280; (4) H. Zouari-Mechichi, T. Mechichi et al. Enzyme and microbial. Technology, 2006, 31 (1) 141-148; (5) Lalaoui N et al, Chemical Communications, 2013.

Acknowledgements

We Thank greatly Mrs. Nadege BLON for her help in HPLC analysis and Mr.Romain MALLET and Mrs. Florence MANERO from SCIAM Angers for SEM images. Thanks a lot to Dr. C. Innocent (European Membrane House), Montpellier, France and Pr. L. Lebrun (Rouen University) France, for fruitfull discussions.

New Tubulin Binding Sulfonamides with application as antileishmanial agents

Myriam González[(1)]*, Laura Gallego[(1)], Alba Vicente[(1)], Serio Ramos[(1)], Raúl Fuentes[(1)], Cristina Sanz[(1)], Miguel Marín[(1)], Sara del Mazo[(1)], Raquel Álvarez[(1)], Manuel Medarde[(1)] and Rafael Peláez[(1)]

(1) Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno. Salamanca, Spain

Restricted to organizers

Tubulin, heterodimeric protein formed by two subunits, α and β; is responsible for the formation of microtubules, which support the cytoskeleton, intervene in cell motility, cell division, transport of organelles, maintenance of cell morphology and signal transduction. Although tubulin is a highly conserved protein, the trypanosomatid microtubules present up to eleven different amino acid substitutions at the colchicine binding site, compared to mammals.

Based on the sulfonamide privileged scaffold, a new family of Tubulin Binding Sulfonamides has been designed, synthesized and modified at strategic points, according to the colchicine tubulin binding site in *Leishmania spp.* with respect to mammals, in order to find novel molecules with possible applications as antileishmanial agents without affecting mammalian host cells (Fig. 1).

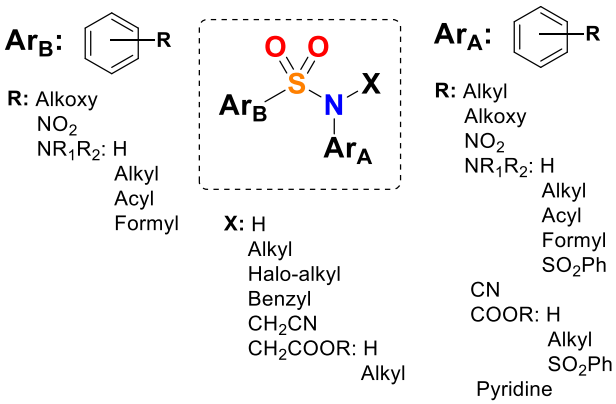

Fig. 1: General structure of new Tubulin Binding Sulfonamides.

The sulfonamide moiety is considered a privileged scaffold in medicinal chemistry due to a combination of favorable and highly tunable physical–chemical and biological properties, including a suitable combination of accessibility, stability, hydrogen bonding capability, polarity, hydrophobic-hydrophilic balance and conformational preferences, among others.[a]

The *in vitro* antileishmanial activity against promastigotes and axenic and intracellular amastigotes together with the antiproliferative activity in human cells will be presented.

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Financial support came from Consejería de Educación de la Junta de Castilla y León SA030U16, SA262P18 and predoctoral fellowship (ORDEN EDU/529/2017 de 26 de junio), FEDER funds, European Social Fund and Operational Program of Castilla y León, Spanish Ministry of Science, Innovation and Universities (RTI2018-099474-BI00).

* Correspondence: mygondi@usal.es

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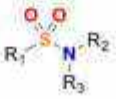
Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca. Spain.

* Correspondence: mygonda@usal.es

INTRODUCTION

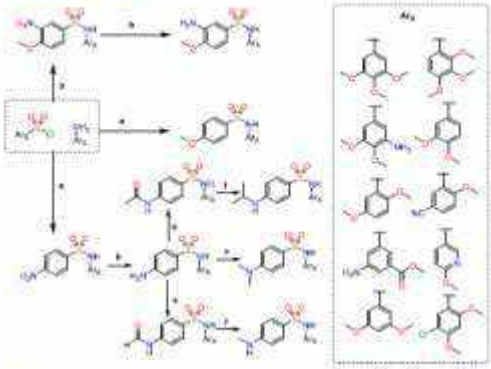
Leishmaniasis, caused by several species of the protozoan *Leishmania* parasite is the world's second-deadliest parasitic disease after malaria and has been classed as an emerging and uncontrolled disease by the World Health Organization. High toxicity, adverse effects, resistance issues, prohibitive prices, long treatment regimen and mode of administration of current leishmaniasis treatments, including amphotericin B and first-line antimony derivatives, require an alternative route with novel drugs capable of fighting the disease. Tubulin is a well-established target in antitumor, antiparasitic, herbicidal, and antifungal chemotherapy.

Based on the sulfonamide privileged scaffold, a new family of Tubulin Binding Sulfonamides has been designed, synthesized and modified at strategic points, according to the colchicine tubulin binding site in *Leishmania spp.* with respect to mammals, in order to find novel molecules with possible applications as antileishmanial agents without affecting mammalian host cells.



- ✓Synthetic accessibility
- ✓Stability
- ✓Hydrogen bonding capacity
- ✓Polarity
- ✓Hydrophobic-hydrophilic balance
- ✓Conformacional preferences

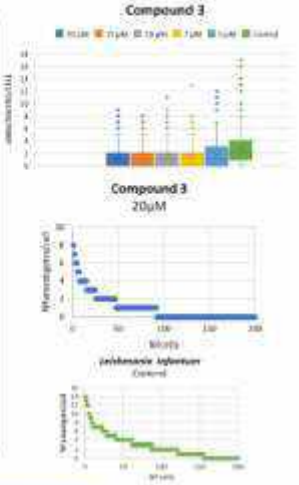
SYNTHESIS

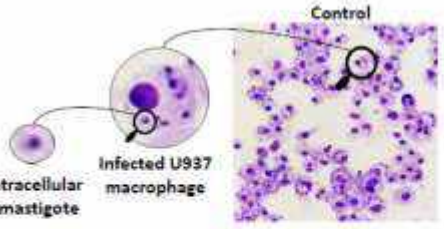


(a) Py, CH_2Cl_2, rt, 1h (b) H_2, Pd/C, EtOAc, rt, 48h (c) *p*-formaldehide, $NaBH_3CN$, AcOH, MeOH, rt, 2h (d) Acetic anhydride, CH_2Cl_2, rt, 30min (e) Formic acid, CH_2Cl_2, rt, 30min (f) Trichloroacetic acid, $NaBH_4$, THF, rt, 24h

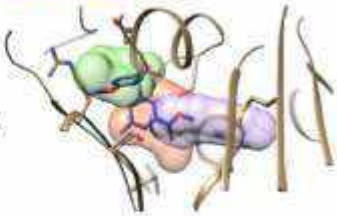
BIOLOGICAL ACTIVITY

Compound	*Leishmania infantum*			Citotoxicity 10µM (HeLa, HT-29, MCF7, U937)
	Promastigotes 10µM	Axenic amastigotes IC_{50} (µM)	Intracellular amastigotes 5-20µM	
1	Non Active	2.7	Active	Non Citotoxic
2	NA	6.9	A	NC
3	NA	1.1	A	NC
4	NA	2.2	A	Citotoxic
5	NA	4.5	A	C
6	NA	8.2	A	C
7	NA	9.2	A	C
8	NA	>10	A	NC
9	NA	>10	A	NC
10	NA	>10	A	NC
11	NA	>10	A	NC
12	NA	>10	A	NC
13	NA	>10	A	NC
14	NA	12	NA	NC
15	NA	5.75	NA	NC
16	NA	>10	A	NC
17	NA	>10	A	NC
18	NA	>10	A	NC
19	NA	>10	A	NC
20	NA	>10	A	NC
21	NA	>10	A	NC
22	NA	>10	A	NC
23	NA	>10	A	NC
Miltefosine	17.5	6.4	23.7	NC





DOCKING



CONCLUSIONS

Synthesis of a wide variety of compounds with a sulfonamide common structure was achieved. Non-cytotoxic compounds on human tumor cells lines have been evaluated as antileishmanial agents *in vitro* against *Leishmania infantum* promastigotes, axenic amastigotes (with IC_{50} in the micromolar range) and intracellular amastigotes (clearly interrupting the course of infection of U937 infected macrophages in the micromolar range). Docking studies and rational design of the compounds reveal a tubulin binding mechanism of action.

ACKNOWLEDGMENTS

Financial support came from Consejería de Educación de la Junta de Castilla y León (SA030U16, SA262P18 and SA116P20), co-funded by the EU's European Regional Development Fund-FEDER, the Spanish Ministry of Science, Innovation and Universities (RTI2018-099474-B00) and predoctoral fellowship from the Junta de Castilla y León (ORDEN EDU/529/2017)

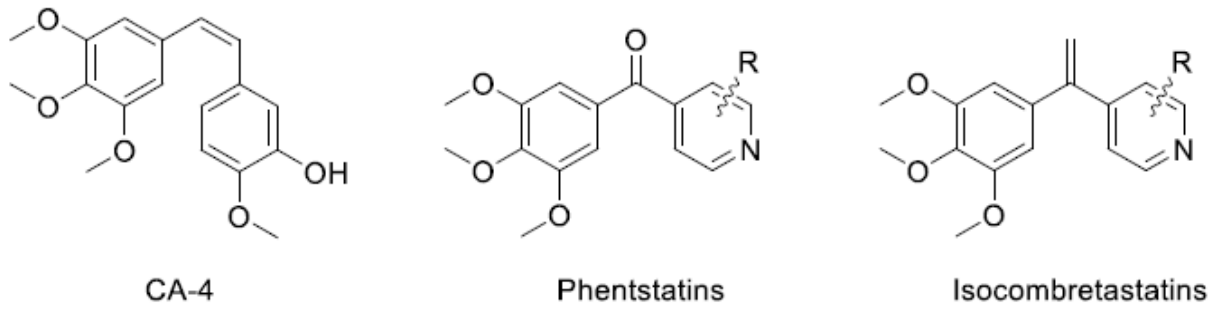
SYNTHESIS AND EVALUATION OF NOVELS CA-4 ANALOGS BASED ON PYRIDINE

Miguel Marín*, Raúl Fuentes, Youes Ellahioui, Laura Gallego-Yerga, Cristina Sanz, Myriam González, Alba Vicente-Blázquez, Sara del Mazo, Manuel Medarde, Esther Caballero, Raquel Alvarez, Rafael Peláez.

(1) Pharmaceutical Sciences Department, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno, 37007. Salamanca, Spain.

Restricted to organizers

The microtubule filament system of eukaryotic cells is an important drug target for anticancer agents. Combretastatin A4 is the most prominent member of a family of products which bind to the colchicine site of tubulin, destabilizing microtubules thus inhibiting polymerization.
CA-4 suffers from low water solubility; therefore, many modifications have been explored to provide new drugs with improved therapeutic profiles. In this work, we have replaced the olefinic bridge into nonisomerizable one-atom bridges such as the isocombretastatins or the phenstatins.



Synthesis and activity results will be presented.

Acknowledgements

Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA262P18 and SA0116P20).
Ministerio de Ciencia, Innovación y Universidades, Proyectos I+D+i «Retos Investigación» del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad RTI2018-099474-B-I00.
Fundación Memoria D. Samuel Solórzano Barruso FS/9-2018 and FS/18-2019

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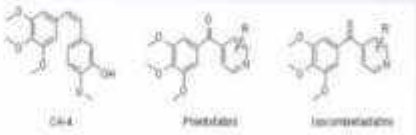
Introduction

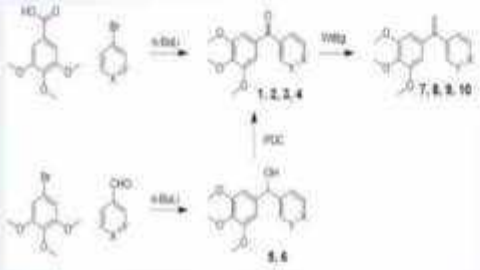
The microtubule filament system of eukaryotic cells is an important drug target for anticancer agents. Combretastatin A4 is the most prominent member of a family of products which bind to the colchicine site of tubulin, destabilizing microtubules thus inhibiting polymerization.

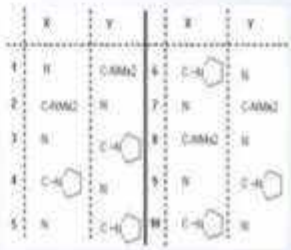
However, most of those products present different drawbacks such as low aqueous solubility or configurational instability. In this communication we have synthesized and evaluated novel CA4 analogs including a pyridine moiety. Also, we have replaced the olefinic bridge by nonisomerizable one-atom bridges in order to improve the pharmacokinetic profile.



Syntesis

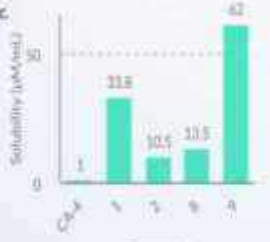
Performing the following synthetic route, several compounds were obtained:





Solubility and biological activity

The aqueous solubility of these compounds was measured spectrophotometrically in a pH=7.0 buffer and compared to Combretastatin A-4. The most remarkable results are shown in the following graphic.



Tubulin polymerization inhibition (TPI) assays were performed in order to compare their potency at a concentration of 5 μM.

COMP	INHIB (%)
1	0
2	0
3	35
4	23
7	0
8	11
9	16
10	25

Conclusions

The proposed synthetic route proved to be efficient in obtaining the desired compounds in good yields. These novel analogs showed a better solubility than CA-4 and some of them are potent tubulin inhibitors.

Acknowledgements:

Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA030U16 and SA262P18).
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Synthesis of edelfosine-loaded nanoparticles and effects on gastric cancer cells	***Restricted to organizers***

Julia Mayor-Pillado(1)*, Marzia Marciello (2), Marco Filice (2), Faustino Mollinedo (1).

(1) Laboratory of Cell Death and Cancer therapy, Biological Research Center Margarita Salas CSIC, E-28040 Madrid, Spain.
(2) Nanobiotechnology for Life Sciences´ Lab, Faculty of Pharmacy, Complutense University of Madird, E-28040 Madrid, Spain.

Although its incidence and mortality have declined over the last decade, gastric cancer (GC) is still one of the most frequently diagnosed and is the third leading cause of cancer death. Therefore there is an urgent need to find new therapeutic approaches that improve GC outcome.

The ether phospholipid edelfosine (EDLF), prototype of a promising family of anti-cancer drugs called alkylphospholipid analogs, induces apoptosis in a wide number of cancer cells (including cells from solid tumors), whereas normal cells are scarcely affected [1]. In order to increase EDLF bioavailability, improve its pharmacokinetic and pharmacodynamic parameters, as well as reducing off-target effects *in vivo*, EDLF encapsulation in nanoparticles (NPs) could be promising for its application in gastric cancer therapy.

Herein we present the design and synthesis of two different types of NPs containing EDLF (Figure1). Firstly, Solid Lipid Nanoparticles (SLNs), where EDLF is embedded inside the NPs in their solid lipid core and surrounded by a surfactant. Secondly, Superparamagnetic Iron Oxide Nanoparticles (SPIONs), composed by an iron oxide core, surrounded by oleic acid and where EDLF is conjugated on the surface of the NP. In this case we synthesized three formulations based on different initial core sizes and EDLF/Fe ratio. Thus, we generated different and new formulations of EDLF and studied their effects on two different gastric cancer cell lines (AGS and SNU-1), considered as situated at two ends of the spectrum of EDLF response. Our results show that SLNs need longer incubation times to achieve the same effect as free EDLF when used at the same drug concentration. On the other hand, our work have demonstrated that EDLF encapsulated on SPIONs is at least as effective as the free drug, and it could be more effective if formulated with certain core size and ratio EDLF/Fe. These results suggest that EDLF loaded in NPs could be a promising new approach for gastric cancer treatment.

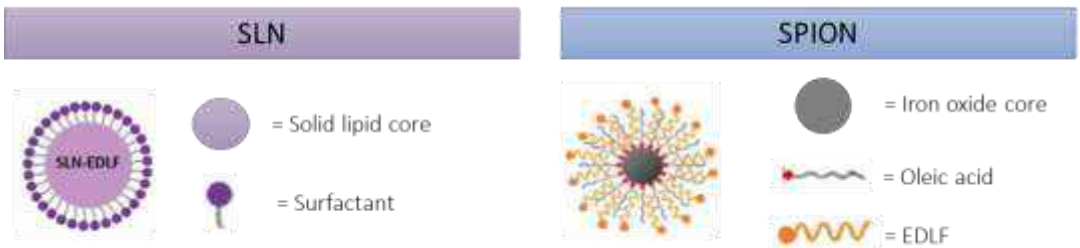

Figure 1 : Structure of two different–EDLF-nanoparticles.

Bibliographic references:

1. Gajate, C., Mollinedo, F. Blood 2007, 109, 711-719.

* Correspondence: juliamayorpillado@gmail.com

Synthesis of edelfosine-loaded nanoparticles and effects on gastric cancer cells

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(1) Laboratory of Cell Death and Cancer Therapy, Biological Research Center, CSIC, E-28040 Madrid, Spain
(2) Nanobiotechnology for Life Sciences' Lab, Faculty of Pharmacy, Complutense University of Madrid, E-28040 Madrid, Spain.

INTRODUCTION

The ether phospholipid **edelfosine** (EDLF), prototype of a promising family of anti-cancer drugs called alkylphospholipid analogs, induces apoptosis in a wide number of cancer cells whereas normal cells are scarcely affected [1]. In order to increase EDLF bioavailability, improve its pharmacokinetic and pharmacodynamic parameters, as well as reducing off-target effects *in vivo*, EDLF encapsulation in **nanoparticles** (NPs) could be promising for its application in gastric cancer therapy.

RESULTS

1. Design of different EDLF-loaded nanoparticles

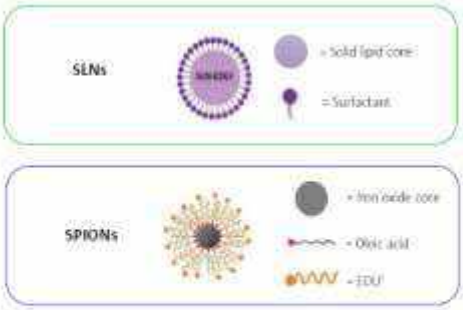

Figure 1: Structure of two different types of EDLF loaded nanoparticles: Solid Lipid Nanoparticles (SLNs) and Superparamagnetic Iron Oxide Nanoparticles (SPIONs).

2. Nanoparticle characterization

	EDLF-SLNs	EDLF-SPIONs
Size	71.08 nm	169.6 nm
PDI	0.249	0.142
EDLF	Inside	Outside
Use	Freeze-dried	Fresh

Figure 2: Summary of several characteristics of two types of edelfosine loaded nanoparticles.

3. Empty NPs cause no significant effect on gastric cancer cell lines

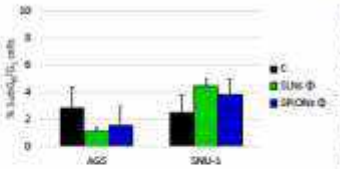

Figure 3: Cells were untreated (control) or incubated with empty nanoparticles (NPs). After 72 hours incubation cell death was measured by flow cytometry using percentage of subG$_0$/G$_1$ cells as an apoptosis indicator. There were no significant effect when using empty NPs, comparing to the control situation. Therefore effect caused by EDLF loaded NPs will be due to the drug, not the carrier.

4. EDLF-SLNs need longer incubation compared to free EDLF

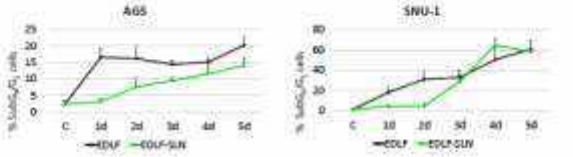

Figure 4: Cells were incubated either with free EDLF or EDLF-loaded in SLNs. Same dosage of EDLF was used in any of the formulations (free or encapsulated), 30 μM for AGS cells and 10 μM for SNU-1 cells. Cell death was measured daily by determining the percentage of subG$_0$/G$_1$ cells as an apoptosis indicator by flow cytometry. EDLF-SLNs need longer incubation times to cause the same effect as the free drug. EDLF-SLNs in AGS cells never equals free EDLF effect, but in SNU-1 cells, EDLF-loaded lipid NPs show a similar effect to EDLF after 3 days of incubation.

5. EDLF-SPIONs treatment is more effective than free EDLF

Figure 5: Cells were incubated either with free EDLF or EDLF-loaded SPIONs. Same dosage of EDLF was used in any of the formulations (free or encapsulated), 30 μM for AGS cells and 10 μM for SNU-1 cells. Cell death was measured after 72 hours incubation by flow cytometry (determining the percentage of subG$_0$/G$_1$ cells as an apoptosis indicator). EDLF-SPIONs are more effective than free EDLF, inducing a higher percentage of apoptotic cells at a given time than the free drug.

CONCLUDING REMARKS

1. Successful design and synthesis of two different types of nanoparticles loaded with EDLF. Empty nanoparticles have no effect on two different gastric cancer cell lines (AGS and SNU-1).
2. EDLF-SLNs need longer incubation times in order to be as effective as the free drug.
3. EDLF-SPIONs are more effective than free EDLF at a given time. Thus, these results suggest that a higher proapoptotic effect is obtained when EDLF is conjugated on the surface of the NP.

AKNOWLEDGEMENTS AND REFERENCES

This work was founded by Ministerio de Ciencia, Innovación y Universidades (SAF2017-89672-R).

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Conditional generation of free radicals by selective activation of alkoxyamines: towards more efficient and less toxic targeting of brain tumors.

P. Piris[(1)*], D. Buric[(1)], T. Yamasaki[(2)], A. Rodallec[(1)], M.-P. Montero[(1)], M. Rossi[(1)], C. Chacon[(1)], N. André[(1)], S. Combes[(1)], P. Brémond[(1)] and M. Carré[(1)]

(1) Aix-Marseille Université, CRCM, Inserm UMR1068, CNRS UMR7258, IPC, Faculté de Pharmacie, Marseille, France
(2) Aix-Marseille Université, ICR, CNRS UMR7273, Faculté des Sciences, Marseille, France

Restricted to organizers

Glioblastoma multiform (GBM) is the most common intracranial tumor observed in adults. Despite a significant progress in their clinical management, these tumors have one of the poorest prognoses for survival due to a poor response to therapeutic strategies. GBMs thus present unique challenges for identification of new drugs. Our previous results[(a),(b)] revealed the theranostic properties of alkoxyamines (R^1–ONR^2R^3), which undergo homolysis to generate: i) an alkyl radical (R^1•) that triggers the death program in GBM cells and ii) a nitroxide (R^2R^3NO•) that can enhance the MRI signal.

In the present project, we propose to improve the efficacy of alkoxyamines while controlling their homolysis by matrix metalloproteases (MMPs)-mediated hydrolysis.

We have synthesized a library of 85 novel alkoxyamines, and selected the most efficient molecule, *i.e.* K1, in inhibiting both GBM cell survival and migration in 2D cultures and 3D spheroids models. Results from a PBPK modeling supported that K1 is able to pass through the plasma membrane. Moreover, we showed that K1 early accumulated in GBM cells, triggered the generation of reactive oxygen species, and induced the fragmentation of the mitochondrial network that results in apoptosis. K1 was then bioconjugated to a peptide selectively recognized by MMPs[(c)]. This bioconjugate successfully inhibited survival, proliferation and invasion of the GBM spheroids. To further characterize its activity, we developed an innovative organotypic model based on the graft of stably-fluorescent GBM spheroids in *ex vivo* mice healthy brain slices. Response to treatment was daily monitored by live cell microscopy and fluorescence well scanning. It confirmed that K1-bioconjugate significantly impaired GBM progression, while no severe side-effects to the healthy tissue was observed.

Most of these results have been also extended to medulloblastoma, which is the most common malignant brain tumor in children. Based on structure-activity relationships, new derivatives are being synthesized to further increase the selective activation of alkoxyamines.

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* Correspondence: patricia.piris@etu.univ-amu.fr

CONDITIONAL GENERATION OF FREE RADICALS BY PEPTIDE-BIOCONJUGATED ALKOXYAMINES: TOWARDS MORE EFFECTIVE AND LESS TOXIC TARGETING OF BRAIN TUMOURS.

P. Piris[1], D. Buric[1], T. Yamasaki[2], A. Rodallec[1], MP. Montero[1], M. Rossi[1], C. Chacon[1], N. André[1], S. Combes[1], P. Bremond[1] and M. Carré[1]

[1]Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm UMR1068, CNRS UMR7258, Aix-Marseille Université UM105, Institut Paoli-Calmettes – Faculté de Pharmacie, Marseille, France
[2]Institut de Chimie Radicalaire, CNRS UMR7273, Aix-Marseille Université – Faculté des Sciences, Marseille, France

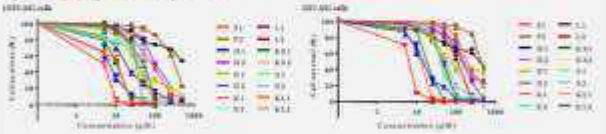
Biological and chemical context

Glioblastoma multiforme (GBM) is the most common intracranial tumor observed in adults. Despite a significant progress in clinical management over the last decade, these tumors have one of the poorest prognoses for survival due to a poor response to therapeutic strategies. GBMs are thus aggressive tumors that present unique challenges for identification of new drugs.
Our previous results showed fluorogenic properties for alkoxyamines TF-CHAMP (Marchal D. et al, Mol Pharmacol 2014; Yamasaki T. et al, Bioorg Med Chem 2019). These molecules can undergo homolysis to generate: 1) a highly toxic alkyl radical (R•), which trigger the cell death process in GBM cells; 2) a non-toxic nitroxide (TEMPO•), which can be used to enhance the MRI signal.
In the present project, we propose to improve the efficacy of the alkoxyamine while triggering their homolysis through peptide bioinducts by matrix metalloproteinases (MMP). Therefore, we have synthesized a range of 15 novel alkoxyamines as putative prodrugs for GBM. End-point and real-time cytotoxicity assays in 2D cultures as well as in 3D spheroids of GBM human cells led to a selection of the most efficient molecule, i.e. K1.

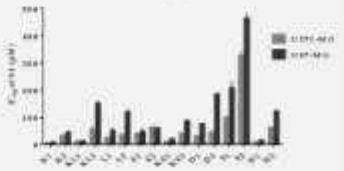
The latter has shown better efficacy in transformed cancer cells (including GBM stem cells) than in cells from other cancer types. K1 was then bioconjugated to a peptide selectively recognized by MMP (Chow T et al, Mol Pharm 2014). This bioconjugate successfully inhibited survival, proliferation, migration and invasion of the GBM cells.
To further characterize its activity, we developed an innovative organotypic model based on the graft of stably-fluorescent GBM spheroids in ex vivo mice normal brain slices. Response to treatment was daily monitored by live cell microscopy and high-resolution fluorescence well scanning, and showed that K1-bioconjugate was promising in inhibiting GBM progression in this co-culture system. No severe side-effects to the healthy tissue were observed at experiment completion.
Lastly, results from a Physiologically-Based Pharmacokinetics analysis (PBPK) supported that K1 was a good candidate for further preclinical tests in brain tumors. Moreover, the results obtained are being transposed on **medulloblastoma**, the most common malignant brain tumor in children.

I. Screening and selection of alkoxyamines for bioconjugation

Cytotoxicity of a panel of alkoxyamines

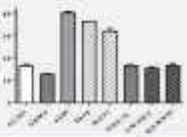


In alkoxyamines were selected to be tested for cytotoxicity (MTT assay) in U251-MG and U87-MG glioblastoma cell lines 2D cultures. IC_{50} values were determined and compared to the current standard of care Temozolomide (TMZ). **The alkoxyamines K1, K11, K31 and H1 showed the highest efficacy in the two GBM cell lines and was selected for further investigation.**

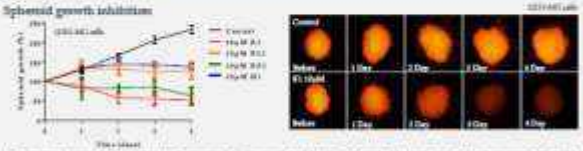


K1 efficacy in other cancer cell types

K1 was also highly active in murine GBM cells, in **GBM stem-like** cells, as well as in **medulloblastoma** cells (ONS76, UW228 and HDMB03) of cerebellar origin.
An invasive cells from **lung** (A549) and **breast** (MCF7) adenocarcinomas or from **neuroblastoma** (SHEP) were 2 to 5 times less responsive to K1.

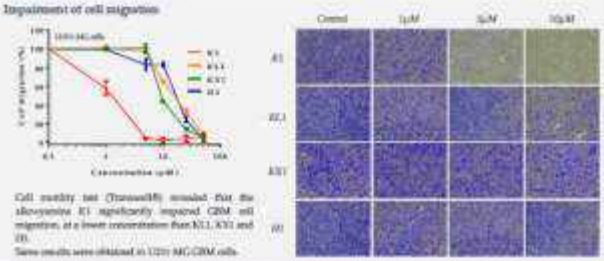


Spheroid growth inhibition



MB spheroid growth was monitored by measuring fluorescence over time. Treatment of these tumor 3D microtissues confirmed the efficacy of the alkoxyamines K1, K11, K31 and H1 in GBM models. Once again, K1 showed the highest efficacy.
Same results were obtained in U87-MG glioblastoma models.

Impairment of cell migration

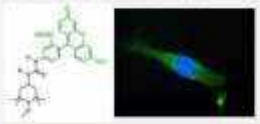


Cell motility test (Transwell®) revealed that the alkoxyamine K1 significantly impaired GBM cell migration, at a lower concentration than K11, K31 and H1.
Same results were obtained in U251-MG GBM cells.

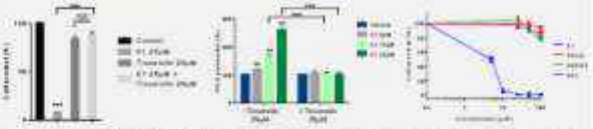
II. Mechanism of action

Intracellular incorporation of alkoxyamines

GBM cells were exposed to a highly stable **alkoxyamine coupled with the fluorochrome** FITC, which showed a **cytoplasmic accumulation** after only 2h.
Same results have been obtained with K1-FITC.

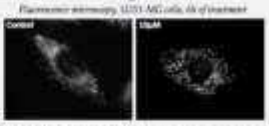


Increase in ROS production as a result of alkyl radicals release



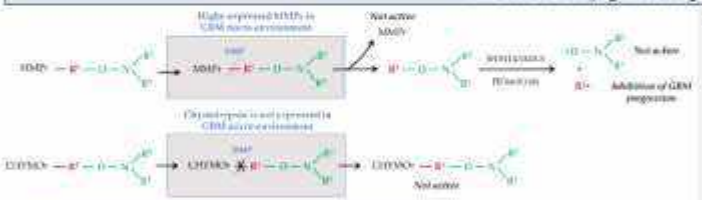
The alkoxyamine caused a dose-dependent increase in endogenous superoxide production (at 6h) by GBM cells. Inactivation of the alkyl radical (using the scavenger Tirosantin or HEOBS/PEOH2 derivatives) had a reverse effect on the cytotoxic activity of K1, by preventing the increase in superoxides. The nitroxyl radical (SG1) was not involved in K1 activity.

Induction of mitochondrial fragmentation

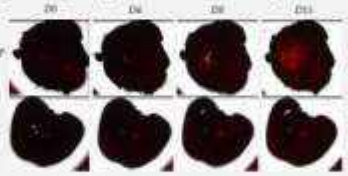


Treatment with K1 results in an **early fragmentation of the mitochondrial network**, suggesting a disruption of mitochondrial functions that triggers generation of ROS, key step in the alkoxyamine-induced apoptotic cell death.
Similar results were obtained in U87-MG GBM cells and both in ONS-76 and UW-228 medulloblastoma cells.

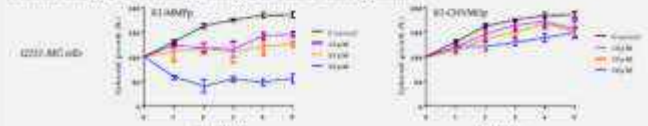
III. K1 bioconjugation to peptides



Selective targeting of brain organotypic GBM models by K1-MMPy

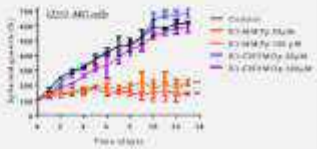


Selective activity of the K1-MMPy bioconjugate towards GBM spheroids



The alkoxyamine **K1, conjugated to a target peptide of MMPs** (MMPy), showed significantly a selective activity that was not observed when K1 was conjugated to a control peptide recognized by chymotrypsin (CHYMOp). Similar results were obtained in U87-MG, ONS-76, UW228-3 and HDMB-03 cells.

We developed an organotypic model based **on the grafting of stably-fluorescent GBM spheroids in ex vivo mice normal brain slices.**
Response to treatment was daily monitored by live microscopy and high resolution well scanning, showing that K1-MMPy was highly effective in inhibiting GBM progression. No severe side-effects to the healthy tissue were observed at experiment completion (data not shown). Same results were obtained in U87-MG model.



Conclusion and perspectives

This work led to the selection of the alkoxyamine K1 for its cytotoxic and anti-invasive properties, and to its bioconjugation to a peptide specifically recognized by MMPs to be activated in the tumor microenvironment.
The project is now focused on : (i) the synthesis of halogen derivatives of K1 to increase the selectivity of alkoxyamines for MMPs (Tranchant I. et al., 2014)
(ii) the generation of conditional fluorescent alkoxyamines (redox-sensitive) to monitor, in real time, the homolytic process

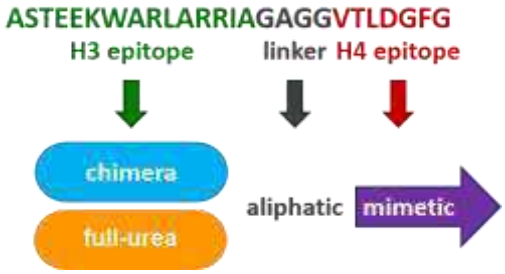
Application of urea-based foldamers to the design of ligands targeting histone chaperone proteins.	***Restricted to organizers***

Marie E. Perrin (1)*, May Bakail (1), Johanne Mbianda (2), Bo Li (2), Gwenaëlle Moal (1), Céline Douat (3), Raphaël Guérois (1), Gilles Guichard (2), Françoise Ochsenbein (1).

(1) CEA Saclay/UMR9198/I2BC/CNRS/UPSud, 91191 Gif-sur-Yvette
(2) IECB/CBMN/UMR5248/CNRS/UBordeaux, 33607 Pessac
(3) Department Pharmazie, LMU, D-81377 Münich, Germany

Histone chaperones are key actors in genome integrity maintenance, they escort histones and assist their deposition on DNA, thus contributing to chromatin dynamics.[a] Among them, the histone chaperone ASF1 (Anti-Silencing Function 1), which handles the histone H3-H4 dimer, was recently shown to constitute a new target in cancers.[b-c] Consistently, ASF1 plays an important role in cell growth and proliferation.[d] Its depletion sensitizes cells to doxorubicin, a drug currently used in chemo- and radiotherapies.

Our team initiated the design of ASF1 inhibitors competing with its association with the H3-H4 dimer. Taking advantage of the high resolution structure of the human ASF1A-H3-H4 complex, a first generation of inhibitory peptides was designed on a rational based strategy, combining epitope tethering and optimization of interface contacts.[e] The designed peptides reached binding affinities in the nanomolar range for ASF1 and showed anti-tumoral properties on cancer cell lines and in mouse allograft models.[f] However, their biological activity was largely impaired by their poor bioavailability and significant sensitivity to protease degradation. The objective of my thesis project is to generate a second generation of inhibitors based on urea-based foldamers/chimeras that showed improved resistance to proteolysis.[g] The last generations of ASF1 inhibitors, integrating full-length peptidomimetics as well as full-urea helixes, will be presented.



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[f] Bakail, M., et al., Cell Chem Biol, 2019. 26(11) : p. 1573-1585.e10
[g] Mbianda, J. et al., ChemRxiv, 2020

* Correspondence: Marie.PERRIN@cea.fr

Application of Urea-Based Foldamers to the Design of Ligands Targeting the Histone Chaperone ASF1

Marie E. Perrin[1], Moy Bokoll[1], Johanne Mbonda[2], Bo Li[1], Gwenaëlle Mool[1], Céline Douat[1], Raphaël Guérois[1], Gilles Guichard[2], Françoise Ochsenbein[1]

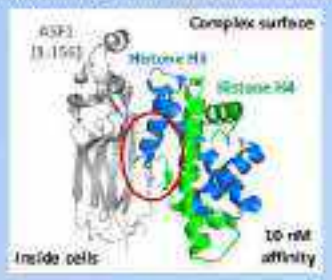
[1]CEA Saclay/Joliot/SB2SM, UMR9198/I2BC/CNRS/Université Paris-Sud – 91191 Gif-sur-Yvette Cedex, France

[2]IECB, CBMN UMR5248 – CNRS/Université de Bordeaux, 2 rue Robert Escarpit – 33607 Pessac, France

[3]Department Pharmazie, Ludwig-Maximilians-Universität München, Butenandtstraße 5-13 – D-81377 Munich, Germany

Histone chaperones play a central role in the maintenance of the chromatin landscape. They are key actors in maintaining genome integrity: they escort histones and ensure their provision and placement onto DNA, thus contributing to chromatin dynamics. In this family, the histone chaperone ASF1 (Anti-Silencing Function 1) handles the histone H3–H4 dimer. **This protein constitutes a new epigenetic target for cancer treatment.**

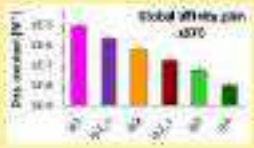
Our team is focusing on vectorized therapeutic inhibitors of the ASF1 protein. As the interaction between ASF1 and its histone dimer is complex, several requirements should be met for the design of inhibitors: **(i) nanomolar affinity (competition with PPI), (ii) cell penetration and (iii) stability in intra- and extracellular conditions.**

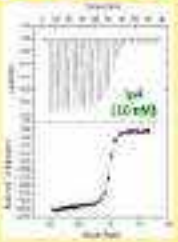


1st generation: interface optimization of high-affinity peptides

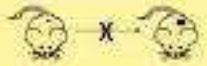
Starting point
High resolution structure of hASF1–H3–H4 complex (PDB: 2I0S)

Rational design strategy
- Epitope tethering
- Optimization of interface contacts





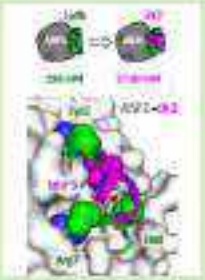
✓ Significant results
Conjugated to CPP
→ anti-proliferative action
Aggressive breast cancer cells
→ anti-tumoral properties



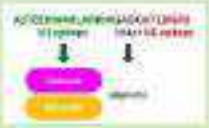
✗ **Two drawbacks using peptides**
Poor bioavailability, high sensitivity to protease degradation

2nd and 3rd generations: An original ip4-peptidomimetic strategy based on urea foldamers

Properties of foldamers
→ high stability
→ low immunogenicity
→ well-defined structure
(mimic side-chains of peptides)



ASF1-ch2 complex: striking plasticity of the urea backbone
→ adaptation of ch2 to ASF1 surface for optimal interaction
Resistance of the chimera to proteolysis was highly improved



Full-urea helices

Addition of H4 epitope

Synthesis of antiplasmodial thienopyrimidinone hit analogs using C6-functionnalization or scaffold hopping strategy.

R. Mustière[1], V. Dell'Orco [1], S. Hutter [2], S. Tajeri [3], N. Amanzougaghene [3], N. Azas [2], D. Mazier [3], P. Verhaeghe [4], P. Vanelle [1]*, N. Primas [1].

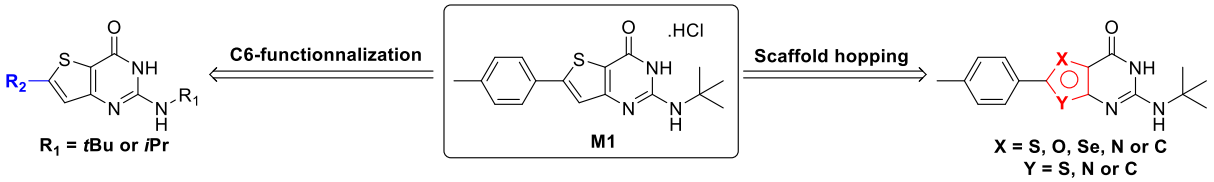
(1) Aix Marseille Univ, CNRS, ICR UMR 7273, PCR, Faculté de Pharmacie, 13385 Marseille, France.
(2) Aix Marseille Univ, IHU Méditerranée Infection, UMR VITROME, 13005 Marseille, France.
(3) Sorbonne Université, CNRS/INSERM, CIMI, 75005 Paris, France.
(4) Université Paul Sabatier, CNRS UPR 8241, LCC, 31077 Toulouse, France.

Restricted to organizers

Malaria is the deadliest parasitic infection with an estimate of 409 000 deaths and 229 million cases worldwide in 2019.[(a)] The disease is caused by a parasite of the *Plasmodium* genus and transmitted by mosquitoes of the *Anopheles* genus.
The lead compound of a series of 2-aminothienopyrimidinones, called M1, showed an antiplasmodial multi-stage activity (liver, blood and sexual stages) *in vitro* with nM values associated with a low cytotoxicity.[(b)] Its mechanism of action is unknown and differs from the commercial antimalarial drugs. *In vivo* activity on *P. yoelii* infected mouse model was achieved but only with the use of CYP450 metabolism inhibitors (1-aminobenzotriazole, ABT).
The goals of this project are to obtain analogs of M1 with improved solubility and metabolic stability while keeping the same activity, and to continue the initial Structure-Activity Relationships (RSA) study. The synthesis work is divided into two parts:

1. Modulation of position 6 of the thienopyrimidinone core using Suzuki-Miyaura, Sonogashira or Buchwald-Hartwig palladium-catalyzed couplings.
2. A scaffold hopping strategy focused on the thiophene cycle to explore different five-membered aromatic rings.

Obtained analogs are tested on *in vitro P. falciparum* blood stage and the best ones are tested on *in vitro* hepatic stage (in murine and human hepatocytes). Synthetic details and biological results will be presented in the communication.



This work is supported by the "Fondation pour la Recherche Médicale" (FRM) – Project code DCM20181039565

Bibliographic references:

[(a)]World Health Organization. (page consulted on 15/12/20). World Malaria Report 2020. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020

[(b)]Cohen, A. *et al*. Discovery of new thienopyrimidinone derivatives displaying antimalarial properties toward both erythrocytic and hepatic stages of *Plasmodium*. *Eur. J. Med. Chem*. **2015**, 95, 16 28.

* Correspondence: patrice.vanelle@univ-amu.fr

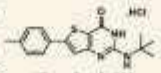
Synthesis of antiplasmodial thienopyrimidinone hit analogs using C6-functionnalization or scaffold hopping strategy

Romain Mustière,[a] Viviana Dell'Orco,[a] Sébastien Hutter,[b] Shahin Tajeri,[c] Nadia Amanzougaghene,[c] Nadine Azas,[b] Dominique Mazier,[c] Pierre Verhaeghe,[d] Patrice Vanelle[a,*] and Nicolas Primas[a]

[a]Aix Marseille Univ, CNRS, ICR UMR 7273, PCR, Faculté de Pharmacie, 13385 Marseille, France.
[b]Aix Marseille Univ, IHU Méditerranée Infection, UMR VITROME, 13005 Marseille, France.
[c]Sorbonne Université, CNRS/INSERM, CIMI, 75005 Paris, France.
[d]Université Paul Sabatier, CNRS UPR 8241, LCC, 31077 Toulouse, France.
Email : patrice.vanelle@univ-amu.fr

Global fight against malaria is endangered by multiple factors including the spread of artemisinin-resistant *P. falciparum* strains.[1] Discovery of new antimalarial drugs with novel mechanisms of action is critical for this fight.

Following a chemical library screening, hit molecule M1 was discovered after an initial round of pharmacomodulations.[2] More pharmacomodulations are needed to improved aqueous solubility and metabolic stability, while maintaining the same phenotypic profile.



Hit molecule M1

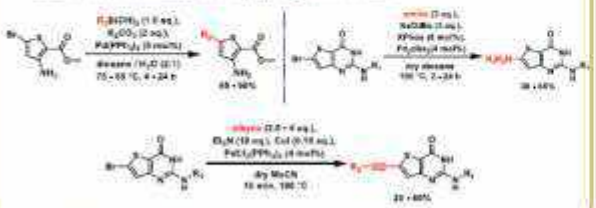
- Multi-stage activity
  - *Pf*K1 IC_{50} = 45 nM / CC_{50} HepG2 = 24 µM
  - *P. yoelii* IC_{50} (HepG2) = 35 nM
  - % inhib exflagellation (at 10 µM) = 90%
- In vivo activity
- Active against artemisinin-resistant strain F32-ARTS
- Non-mutagenic / Non-genotoxic

- Metabolic liabilities
  - $t_{1/2}$ = 10 min
- Poor solubility

1. C6-Functionnalization

Starting from methyl 3-amino-5-bromothiophene-2-carboxylate, the thieno[3,2-*d*]pyrimidinone core is obtained by a 3-steps one pot synthesis.

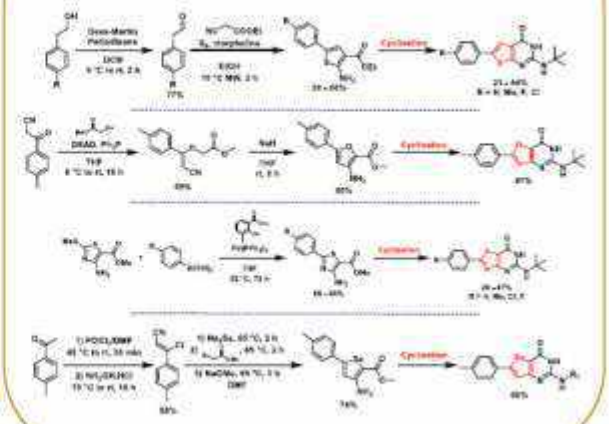
Occurring before or after this step, the bromine atom is used for pallado-catalyzed couplings (Suzuki-Miyaura, Sonogashira and Buchwald-Hartwig) to obtain C6-functionnalized thieno[3,2-*d*]pyrimidinones.



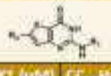
This process (including further steps if needed) led to the synthesis of 58 new compounds.

2. Scaffold hopping

The five membered aromatic ring in the thieno[3,2-*d*]pyrimidinone core was modified for SAR study purpose. Four new cores were synthesized.

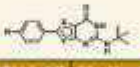


3. Biological results



R1	R2	IC_{50} PfK1 (µM)	CC_{50} HepG2 (µM)	SI
tBu		0.4	> 12.5	> 31.2
		3.1	25.3	8.1
		2.5	> 12.5	> 5
		0.8	9.2	11
iPr		2.5	19.5	7.8
tBu		6	52	8,7
iPr		10	> 50	> 5
tBu		0.7	> 12.5	> 17.9
		0.8	> 12.5	> 15.6
		1.1	29.7	27
iPr		0.5	23.6	47.2
M1		0.035	24	685
Chloroquine		0.8	30	37.5
Atovaquone		0.013	> 15.6	> 12000
Doxorubicine		-	0.2	-

R1	R2	IC_{50} PfK1 (µM)	CC_{50} HepG2 (µM)	SI
tBu		2.35	> 12.5	> 5.3
iPr		4.6	3.1	0.7
tBu		1.9	20.8	10.9
		> 10	> 50	< 5



R	X	Y	IC_{50} PfK1 (µM)	CC_{50} HepG2 (µM)	SI
H	C	S	33.8	> 12.5	> 0.4
Me			32.4	20.5	0.63
Cl			> 50	21.4	< 0.4
Me	O	C	0.8	10.9	13.6
	Se		1.1	> 7.8	> 6

Compounds below 1 µM activity on *Pf*K1 will be further assessed on hepatic stage malaria (*P. yoelii*).

Regarding Suzuki-Miyaura series, many compounds' tests are ongoing including substituted vinylogues and phenyl derivatives with sulphur functions.

Further developments on the alkyne derivatives may be stopped if the upcoming results are not satisfying. Work is ongoing on copper-catalyzed azide-alkyne cycloaddition reactions to provide triazoles.

Scaffold hopping strategy exposed the important role for activity of a heteroatom at the position 5. Tests are ongoing for thiazolo[4,5-*d*]pyrimidinone series. Work is continued on the furo[3,2-*d*]pyrimidinone series and the synthesis of the thiazolo[5,4-*d*]pyrimidinone analog of M1.

This work is supported by the "Fondation pour la Recherche Médicale" (FRM) – Project code DCM20181039565

References :
1. World Health Organization. (page consulted on 22/01/21). World Malaria Report 2020. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020
2. Cohen, A. *et al.* Discovery of new thienopyrimidinone derivatives displaying antimalarial properties toward both erythrocytic and hepatic stages of Plasmodium. *Eur. J. Med. Chem.* 2015, 95, 16-28.

Design and synthesis of new pyrazole and imidazo-pyrazole compounds with dual activity.

Federica Rapetti*, **Chiara Brullo, Olga Bruno**

University of Genoa, Department of Pharmacy, V.le Benedetto XV, 3-16132 Genova-Italy

Restricted to organizers

To obtain anti-inflammatory compounds with dual mechanisms, we combined, by an acylhydrazone linker, the pyrazole and imidazo-pyrazole scaffolds, typical of compounds previously synthesized[(a)] and able to block IL-8 and fMLP-induced chemotaxis respectively, with the catecholic groups of our Phosphodiesterase 4 inhibitors (PDE4Is)[(b)]. New compounds **1** and **2** (Fig. 1) showed many and interesting activities: pyrazole derivatives **1** evidenced a good ROS production and platelet aggregation inhibition[(c)], whereas imidazo-pyrazole **2** have a great antioxidant and potent antiproliferative activity (on A2780 human ovarian carcinoma cell line, A549 adenocarcinomic human alveolar basal epithelial cell line and IMR-32 human neuroblastoma cell line with a LC_{50} in the micromolar range), probably by interaction with microtubular system[(d)].

Starting from these great and multiple results, after deeply SAR investigations, we design and synthesize other chimeric compounds, making some chemical changes, particularly:

- insertion of an additional methyl group (compounds **3** and **4**);
- replacement of the phenyl ring on N1 chain with a more flexible and linear butyl chain (compounds **5**);
- moving the acylhydrazone linker to obtain specular derivatives (compounds **6** and **7**).

Synthetic procedures and detailed biological results will be presented during the oral presentation.

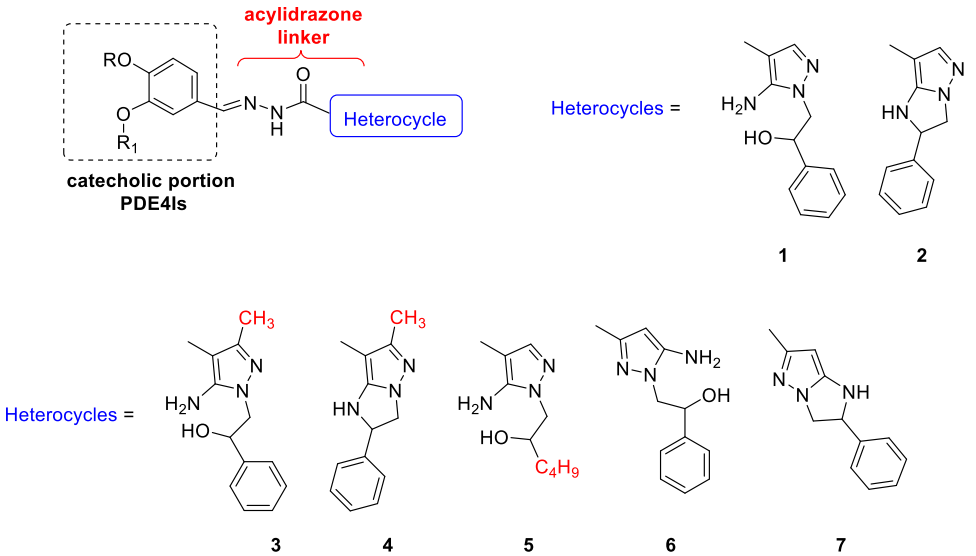


Figure 1. Chemical structures of compounds **1-7**.

Bibliographic references:

[(a)] Brullo C. et al. *Eur. J. Med. Chem.*, **2012**, *47*, 573–579.

[(b)] Prosdocimi T. et al. *Biochemistry*, **2018**, *57*, 2876−2888.

[(c)] Brullo C., Massa M., Rapetti F. et al. *Molecules*, **2020**, *25*, 899-917.

[(d)] Brullo C., Rapetti F. et al. *ChemMedChem*, **2020**, *15*, 1–10.

* Correspondance: federica.rapetti@edu.unige.it

XXVIIIth Young Research Fellows' Meeting Società Italiana Chimica Farmaceutica

Design and synthesis of new pyrazole and imidazo-pyrazole compounds with dual activity.

Federica Rapetti[1]*, Chiara Brullo[1], Olga Bruno[1]

[1] University of Genoa, Department of Pharmacy, V.le Benedetto XV, 3-16132 Genova-Italy; *Correspondance: federica.rapetti@edu.unige.it

1 Introduction

Inflammation is a non-specific innate defense mechanism and a protective response to a cellular or tissue damage, involved in many diseases; it removes the cause of the damage and start the reparative processes.

Inflammation involves many **cell types** (e.g. macrophages, lymphocytes, platelets) and **chemical mediators** (e.g. cytokines, coagulation factors, neurotransmitters, ROS and NO). Moreover, **chronic inflammation** caused several and different diseases, such as diabetes, hypertension, heart disease, rheumatoid arthritis, and even cancer. Among these, **neuroinflammation**, characterize neurodegenerative diseases, such as Alzheimer's disease (AD) and Multiple Sclerosis (MS).

2 Background

In the past years, we synthesized different **Phosphodiesterase 4 inhibitors** (PDE4is), characterized by catecholic groups variously substituted[2], which inhibited selectively PDE4D3 with significant increase in memory and cognitive function; moreover, some of them, reduce superoxide anion and increase intracellular cAMP level. At the same time, we obtained a large library of compound with **pyrazole and imidazo-pyrazole** scaffolds[3], able to block IL-8 and fMLP-induced chemotaxis respectively, showing a great activity.

To obtain compounds with dual **anti-inflammatory activity** by both inhibition of ROS production and inhibition of PDE4, we decided to combine, by an acylhydrazonic linker, the pyrazole and imidazo-pyrazole scaffolds, previously synthesized[3], with the catecholic groups of our PDE4Is[2].

New compounds 1 and 2 showed many and interesting activities: **pyrazole** derivatives 1 evidenced a good **ROS production** and **platelet aggregation inhibition**[4], whereas **imidazo-pyrazole** ones 2 have a great **antioxidant** and **potent antiproliferative activity** (on A2780 human ovarian carcinoma cell line, A549 adenocarcinomic human alveolar basal epithelial cell line and IMR-32 human neuroblastoma cell line with a IC_{50} in the micromolar range), probably by interaction with **microtubular system**[4].

3 New Compounds

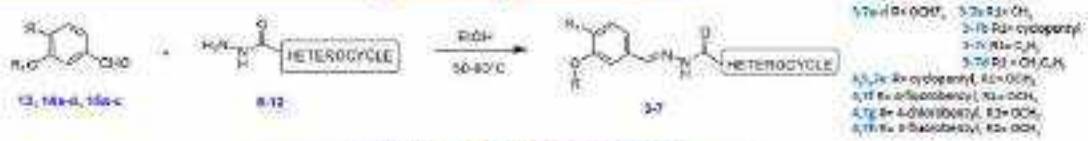
Starting from these results we design and synthesize other chimeric compounds, making some chemical changes:

-in compounds 3 and 4, we inserted an additional **methyl group**;

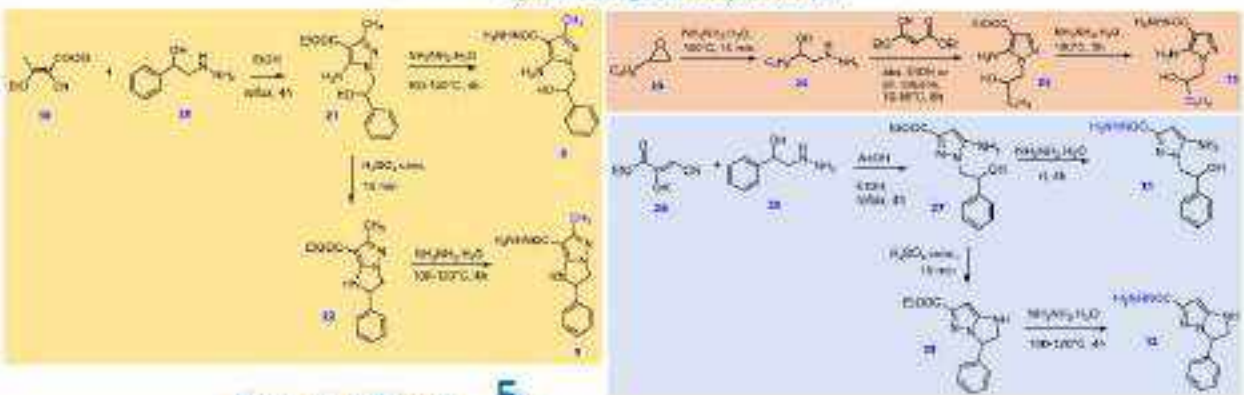
-in compounds 5, we replaced the phenyl ring on N1 with a **butyl chain**;

- in compounds 6 and 7,we moved the acylhydrazone linker to obtain **specular derivatives**.

4 Synthetic procedures



Synthesis of carbohydrazides



5 Biological Results

All compounds will be tested to evaluate:

- ROS inhibition on platelet;
- antiproliferative activity on different cancer cell lines;
- PDE4 inhibition.

The most active compounds will be submitted on molecular docking study on tubulin system.

References

[1] Prosdocimi T. et al. Biochemistry, 2018, 57, 2876–2888.
[2] Brullo C. et al. Eur. J. Med. Chem., 2012, 47, 573–579.
[3] Brullo C., Masse M., Rapetti F. et al. Molecules, 2020, 25, 899-917.
[4] Brullo C., Rapetti F. et al. ChemMedChem, 2020, 15, 1–10.

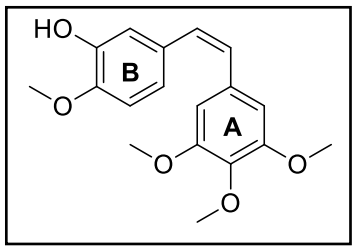
Synthesis and evaluation of new combretastatin analogues.	***Restricted to organizers***

Raúl Fuentes *, Myriam González, Alba Vicente-Blázquez , Laura Gallego-Yerga, Sara del Mazo, Sergio Ramos, Miguel Marín, Cristina Sanz, Younes Ellahioui, Esther Caballero, Raquel Alvarez, Manuel Medarde, Rafael Peláez.

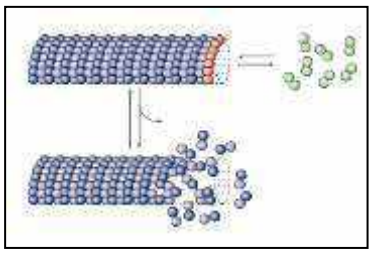
Pharmaceutical Sciences Department, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno, 37007. Salamanca, Spain.

Microtubules, polymers of α-tubulin and β-tubulin, are key components in the process of mitosis. Because of that, microtubules disrupting agents are used in anticancer chemotherapy. This kind of drugs interrupts the polymerization dynamic of tubulin, crucial for the cell division. Ligands which bind to colchicine domain, with destabilizing effect over the microtubule polymerization, are one of the most important groups.[1]

Combretastatins are an aggrupation of natural compounds with these characteristics, among which the combretastatin A-4 (CA4) stands out as the reference ligand. CA4 consists of a 1,2-diarylethene scaffold with a cisoid disposition, a structural point that preserves the antimitotic activity.[2]



Combretastatin A-4



Polymerization dynamics

Colchicine site ligands have not only antimitotic activity, but also a vascular disrupting effect.[2] Despite trimethoxyphenyl ring (A-ring) confers high potency to combretastatin derivatives, it is also responsible for low aqueous solubility. For this reason, this project is focused on finding an A-ring replacement that increase water solubility of antimitotic compounds. A methoxy group has been replaced by several nitrogenated substituents. Synthesis methodology, physicochemical assays and results on tubulin polymerization inhibition and cytotoxicity will be analysed.

Acknowledgements:

Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA262P18 and SA0116P20) Ministerio de Ciencia, Innovación y Universidades. Proyectos I+D+i "Retos Investigación" del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad RTI2018-099474-B-100.
Fundación Memoria D. Samuel Solórzano Barruso (FS/9-2018 and FS/18-2019)

Bibliographic references:

(a) Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004; 4(4):253-65

(b) Tron GC, Pirali T, Sorba G, Pagliai F, Busacca S, Genazzani AA. Medicinal chemistry of combretastatin A4: present and future directions. J Med Chem. 2006; 49(11):3033-44.

* Correspondence: raulfuentes@usal.es

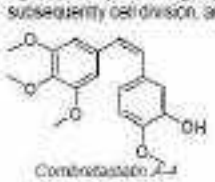
Synthesis and evaluation of new combretastatin analogues

Raul Fuentes [1], Myriam González [1], Alba Vicente-Blanques [1], Laura Gallego-Yerga [1], Sara del Moro [1], Sergio Ramos [1], Miguel Marín [1], Cristina Sanz [1], Younes Ellahioui [1], Esther Caballero [1], Raquel Alvarez [1], Manuel Medarde [1], Rafael Peláez [1].

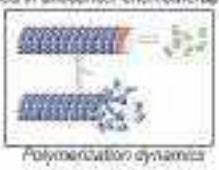
(1) Pharmaceutical Sciences Department, Faculty of Pharmacy, University of Salamanca, Campus Miguel de Unamuno, 37007, Salamanca, Spain.

INTRODUCTION

Microtubules, polymers of α-tubulin and β-tubulin, are key components in the process of mitosis. Because of that, antimitotic agents, that prevent right polymerization dynamic of tubulin and subsequently cell division, are used in anticancer chemotherapy.



Combretastatin A-4

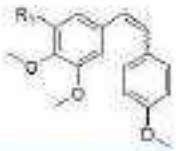

Polymerization dynamics

Combretastatin A-4 (CA4) stands out as the reference ligand from a group of drugs which destabilize the microtubule polymerization by binding to colchicine domain. Despite providing strong cytotoxicity, the trimethoxyphenyl ring (A-ring) of CA4 is the main responsible for the low aqueous solubility of the molecule.

In this work we have explored A-ring replacements that increase water solubility of antimitotic compounds. We have synthesized and evaluated novel antimitotic agents in which replacement of one of the methoxy groups by several nitrogenated substituents was carried out.

SYNTHESIS

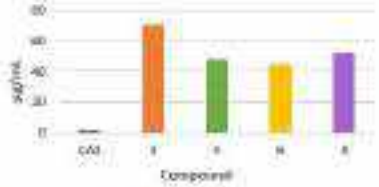
By means of Wittig reaction between the corresponding aldehyde and the phosphonium salt, the following compounds were obtained:



Compound	R_1	Isomer
1	$-NO_2$	Z+E
2	$-NH_2$	Z+E
3	$-N(CH_3)_2$	Z+E
4	$-N^+(CH_3)_3$	Z
5	-NHAc	Z+E
6	$-NCH_2Ac$	Z+E
7	$-NCH_2Boc$	Z+E
8	$-NHCH_3$	Z
9	$-NHCH_3$	E

SOLUBILITY

The aqueous solubility of the analogues synthesized was measured spectrophotometrically in a pH=7.0 buffer and compared to Combretastatin A-4.



BIOLOGICAL ACTIVITY

Tubulin polymerization inhibition (TPI) assay and the half maximal inhibitory concentration (IC_{50}) of this test were studied in order to compare their potency with the Combretastatin A-4 (IC_{50}= 2 μM).

Compound	TPI Conc. (μM)	%	IC_{50} (μM)
2	5	74	6,2
3	5	92	1,1
4	5 20	16 82	- -
5	5	5	-
6	5	25	-
7	5	14	-
9	20 5	43 85	- 0,8

CONCLUSIONS

In general, replacement of a methoxy group in the trimethoxyphenyl ring (A-ring) of CA4 by several nitrogenated substituents leads to an increase in the water solubility profile. Regarding the ammonium salt (4), increase considerably the aqueous solubility of their neutral analogue, showing a solubility of 1mg/mL.

In general, combretastatins in which a methoxyl group of the A-ring has been replaced decrease the inhibition of the microtubule polymerization, in comparison with CA4. However, when has been replaced by a free or methylated amino group maintained or even improved the activity.

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ACKNOWLEDGEMENTS

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Synthesis and evaluation of new antimitotic compounds with a tetrazole ring

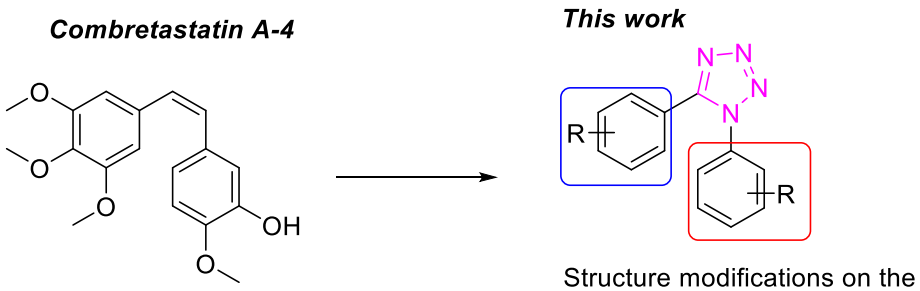
Sara del Mazo[(1)*], Myriam González[(1)], Alba Vicente-Blázquez[(1)], Sergio Ramos[(1)], Miguel Marín[(1)], Cristina Sanz[(1)], Raúl Fuentes[(1)], Pilar Puebla[(1)], Laura Gallego[(1)], Raquel Álvarez[(1)], Manuel Medarde[(1)], Rafael Peláez[(1)].

(1) Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno, E-37007 Salamanca, Spain.

Restricted to organizers

Microtubules, composed of α and β tubulin heterodimers, are involved in numerous cellular processes such as cell division, as they form the highly dynamic mitotic spindle through a process of polymerization and depolymerization. Perturbation of these tubulin dynamics makes microtubules an attractive target for anticancer therapeutics. Combretastatins are natural products that bind to the colchicine site of tubulin and strongly inhibit tubulin polymerization. One compound of this family, combretastatin A-4 (CA-4), shows potent cytotoxic activity, although its main disadvantages are the isomerization of its *cis*-configured double bond, essential for the antitumor activity, and its low aqueous solubility.

We have been focused on replacing CA-4 double bond with a heterocyclic tetrazole ring to restrict the *cis* conformation. Structural modification on the aryl groups were carried out to explore the effect on antitumor activity. In this work the synthetic route leading to a new series of compounds will be presented, as well as their biological evaluation.



Structure modifications on the aryl groups and the bridge

Bibliographic references :

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Acknowledgements

Financial support came from Consejería de Educación de la Junta de Castilla y León and FEDER funds (SA030U16, SA262P18, SA116P20), Ministerio de Ciencia, Innovación y Universidades, Proyectos I+D+i «Retos Investigación» del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad. (RTI2018-099474-B-100) and Fundación Memoria Samuel Solórzano Barruso (FS/9-2018, FS/18-2019). S.M, M.G and M.M acknowledge Consejería de Educación de la Junta de Castilla y León and European Social Fund for a predoctoral fellowship. A.V, the Spanish MINECO for a predoctoral FPU fellowship. S.R and R.F, the University of Salamanca for a predoctoral fellowship.

* saradelmazo@usal.es

Synthesis and evaluation of new antimitotic compounds with a tetrazole ring

Sara del Mazo[1*], Miriam González[1], Alba Vicente-Blázquez[1], Sergio Ramos[1], Miguel Martín[1], Cristina Sanz[1], Raúl Fuentes[1], Pilar Puebla[1], Laura Gallego[1], Raquel Álvarez[1], Manuel Medarde[1], Rafael Peláez[1].

[1] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno, E-37007 Salamanca, Spain.

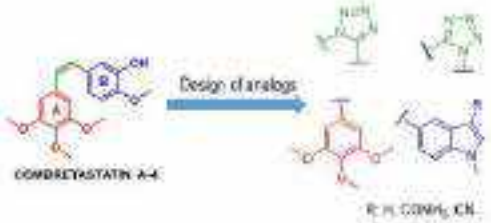
* e-mail: saradelmazo@usal.com

1. Introduction and objectives

Combretastatins are natural products that bind at the colchicine site of tubulin inhibiting the polymerization of tubulin. Combretastatin A-4 (CA-4) is a potent cytotoxic agent that strongly inhibits tubulin polymerization.

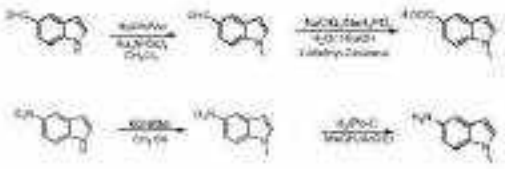
In spite of its benefits, CA-4 has a low aqueous solubility and its cis double bond is susceptible to isomerize to the more stable trans isomer, resulting in a dramatic loss in antitumor activity.

In this work, with CA-4 used as a structural template for the design of antimitotic agents, we replaced the CA-4 double bond with a heterocyclic tetrazole ring in order to locate the cis disposition between both aromatic systems. In addition, B ring of CA-4 has been replaced with a methylated indole ring, highly studied in our group and in which the introduction of polar groups at the 3-position could serve for activity modulation and solubility improvement.



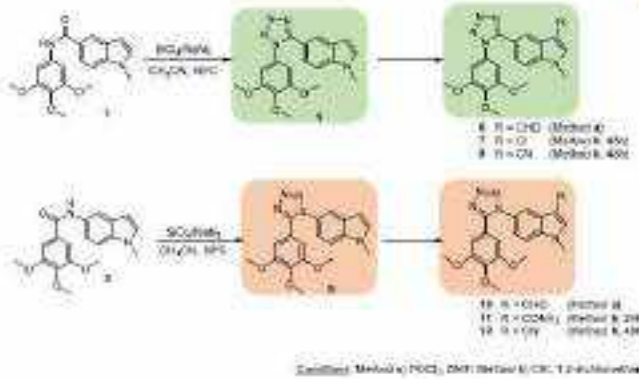
2. Synthesis

Starting materials



Amide derivatives

Tetrazole derivatives



3. Biological activity

Cytotoxic assays against HeLa cancer cell line were carried out for the synthesized compounds in order to determine their antiproliferative activity. The initial compound concentration was 1μM.

4. Conclusions

1. Comparing amides derivatives with tetrazoles, it clear that amides are less cytotoxic than their correlative tetrazoles. On amides, orientation of amide bridge seems to be important for biological activity, as compounds 1 and 2 do not display cell proliferation inhibition at 1μM concentration.

2. Tetrazole derivatives 5 and 9, without substituents at indole 3-position, exhibit similar potency in HeLa cells, showing that in these compounds the orientation of the tetrazole core doesn't seem to be important for the activity. Moreover, they strongly inhibit tubulin polymerization at 10 μM concentration (more than 50%).

3. Overall, introduction of substituents on indole ring results in a decrease in cytotoxic potency. Comparing similar substituents on the indole for tetrazoles, one orientation shows better potency than another, as noted for compounds 9 and 6 versus 10 and 12.

5. References & Acknowledgments

Álvarez, R.; Medarde, M.; Peláez, R. Curr. Top. Med. Chem. 2016, 16, 2251-2252.

Financial support came from Consejería de Educación de la Junta de Castilla y León and FEDER funds (SA162P18, SA114P20), Ministerio de Ciencia, Innovación y Universidades, Proyectos I+D+i «Retos Investigación» del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad, (RTI2018-099474-B-100) and Fundación Memoria Samuel Solórzano Barruso (FS/9-2018, FS/15-2019). S.M, M.G and M.M acknowledge Consejería de Educación de la Junta de Castilla y León and European Social Fund for a predoctoral fellowship. A.V. the Spanish MINECO for a predoctoral FPU fellowship. S.R and R.F, the University of Salamanca for a predoctoral fellowship.

EXPLORING NEW AROMATIC RING SUBSTITUTION PATTERNS IN PHENSTATIN ANALOGUES AS ANTIMITOTIC AGENTS

Sergio Ramos*, Myriam González, Alba Vicente-Blázquez, Laura Gallego-Yerga , Sara del Mazo, Miguel Marín, Raúl Fuentes, Cristina Sanz, Younes Ellahioui, Esther Caballero, Raquel Alvarez, Manuel Medarde, Rafael Peláez·

Pharmaceutical Sciences Department, Faculty of Pharmacy, University of Salamanca. Campus Miguel de Unamuno, 37007. Salamanca, Spain.

Restricted to organizers

Microtubules are built up by polymerization of α-β tubulin heterodimers and they play an essential role in several cell functions such as formation of the mitotic spindle or vesicle transport. The anti-mitotic natural product combretastatin A4 and its benzophenone analogues (phenstatins) are both potent inhibitors of tubulin polymerization and cytotoxic compounds. Although phenstatins avoid configurational instability, they still present low aqueous solubility and their structure-activity relationships differ due to a distinct arrangement of the phenyl rings.

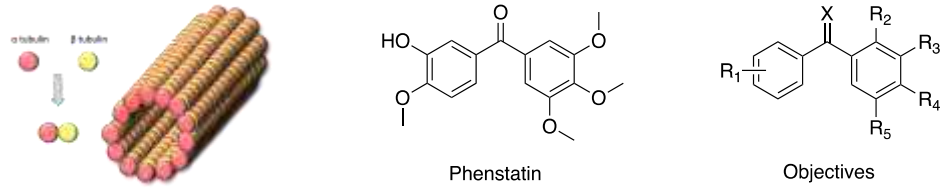


Figure 3. Structure of microtubules, reference compounds and target new compounds.

In this communication we have synthesised and evaluated novel CA-4 analogues in order to improve the pharmacokinetic profile by replacing the original 1,2-substituted olefinic bridge by different moieties such as oximes. On the other hand, modifications on the methoxy group substitution patterns with replacements of the methoxy groups by alternative moieties were carried out. Results of the biological evaluation and aqueous solubility will also be presented.

Acknowledgements

Consejería de Educación de la Junta de Castilla y León and FEDER Funds (SA262P18 and SA0116P20).

Ministerio de Ciencia, Innovación y Universidades, Proyectos I+D+i «Retos Investigación» del Programa Estatal de I+D+i Orientada a los Retos de la Sociedad RTI2018-099474-B-I00.

Fundación Memoria D. Samuel Solórzano Barruso (FS/9-2018 and FS/18-2019)

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* Correspondence: sergio.rvarela@usal.es

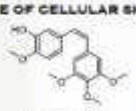
EXPLORING NEW AROMATIC RING SUBSTITUTION PATTERNS IN PHENSTATIN ANALOGUES AS ANTIMITOTIC AGENTS

SERGIO RAMOS(1)*, MYRIAM GONZÁLEZ(1), ALBA VICENTE-BLÁZQUEZ(1), LAURA GALLEGO-YERGA (1), SARA DEL MAZO(1), MIGUEL MARÍN(1), RAÚL FUENTES (1), CRISTINA SANZ(1), YOUNES ELLAHIOUI(1), ESTHER CABALLERO(1), RAQUEL ÁLVAREZ(1), MANUEL MEDARDE(1), RAFAEL PELÁEZ(1).

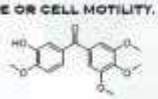
(1)Pharmaceutical Sciences Department, Faculty of Pharmacy, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain.

INTRODUCTION

MICROTUBULES ARE BUILT UP BY POLYMERIZATION OF α Α-β TUBULIN HETERODIMERS. THEY PLAY AN IMPORTANT ROLE IN SEVERAL CELL FUNCTIONS SUCH AS FORMATION OF THE MITOTIC SPINDLE, VESICLE TRANSPORT, MAINTENANCE OF CELLULAR SHAPE OR CELL MOTILITY.



Combretastatin A4

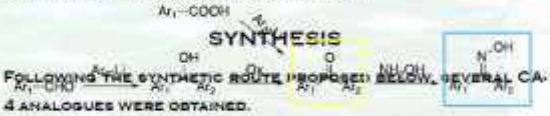


Phenstatin

COMBRETASTATIN A-4, ALSO KNOWN AS CA-4, IS A NATURAL PRODUCT THAT STRONGLY INHIBITS TUBULIN POLYMERIZATION BY BINDING AT THE COLCHICINE SITE OF TUBULIN. HOWEVER, DESPITE THE PROMISING POLYMERIZATION INHIBITION AND CYTOTOXICITY, CA-4 PRESENTS DIFFERENT DRAWBACKS SUCH AS LOW AQUEOUS SOLUBILITY OR CONFIGURATIONAL INSTABILITY, WHICH PREVENT IT FROM BEING USED AS A DRUG.

THUS, IN THIS COMMUNICATION WE HAVE SYNTHESIZED AND EVALUATED NOVEL CA-4 ANALOGUES IN ORDER TO IMPROVE THE PHARMACOKINETIC PROFILE BY REPLACING THE ORIGINAL 1,2-SUBSTITUTED OLEFINIC BRIDGE BY DIFFERENT MOIETIES SUCH AS OXIMES OR A CARBONYL GROUP (PHENSTATINS).

SYNTHESIS



FOLLOWING THE SYNTHETIC ROUTE PROPOSED BELOW, SEVERAL CA-4 ANALOGUES WERE OBTAINED.

FURTHERMORE, REACTION OF THE SYNTHESIZED COMPOUNDS WITH CH_3I LED TO THE CORRESPONDING AMMONIUM SALTS DERIVATIVES.

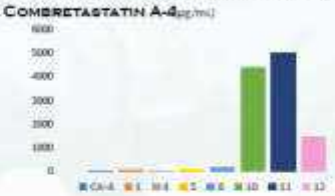
THE SYNTHESIZED COMPOUNDS ARE PRESENTED IN THE FOLLOWING TABLE:

	X	R_3	R_4	R_5	R_6	R_7
1	O	H	N-$(CH_3)_2$	O-CH_3	O-CH_3	O-CH_3
2	O	H	N-$(CH_3)_2$	O-CH_3	H	H
3	O	H	N-$(CH_3)_2$	O-CH_3	H	O-CH_3
4	O	N-$(CH_3)_2$	H	H	O-CH_3	O-CH_3
5	N-OH	H	N-$(CH_3)_2$	O-CH_3	O-CH_3	O-CH_3
6	N-OH	H	N-$(CH_3)_2$	O-CH_3	O-CH_3	H
7	N-OH	H	N-$(CH_3)_2$	O-CH_3	H	H
8	N-OH	N-$(CH_3)_2$	H	H	O-CH_3	O-CH_3
9	O	H	N$^+$-$(CH_3)_3$	H	O-CH_3	O-CH_3
10	O	H	N$^+$-$(CH_3)_3$	O-CH_3	O-CH_3	O-CH_3
11	O	H	N$^+$-$(CH_3)_3$	O-CH_3	H	H
12	N-OH	H	N$^+$-$(CH_3)_3$	H	O-CH_3	O-CH_3

sergio.varela@usal.es

SOLUBILITY

THE AQUEOUS SOLUBILITY OF THESE COMPOUNDS WAS MEASURED SPECTROPHOTOMETRICALLY IN A PH=7.0 BUFFER AND COMPARED TO COMBRETASTATIN A-4 (μg/mL)



Compound	Solubility
CA-4	1
1	48
4	11
5	74
8	153
10	4400
11	5000
12	1500

BIOLOGICAL ACTIVITY

Tubulin polymerization inhibition (TPI) assays and cytotoxicity against HeLa and HT-29 cancer cell lines of the synthesized compounds were performed in order to compare their potency. The most remarkable results are shown in the following table.

	TPI Conc.	TPI %	HeLa IC_{50} (nM)	HT-29 IC_{50} (nM)
1	5	25	90	400
2	20	30	413	500
3	20	51	>1000	>1000
4	10	16	160	460
5	5	7	800	1000
	10	23		
6	-	-	373	320
7	-	-	>1000	>1000
8	5	0	>1000	>1000
9	5	2	>1000	>1000
10	5	13	>1000	>1000
11	5	12	>1000	>1000
12	5	22	>1000	>1000

CONCLUSIONS

THE PROPOSED SYNTHETIC ROUTE PROVED TO BE EFFICIENT IN OBTAINING THE DESIRED COMPOUNDS IN GOOD YIELDS.

THE SYNTHESIZED COMPOUND IMPROVED THE AQUEOUS SOLUBILITY IN ALL CASES, SHOWING THE BEST RESULTS THE AMMONIUM SALTS 10, 11 AND 12

THE OBTAINED RESULTS FROM TPI SHOWED THAT THE DIMETHYLAMINOSUBSTITUTED ANALOGUES SHOW HIGHER POTENCY THAN THE CORRESPONDING AMMONIUM SALTS. ON THE OTHER HAND, COMPOUNDS MAINTAINING THE ORIGINAL 3,4,5-TRIMETHOXY AROMATIC RING SHOWED THE HIGHEST CYTOTOXICITY AGAINST BOTH HELA AND HT-29 CELL LINES.

ACKNOWLEDGEMENTS

CONSEJERÍA DE EDUCACIÓN DE LA JUNTA DE CASTILLA Y LEÓN AND FEDER FUNDS (SA262P18 AND SA0116P20).

MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES. PROYECTOS I+D+I «RETOS INVESTIGACIÓN» DEL PROGRAMA ESTATAL DE I+D+I ORIENTADA A LOS RETOS DE LA SOCIEDAD RTI2018-099474-B-I00.

... ARIANO BARRIUSO (FS/9-2018 AND FS/18-2019)

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Interactions of biologically active peptides with the membrane environment

Monika Wojciechowska*, Joanna Miszkiewicz, Joanna Trylska

Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097, Warsaw, Poland

Restricted to organizers

One of the features connecting biologically active peptides is their ability to interact with the cell membrane. To understand how these peptides, behave near the membranes, we investigated the structural changes of peptides in different lipid environments. We focused on cell penetrating peptides (CPP) and antimicrobial peptides (AMP) that take on an α-helical structure in the membrane medium. We chose two AMPs: anoplin (GLLKRIKTLL-NH_2) and W-MreB$_{1-9}$ (WMLKKFRGMF-NH_2) and one CPP - (KFF)$_3$K-NH_2. The aim of this project was to analyze and compare conformational changes of these peptides in the presence of various membrane mimics: sodium dodecyl sulfate (SDS) and dodecylphosphocholine (DPC) micelles, small unilameller vesicles, lipopolysaccharides isolated from *Escherichia coli* (*E. Coli*) and live *E. coli* cells. Structural changes of peptides were examined using circular dichroism (CD) spectroscopy. In addition, we investigated whether the structural changes of these peptides correlate with their biological activity. We evaluated how the peptides disorder the cell membrane by monitoring the release of calcein from large unilamellar vesicles. We also studied the antibacterial activity of these peptides against *E. coli* by determining their minimum inhibitory concentrations [(a)].

In the buffer solution the peptides were disordered. However, after binding to the lipid membrane they either took on regular secondary structures with characteristic CD spectra (an α-helix and/or β-strand) or unordered structures whose CD spectra are difficult to interpret. Anoplin and W-MreB$_{1-9}$ tend to adopt an α-helix in the presence of lipids but structural changes of these peptides depend on the charge, concentration and type of particles imitating the membrane. Anoplin and (KFF)$_3$K showed comparable antibacterial activity with minimal inhibitory concentrations of 32 μM, while W-MreB$_{1-9}$ did not show antibacterial properties against *E. coli*. Based on these results, (KFF)$_3$K can be treated not only as a CPP but also as a potential AMP. The comparison of the antibacterial activity of peptides with the structural data obtained in CD experiments showed that conformational changes of peptides, caused by the lipid environment, did not correlate directly with the activity of peptides. However, we proved that the type and charge of the membrane affects the peptide structure and its destructive properties. Positively charged peptides disintegrated to a higher extent the liposomes bearing the negative charge than the zwitterionic but neutral liposomes. From a therapeutic point of view, this is a critical observation suggesting that a positively charged peptide preferentially interacts with a negatively charged bacterial cell membrane and not with the eukaryotic membrane [(a)].

Acknowledgements: This work was supported by National Science Centre (2019/35/D/NZ1/01957, 2016/23/B/NZ1/03198)

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* Correspondence: m.wojciechowska@cent.uw.edu.pl

Interactions of biologically active peptides with the membrane environment

Monika Wojciechowska, Julia Macyszyn, Joanna Miszkiewicz, Joanna Trylska

Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland

m.wojciechowska@cent.uw.edu.pl

Introduction:

We investigated how the interactions of membrane-active peptides with various types of membranes affect the secondary structures of these peptides. We chose two antimicrobial peptides, anoplin (GLLKRIKTLL-NH_2) and W-MreB$_{1-9}$ (WMLKKFRGMF-NH_2), and one cell-penetrating peptide - (KFF)$_3$K. We used **circular dichroism** (CD) spectroscopy to monitor peptide conformational changes in different membrane models: micelles (charged SDS and zwitterionic DPC), small liposomes (SUV) composed of POPC:POPG (3:1) and POPC:POPE (3:1) lipids, lipolysaccharides (LPS) isolated from *E. coli* and live *E. coli* cells (Fig. 1). Next, we used large liposomes (LUV) with **fluorescent dye - calcein**, to assess the destructive properties of these peptides on liposomes. Third, we determined the **antibacterial activity** of the peptides to relate them with peptide structural changes near the membranes.

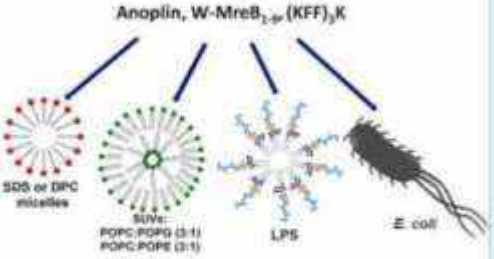


Fig. 1. Schematic representation of membrane mimics used in our work.

Conformation change

In the phosphate buffer, the peptides adopt a random coil and change conformation in membrane environments (Fig. 2). Anoplin and W-MreB$_{1-9}$ adopt helical structures near most types of lipids. The observed spectral shifts depend on the charge, composition and concentration of the model membranes.

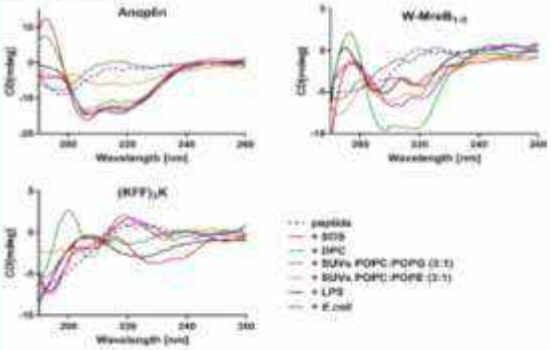


Fig. 2. CD spectra of peptides recorded in the presence of SDS and DPC micelles, POPC:POPG (3:1) and POPC:POPE (3:1) SUVs, LPS, and live *E. coli*.

Dye leakage measurements

The peptides induce the release of a fluorescent probe from both the negatively charged POPC:POPG (3:1) and zwitterionic POPC:POPE (3:1) liposomes, with (KFF)$_3$K being most effective (Fig. 3). Monitoring of fluorescent dye leakage from LUVs upon their incubation with peptides showed different degradation rates of liposomes with different compositions, which confirms peptides' selectivity toward bacterial cells.

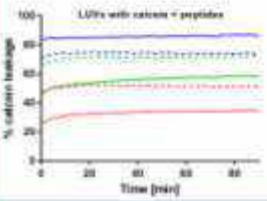


Fig. 3. The percentages of calcein leakage from the negatively charged and neutral LUVs (solid lines) in the presence of different peptides.

Antibacterial activity:

We determined minimum inhibitory concentrations (MIC) of the peptides and conventional antibiotics on two *E. coli* strains: K12 - the wild type strain and BL21(DE3) - a strain deficient in the outer membrane protease OmpT (Tab. 1).

Tab. 1. Antibacterial activity of the peptides and antibiotics.

Peptide	MIC [μM]	
	E. coli K12	*E. coli* BL21(DE3)
Anoplin	32	32
W-MreB$_{1-9}$	128	256
(KFF)$_3$K	32	32
ampicillin	8	4
tetracycline	8	16

Conclusions:

Our observations of peptide structural changes in different membrane environments are a solid step towards understanding the mechanisms of action of antibacterial and cell-penetrating peptides and could help in the design of new, membrane-active peptide sequences. The (KFF)$_3$K peptide showed a comparable antibacterial activity to anoplin, which suggests to consider (KFF)$_3$K not only as a cell-penetrating peptide but also as a potential antibacterial peptide. Most AMPs demonstrate an α-helix structure as the biologically active structure. In order to stabilize the helical structure of an AMP, a **hydrocarbon stapling technique** will be used. In our research we use the hydrocarbon stapling technique to stabilize AMP helical structures and improve their effectiveness in penetration and disintegration of bacterial membranes (see: **flash poster Julia Michalska**).

Acknowledgements: This work was supported by National Science Centre (2016/23/B/NZ1/03198, 2019/35/D/NZ1/01957)

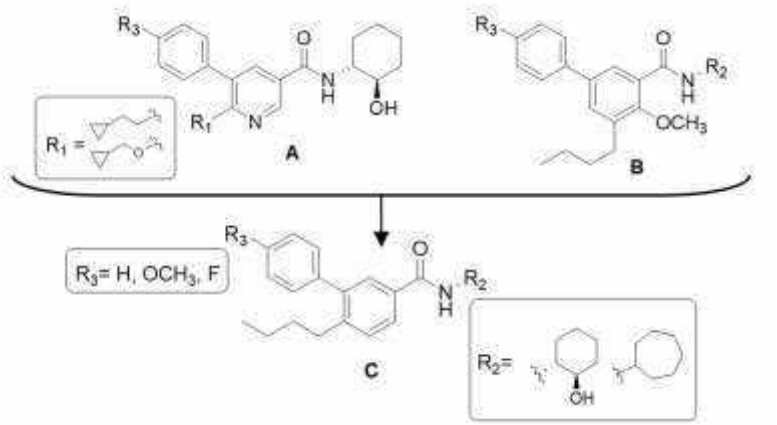
New Biphenylic Derivatives Active On CB1 Cannabinoid Receptor

Ceni C. [(1)*], Gado F. [(1)], Sbrana G. [(1)], Ferrisi R. [(1)], Pertwee R.G. [(2)], Stevenson L.A. [(2)], Macchia M. [(1)] Digiacomo M. [(1)], Bertini S. [(1)], Manera C. [(1)].

(1) Dipartimento di Farmacia, Università di Pisa, Pisa, Italy.
(2) School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK.

Restricted to organizers

In the last decades, a large deal of evidences has shown the involvement of cannabinoid receptor type-1 (CB1R) in metabolic disorders. In particular, the development of CB1R antagonists represents a new therapeutic strategy in the treatment of obesity. However, the central psychiatric side effects such as depression, anxiety and suicidal tendencies, may limit their clinical use. In this contest, the research is particularly active in the identification of antagonists that selectively act on peripheral CB1Rs, with minimal central side effects. A recent study reported a class of peripheral CB1R antagonists characterized by a 5-aryl substituted nicotinamide core (**A**).[a] These derivatives present structural similarities with some biphenylic compounds (**B**),[b] endowed with a CB2R antagonist activity, previously synthesized by our research group. In this work we combined the two structures (**A** and **B**) synthesizing compounds of type **C**, with the aim of obtaining novel CBRs antagonists, but also improving structure-activity relationships studies, highlighting key groups involved in the interaction with the CBRs binding sites. In particular, structural modifications essentially consist in the replacement of N atom with a C atom, the removal of the methoxy group and the shift of the *n*-butyl chain position. Among the synthesized compounds, the derivative characterized by the presence of a *trans*-2-hydroxy cyclohexyl substituent and a methoxy group in R_2 and R_3 respectively, showed the most interesting results. Indeed, rather unexpectedly for this derivative, we did not detect radioligand displacement on CB1R but increased binding, suggesting an allosteric behavior.



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(b) Bertini S. et al., Synthesis, biological activity and molecular modeling of new biphenylic carboxamides as potent and selective CB2 receptor ligands, Eur J Med Chem, 2015, 90: 526-36.

* Correspondence: costanza.ceni@phd.unipi.it

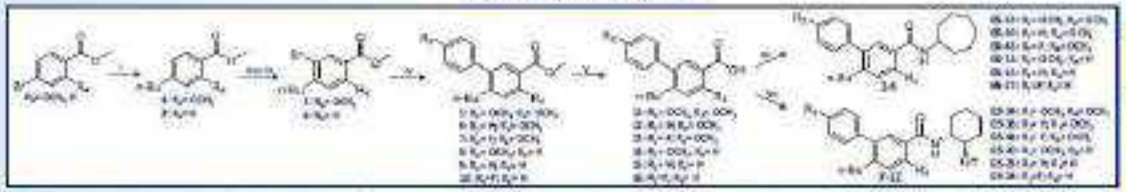
NEW BIPHENYLIC DERIVATIVES ACTIVE ON CB1 CANNABINOID RECEPTOR

Coni C. (1)*, Gado F. (1), Sibrana G. (1), Ferrisi R. (1), Portwoo R.G. (2), Stevenson L.A. (2), Macchia M. (1), Digiacomo M. (1), Bertini S. (1), Manera C. (1).
(1) Dipartimento di Farmacia, Università di Pisa, Pisa, Italy.
(2) School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK.
costanza.coni@phd.unipi.it

INTRODUCTION

In the last decade, cannabinoid receptor type-1 (CB1R) has emerged as a key target in the regulation of several physio-pathological processes, including metabolic disorders. In particular, the development of CB1R antagonists represent a novel therapeutic strategy in the treatment of obesity. However, psychiatric effects associated with a central orthosteric stimulation of CB1R, such as anxiety, depression and suicidal tendencies, may limit their clinical use. Thus, the development of antagonists, able to selectively act on peripheral CB1R, could be a successful choice to avoid these psychiatric side effects. Alternatively, selective CB1R allosteric modulators could represent a beneficial strategy to reduce central side effects arising from orthosteric CB1R stimulation. A recent study reported a class of peripheral CB1R antagonists, characterized by a 5-aryl substituted nicotinamide core[1] (A). These compounds present structural similarities with a class of biphenylic compounds[2] (B), endowed with a CB2R antagonist activity, previously synthesized by our research group. By combining the two structures, we synthesized a new series of compounds (C) with the aim of obtaining novel CBRs antagonists, but also improving structure-activity relationships studies, providing additional information about the key groups involved in the interaction with both CBRs binding site.

GENERAL SYNTHETIC SCHEME



Scheme 1. General synthetic procedure for the preparation of "C" series compounds. Reagents and conditions: …

STRUCTURAL MODIFICATIONS

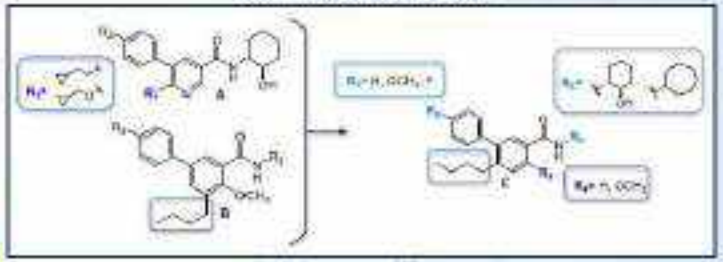


Figure 1.

BIOLOGICAL EVALUATION

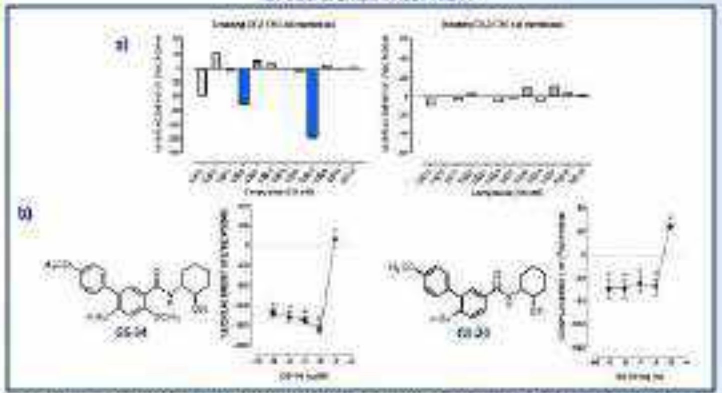


Figure 2.

RESULTS AND DISCUSSION

Among the synthesized compounds, derivatives **GS-34** and **GS-20** showed the most interesting results. Indeed, we expected to detect an antagonist behaviour on CB1R and/or CB2R. CB1R and CB2R screening (at 100 nM) shown that all compounds resulted inactive on CB2R but, rather unexpectedly, **GS-12**, **GS-20** and **GS-34** enhanced the binding of radioligand CBRs unselective agonist [^{3}H]CP55940 on CB1R, suggesting an allosteric behaviour. **GS-20** and **GS-34**, which resulted as the best enhancers in CB1R screening, were tested at different concentrations to construct a dose-response binding curve, revealing to be able to significantly enhance the binding of [^{3}H]CP55940 up to 1μM. Furthermore, we extended the information about the key groups probably involved in the interaction with CB1R binding site. In particular, the replacement of N atom with a C atom and the shift of the n-butyl chain position represent the main structural changes, which led to this new series of derivatives. While the first modification did not really affect CB1R affinity, the second one appear to be the main cause of high selectivity of compounds toward CB1R. Moreover, the presence of a small substituent in R_4 position is well tolerated, while in R_1 and R_3 positions the most promising substituents seems to be the trans-2-hydroxy-cyclohexyl residue and the methoxy group, respectively.

CONCLUSION

In this work we combined the structural features of two classes of compounds already studied (**A** and **B**), with the aim to synthesize a novel series of CBRs ligands and to have additional information about the key groups involved in the interaction with CBRs. Among the synthesized compounds, the CB1R selective ligands **GS-20** and **GS-34** unexpectedly enhanced the binding of the radioligand with CB1R, suggesting an allosteric behaviour. Thus, they have been tested for their functional activity using [^{35}S]GTPγS assays, which are still on going, to demonstrate their allosteric behaviour and to evaluate their profile as positive, negative, or neutral allosteric modulators (PAM, NAM, NAL).

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[1] Rover S. et al., 6-Alkoxy-5-aryl-3-pyridinecarboxamides, a New Series of Bioavailable Cannabinoid Receptor Type 1 (CB1) Antagonists including Peripherally Selective Compounds. J Med Chem, 2013, 56: 9874-96.
[2] Bertini S. et al., Synthesis, biological activity and molecular modeling of new biphenylic carboxamides as potent and selective CB2 receptor ligands. Eur J Med Chem, 2015, 90: 526-36.

Synthesis of new fluorescent chemical probes targeting bacterial efflux to early detect and fight the first barrier antibiotic resistance

J. Revol-tissot ; J. Vergalli ; S. Alibert ; G. Boyer ; J.-M. Pages and J.-M. Bolla

Aix Marseille Univ, INSERM, SSA, MCT, FAC PHARM, Marseille, 13005, France

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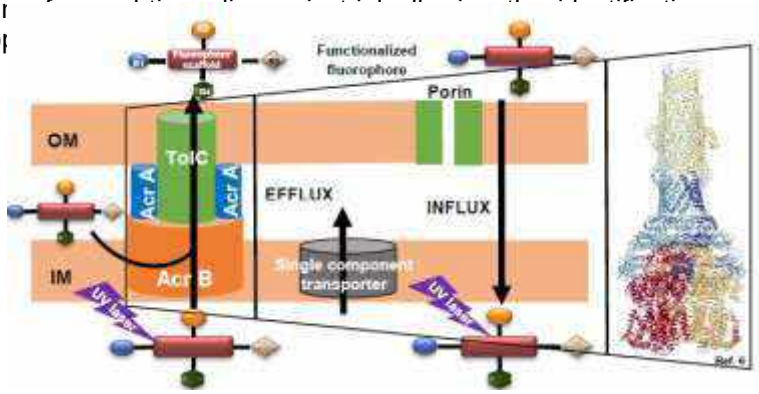
Antimicrobial resistance (AMR) is one of the more serious problem of Public Health. The 2014 WHO's global report[1] and the more recent one of J. O'Neal[2] outlined worrying levels of AMR worldwide involving therapeutic failure of large classes of antibiotics as quinolones, beta-lactams, aminoglycosides, macrolides, and cyclins especially in Gram-negative bacterial diseases. Resistance is a natural response of microorganisms like bacteria, allowing them to counteract pharmacological effects of antibiotic agents. A lot of mechanisms as membrane permeability variations, enzymatic degradations and intracellular target modifications contribute to the Multi-Drug Resistance (MDR) phenotypes. Furthermore, efflux overexpression is a major early stage trigger in the MDR setting up[3]. Especially, Resistance- Nodulation-Cell-Division (RND) efflux pump superfamily constitutes a tripartite protein system which is the resistance first line in Gram-negative bacteria[4]. They can extrude structurally different substances outside the cell space; hence antibiotics struggle to reach effective intracellular concentrations. Thanks to the spectrofluorometric method developed in our laboratory[5], we are working on the design of new natural product derivatives with high fluorescent and good efflux substrate properties useful to detect their capacity to cross membranes and to accumulate in bacteria. These compounds must not have cytotoxic effects on bacteria growth to minimize their resistance impact on new drug discovery. Starting from a screening of chemical libraries of natural products and by a medicinal chemistry approach, pharmacophores and their positions were identified on a fluorescent scaffold. Then, structure activity relationship studies allowed us to highlight physicochemical properties responsible not only for a high fluorescence intensity but also for substrate efflux-pump features. These results will guide targeted pharmacomodulations to improve the desig
minimization and prevention of the early ap
patient exposure to antibiotics.



These issues highlight the required knowledge and need to further develop and improve clinically used diagnosis tests [40] to identify patterns and resistance levels of pathogens in septic patients. These diagnosis assays are crucial in clinical practice for infectious patients, because clinicians are able to use techniques to identify main resistant mechanisms, such as early bacterial efflux, which is essential in infected patient treatments.

Hence, matching pharmacological and structural properties of antibiotics will emphasize the study of their interaction with efflux pumps. This will also be a basis for screening and development of new interesting compounds as efflux biomarkers in Gram-negative bacteria, which will contribute to the design of new methods of Antibiotic Susceptibility Testing (AST; unpublished works)

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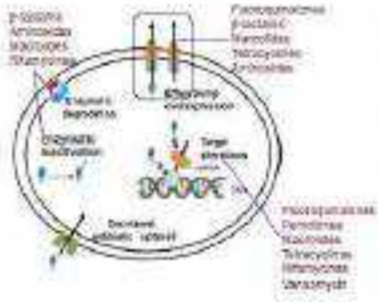
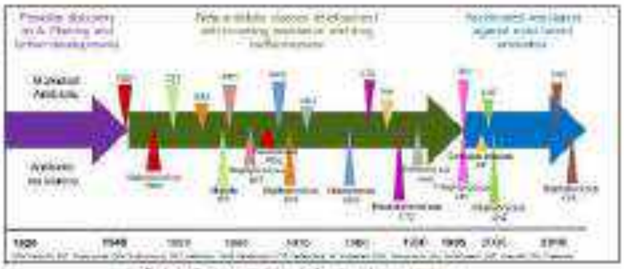
SYNTHESIS OF NEW FLUORESCENT CHEMICAL PROBES TARGETING BACTERIAL EFFLUX TO EARLY DETECT AND FIGHT THE FIRST BARRIER ANTIBIOTIC RESISTANCE

Johan Revol-Tissot, Sandrine Alibert, Gérard Boyer, Jean-Marie Pagès and Jean-Michel Bolla
Aix Marseille Université, UMR-MD1, Inserm U1261, MCT, Marseille, France

Bacterial resistance

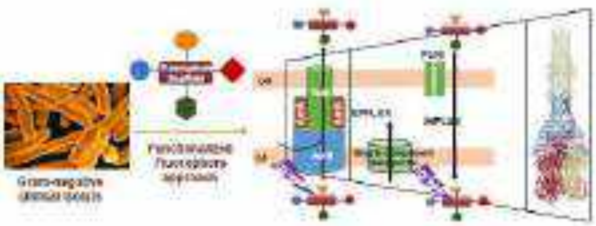


- Various action mechanisms according to ATB classes
- Multiple targets → diverse strategies but no high significant activities
- Efflux plays a major role in MDR phenotype appearance [1]
- 700,000 people die every year from microbial infections mediated by drug resistant species...
- Countries must elaborate new strategies to maintain antibiotic clinical use thanks to early diagnosis tools [2]



- Early in the course of time before resistance appears...
- A specific ATB agent resistance can lead to MDR
- No new classes introduced in the market since several years...

A fluorophore-based design approach

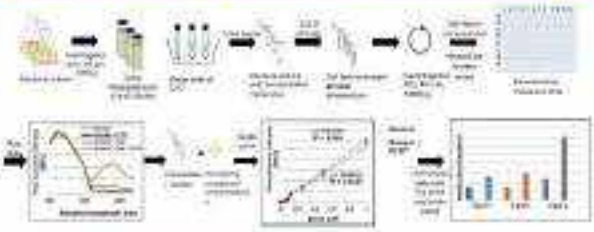


- New fluorophore for Gram-negative bacteria efflux detection → Natural products screening for new derivative compound synthesis with high fluorescence properties
- Highlight E.coli AcrAB-TolC efflux pump substrate affinity to identify a hit compound to promote early efflux clinical detection for other AcrAB-TolC expressing strains (e.g. K.aerogenes, K.pneumoniae, S.enterica...)

New derivatives Design and synthesis

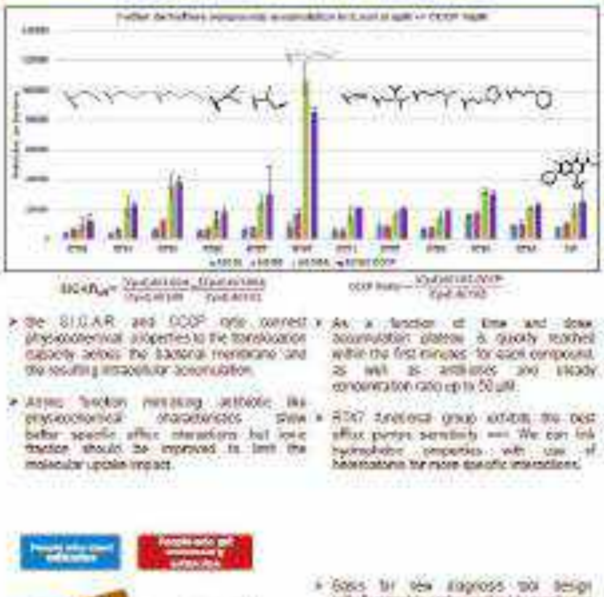
- Chemical library screening identifying pharmacophores for high level fluorescence detection and optimal substrate features.
- New natural product derivatives synthesis: alkyl ether or amino alkyl ether pharmacophores have the best profile.
- S.A.R. studies performing to link physicochemical properties and efflux pump interaction

Biologic properties analysis : accumulation method



- MIC studies show the non-cytotoxicity of our compounds
- Fluorescence intensities are quantified on intracellular lysates according to the pH, excitation and emission wavelengths of the compound tested according to physicochemical properties.
- Accumulation assays [3] lead in E. coli strains population: AG100 (WT), AG100A (AG100 KAN ΔAcrAB gene) & AG102 (AcrAB overexpression) ± Carbonyl Cyanide m-Chlorophenylhydrazone (CCCP; AG102 + proton pump inhibitor).

Hit identification: a S.A.R study for an efflux biomarker



- The S.I.C.A.R. and CCCP ratio connect physicochemical properties to the translocation capacity across the bacterial membrane and the resulting intracellular accumulation.
- Amine function mimicking antibiotic-like physicochemical characteristics show better specific efflux interactions but ionic fraction should be improved to limit the molecular uptake impact.
- As a function of time and dose, accumulation plateau is quickly reached within the first minutes for each compound, as well as antibiotics and steady concentration ratio up to 50 µM.
- RTX7 functional group exhibits the best efflux pumps sensitivity → We can link hydrophobic properties with use of heteroatoms for more specific interactions.

Perspectives on real-time diagnosis trial

- S.A.R. studies to select one hit compound as efflux biomarker in Gram-negative bacteria → Needing for more pharmacomodulation to improve physicochemical efflux uptake with suitable fluorescence for these compounds.
- Competition tests will be conducted in presence of antibiotic to identify efflux substrates features and mode of action and binding on the pump.
- Other AcrAB-TolC Gram-negative producer strains have to be tested with interesting hit to extend this feature to efflux pump expression in clinical and laboratory strains.

- Basis for new diagnosis tool design with fluorimetric probe method to monitoring ATB accumulation and efflux in patient-related clinical isolates [4].
- New method to diagnose antibiotic resistance:
  - For one patient in Gram-negative diseases
  - For the adaptation of antibiotic treatment
  - For the minimization of MDR emergence

Mechanistic insights into the anticancer properties of higher order DNA structure-targeting agents.

Joanna Zell[(1)]*, Katerina Duskova[(1)], Paul Reyes-Guttierez[(2)], Anton Granzhan[(3,4)], Sébastien Britton[(5)], David Monchaud[(1)]

(1) Institut de Chimie Moléculaire de l'Université de Bourgogne (ICMUB), CNRS UMR 6302, UBFC Dijon, 21078 Dijon, France. (2) Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic (3) Institut Curie, CNRS UMR 9187, INSERM U1196, PSL Research University, 91405 Orsay, France. (4) Université Paris-Sud, Université Paris Saclay, CNRS UMR 9187, INSERM U1196, 91405 Orsay, France. (5) Institut de Pharmacologie et de Biologie Structurale (IPBS), CNRS UMR 5089, Université de Toulouse, UPS, Équipe labellisée la Ligue Contre le Cancer, 31077 Toulouse, France.

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DNA damage induction is still the preponderant mechanism of action of most chemotherapeutics. However, classic antiproliferative agents are poorly specific for cell type or genetic sequence. Thanks to the recent characterization of non-B helix structures in cells, particularly G-quadruplexes (G4s) and three-way junctions (TWJs) (Figure 1A), new chemotherapeutics are now being developed, which target DNA structures rather than sequences, with a higher degree of cancer cell selectivity. These structure-targeting agents (G4- and TWJ-ligands) have shown high activity in cancer models, owing to their ability to trigger DNA damage.[(a)] We report here on a family of small molecules (so called azacryptands) that displays high *in vitro* affinity and specificity for TWJ,[(b)] induces DNA damage in cells,[(c)] and exerts extreme synergic cytotoxicity in combinatorial treatment with DNA damage repair (DDR) inhibitors (Figure 1B), a chemotherapeutic technique popularized as *chemically induced synthetic lethality*.[(d)] A clickable analogue is used to study the subcellular localization of TWJs for the first time *via* a bioorthogonal imaging protocol referred to as *in situ* click imaging (Figure 1C).

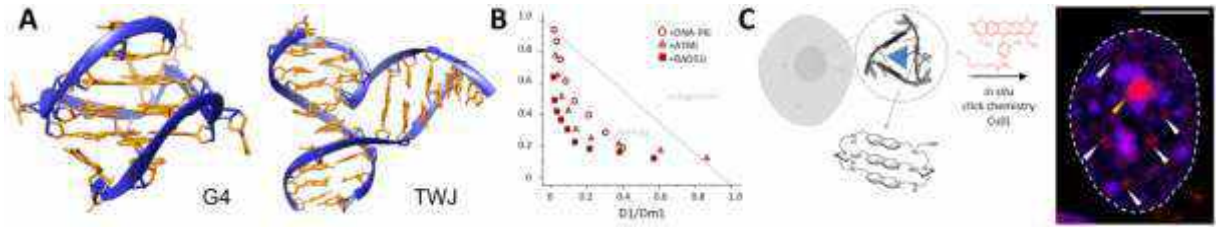

Figure 1. **A**. Higher order DNA structures G-quadruplexes and three-way junctions (TWJ). **B** Synergic cytotoxicity properties of an azacryptand with three DDR inhibitors (DNA-PKi, ATMi and RAD51i). **C** Copper-catalysed click chemistry performed on cells to the localise azacryptand binder.

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[(d)] K. I. E. McLuckie, M. Di Antonio, H. Zecchini, J. Xian, C. Caldas, B. F. Krippendorff, D. Tannahill, C. Lowe, S. Balasubramanian, *G-Quadruplex DNA as a Molecular Target for Induced Synthetic Lethality in Cancer Cells*. *J. Am. Chem. Soc.*, 135, 9640-9643 (2013)

*** Correspondence: joannazell@hotmail.co.uk**

Targeting Three-way DNA junctions with small molecules to induce cytotoxic DNA damage

Joanna Zell,[1] Katerina Duskova,[1] Leila Chouh,[2,3] Paul E. Reyes-Guttierez,[4] Anton Granzhan,[2,3] Sébastien Britton,[5] David Monchaud[1]

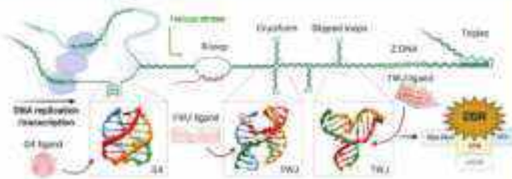
[1]Institut de Chimie Moléculaire de l'Université de Bourgogne (ICMUB), CNRS UMR 6302, UBFC Dijon, 21078 Dijon, France. [2]Institut Curie, CNRS UMR 9187, INSERM U1196, PSL Research University, 91405 Orsay, France. [3]Université Paris Saclay, CNRS UMR 9187, INSERM U1196, 91405 Orsay, France. [4]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, 16610, Prague 6, Czech Republic. [5]Institut de Pharmacologie et de Biologie Structurale (IPBS), CNRS UMR 5089, Université de Toulouse, UPS, Équipe labellisée la Ligue Contre le Cancer, 31077 Toulouse, France

Introduction

Our DNA is constantly being damaged, thus creating lesions which in turn cause blockages in DNA transactions (i.e. replication and transcription), leading to damage signaling and single- (SSB) and double-strand break (DSB) induction. A complex network of repair mechanisms, known as the DNA damage response (DDR), exists in higher organisms to sense this damage, stall the cell cycle and repair the DNA. Cancer cells frequently have DDR defects, and these weaknesses are exploited by some anticancer drugs currently used in the clinic, which trigger irreversible DNA damage, thus promoting replicative stress.

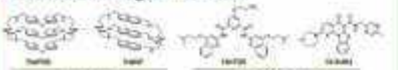
As an alternative to inducing irreversible and poorly-specific DNA strand breaks, the stabilization of non-B DNA structures using small molecules has emerged as a promising way to create DNA damage. One of such non B-DNA structures is the three-way DNA junction (TWJ) that is proposed to fold both upstream and downstream of the replication fork. TWJ loci are associated with genetic instability in cancer and neurodegenerative diseases. The non-covalent small-molecule stabilization of TWJ therefore represents a promising strategy to induce replicative stress in a highly cancer-specific manner.

The main goal of this work is to characterise a new series of compounds capable of creating DNA damage through TWJ stabilization and to apply them either as standalone chemotherapeutic agent or in combination with established anti-cancer drugs that disrupt DDR signaling pathways.



We have characterised promising TWJ ligands, a family of macrocyclic azacryptands, via multiple in vitro techniques (Box A) (FRET-melting, PAGE, ESI-MS); we confirmed that these compounds could induce DNA damage in cells via immunodetection (Box B). Their cytotoxicity fingerprint in 60 cancer cell lines indicates a DNA-damage inducing mechanism of action (Box C). Strong synthetic lethality is observed with known DDR inhibitors (Box D). Finally, we attempt to illuminate the intracellular localisation of TWJ-DNA using in situ click chemistry, for the first time, with a clickable azacryptand (Box E).

A. *In vitro* binding to TWJ-DNA



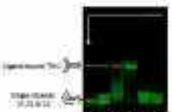
a. FRET-melting assay

Azacryptands stabilise the structure of TWJ-forming DNA. They maintain their affinity in the presence of an excess of double stranded DNA (ds26) and G-quadruplex (G4)-forming DNA (TG4T).

Azacryptands show affinity for G-quadruplex (G4)-forming DNA which is immediately lost in the presence of TWJ-forming DNA.

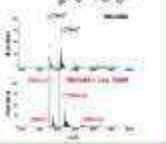
b. Polyacrylamide gel electrophoresis

Azacryptands efficiently act in the formation of TWJ DNA structures, unlike G4 ligands.



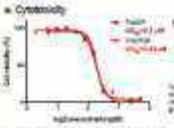
c. ESI-MS

Mass spectrometry shows almost complete formation of the 1:1 DNA/ligand complex upon incubation with 1 molar eq. of azacryptand.



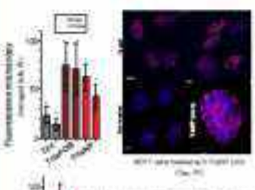
B. Cytotoxicity and DNA damage

a. Cytotoxicity

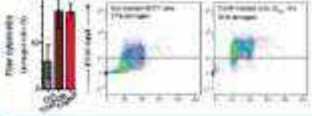


Azacryptands show micromolar cytotoxicity in two breast cancer cell lines.

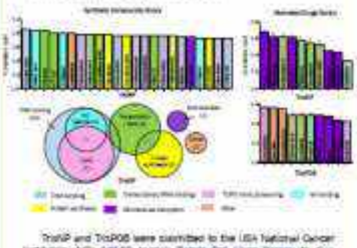
b. DNA damage induction (γH2AX immunodetection)



Azacryptand's toxicity most likely originates in their ability to trigger DNA damage. DNA damage induction is evidenced by a strong γH2AX antibody staining corresponding to the phosphorylation of histone H2AX to γH2AX, an established marker of DSB, which forms upon DNA damage induction.



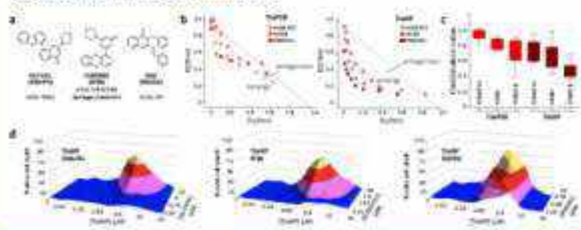
C. NCI-60 correlations give mechanistic prediction



TrisNP and TrisPOB were submitted to the USA National Cancer Institute's (NCI) NCI-60 Human Tumor Cell Lines Screen to gain insights into the mechanism with which they exert their antiproliferative effects. This open-access platform evaluates the toxicity of the submitted compound in 60 tumor cell lines, providing a fingerprint which can be compared with that of other compounds previously tested using the COMPARE algorithm (ranked by a correlation coefficient).

The 25 compounds with the highest correlation coefficients with TrisNP (0.60 to 0.75), from the Synthetic Compounds library, are described as being involved in DNA binding, including G4 binding, transcription and RNA binding, protein synthesis and topoisomerase (TOPO, crucial DNA-unravelling enzyme) inhibition or poisoning.

D. Synergism with DDR inhibitors



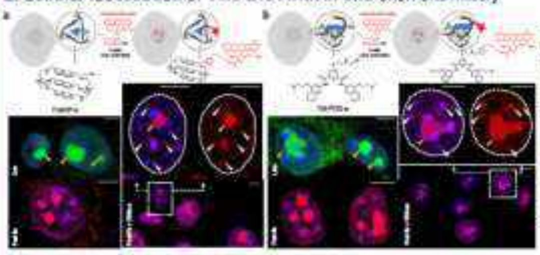
TrisNP shows strong synergistic synthetic lethality properties with inhibitors of DNA-PK, ATM and RAD51 (a), key mediators of DDR, specifically in double-strand break repair mechanisms:

- NHEJ (non-homologous end joining)
- HR (homologous recombination)

TrisPOB shows similar, although weaker trends.

These results indicate important clinical potential.

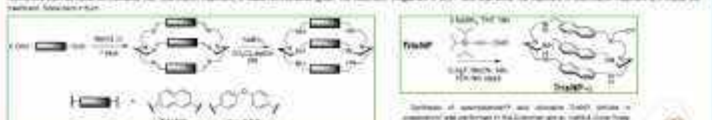
E. Cellular localisation of TWJ-DNA with *in situ* click chemistry



Trackable TrisNP analogue, TrisNP-α (a), localises in the nucleus, and particularly in the nucleoli (yellow arrows), when incubated in live and fixed cells.

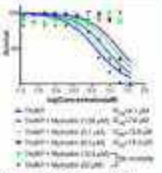
Non-nucleolar foci are also observable (white arrows) in weakly DAPI-labeled zones, in agreement with the formation of TWJs in open chromatin regions.

A similar staining pattern is obtained for the clickable G4 ligand TMPDS-α (b), although with a noticeable cytoplasmic labeling, which presumably corresponds to G4-RNA sites.



Future work

- Myricetin appears to block TrisNP's cytotoxic effects. Myricetin is a long-known cytotoxic flavonoid with multiple mechanisms of action described including TOPO inhibition, Flap Endonuclease (FEN1) inhibition, cell cycle inhibition and even antioxidant activity. We will investigate the importance of such mechanisms to that of TrisNP.
- Test the role of DNA topoisomerases in mediating the cytotoxic effects of TWJ ligands.
- Synthesise and screen for other TWJ-binding molecules and characterise their specificities in vitro and in cells.



- Incorporate synthetic TWJs into cells to show an increase in TrisNP-α binding, thus confirming the TWJ specificity.
- Perform pull-down experiments to isolate TWJ-DNA binding to TrisNP-α and identify sequences via sequencing and/or RT-qPCR, as previously performed in the lab with G4s.

Bibliography

[illegible]

Acknowledgements

Microscopy imaging was carried out on a Leica SP8 confocal microscope, at the DImaCell Platform, Dijon. Flow cytometry data was collected at the Dijon Cytometry platform.

This project has received funding from the Agence Nationale de la Recherche (grant agreement ANR-17-CE17-0010-01) and INSERM Plan Cancer 2014-2019 ... as well as being part of the project "Pharmacoimagerie & agents théranostiques" supported by the Université de Bourgogne and Conseil Régional de Bourgogne (PARI) and the European Union (PO FEDER-FSE Bourgogne 2014/2020 programs).

Anticancer potential of selective COX-2 inhibitors of pyranocoumarin type

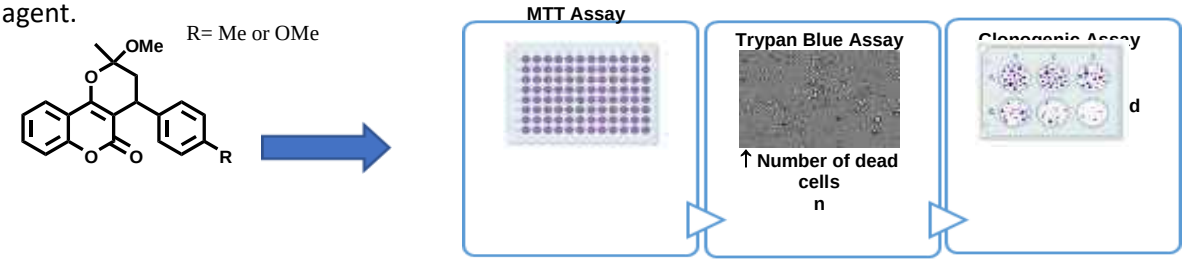
Batoul ROSTOM [1, 2]*, Racha KARAKY [2], Fatima SAAB [2], Joumana AKHADAR [2], Issam KASSAB [2], Maité SYLLA-IYARRETA VEITIA [1].

(1) Equipe de Chimie Moléculaire, Laboratoire GBCM (EA 7528), Conservatoire national des arts et métiers, 2 rue Conté, 75003 Paris, HESAM Université, France.

(2) Faculty of Pharmacy, Lebanese University, Rafik Hariri University Campus, PO Box5, Hadat, Lebanon.

Restricted to organizers

Cancer is a widely spreading disease showing high incidence of morbidity and mortality worldwide despite advances in its management [a]. Cyclooxygenase 2 (COX-2), an enzyme involved in the synthesis of prostaglandins, which are mediators of inflammation, is widely overexpressed in many types of solid cancers (breast, prostate, colon, and lung). Studies have established COX-2 involvement in the promotion of cancer proliferation, angiogenesis, invasion and metastasis. Thus, blocking COX-2 appears as a promising therapeutic approach to prevent or treat cancers [b-c]. Coumarins are organic compounds from natural and synthetic sources, that possess different therapeutic effects including anticoagulant, anti-inflammatory antioxidant, and antitumor effects [d]. A series of pyranocoumarin derivatives was designed and synthesized previously by the GBCM laboratory at Cnam Paris and were found to be selective inhibitors of COX-2 [e]. We describe herein, the biological evaluation of two derivatives characterized by their high anti-COX-2 activity: methylcyclocoumarol and methoxycyclocoumarol. These derivatives were screened for their anticancer activity using hormone-dependent breast cancer MCF-7 cell line. MTT results revealed an antiproliferative effect of methylcyclocumarol on MCF-7 cell line with an IC_{50} at 48 hours of treatment equal to 100 µM. This antiproliferative effect was associated to a cytotoxic effect which was evaluated by Trypan blue exclusion assay. MCF-7 cells treated with methylcyclocoumarol showed an increase in their percentage of dead cells in comparison to control cells. Finally, long term effect of methylcyclocoumarol was evaluated using Clonogenic assay. After 10 days of treatment, a dose-dependent decrease in number of colonies was observed with an IC_{50} of 40 µM significantly lower than the MTT IC_{50}. These preliminary results suggest that this compound may have potential as a novel anticancer agent.



Bibliographic references:

[a] Hausman D.M., *Perspectives in Biol Med*, 62(4), 778-784, 2019.

[b] Mahboubi Rabbani S.M.I. et al, *Expert Opin Ther Pat*, 29(6), 407-427, 2019.

[c] Hashemi Goradel N. et al, *J Cell Physiol*, 234(5), 5683-5699, 2019.

[d] Zhang L. et al, *Eur J Med Chem*, 181, 111587, 2019.

[e] Rayar A. M. et al, *Eur J Med Chem*, 146, 577-587, 2018.

* Correspondence: E-mail batoul_rostom@hotmail.com

Anticancer potential of selective COX-2 inhibitors of pyranocoumarin type

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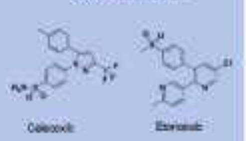
Cancer and COX-2

According to WHO, cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018. About 1 in 6 deaths is due to cancer.

COX-2 Inhibitors

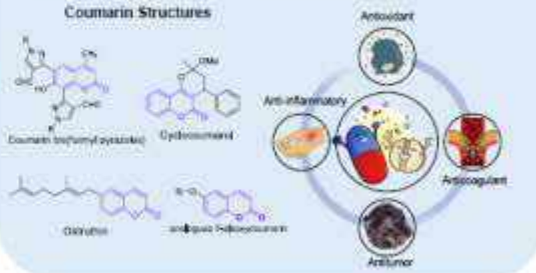


Overexpressed in different types of solid cancers (Breast, prostate, colon and lung) ⇒
- ✓ Inhibiting apoptosis
- ✓ Increasing angiogenesis
- ✓ Promoting tumor invasion

Coumarins: bioactive compounds

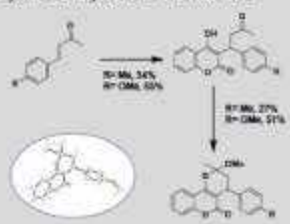
Coumarin scaffold has been considered as a valuable source of inspiration in the design of novel biologically active compounds

Coumarin Structures



Preliminary Studies

Synthesis of cyclocoumarol derivatives



Rayar A. M. et al, Eur J Med Chem, 146, 577-587, 2018

Docking studies with COX-2

✓ Synthesized derivatives were found to inhibit COX-2 enzyme

Do these derivatives have an anticancer effect?

Materials and Methods

Anticancer screening

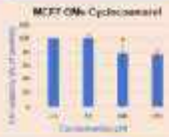
Derivatives were screened for anticancer activity on hormone-dependent breast cancer MCF-7 cell line:

Cyclocoumarol derivatives concentrations: 10, 25, 50, 75, and 150 µM
Ctl = mix DMSO - Acetone (1V/1V)

Results

MTT Assay
Cellular viability and proliferation

IC_{50} = 100 µM
A significant dose dependent decrease in cell viability with increased Me-Cyclocoumarol concentrations.

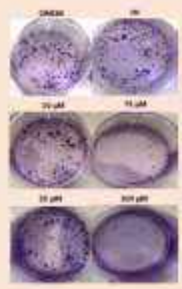


IC_{50} > 150 µM
A significant slight decrease in % of cell survival was shown at dose of 100 µM.

Trypan Blue Assay
Cytotoxicity assessment

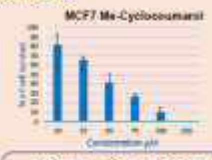
After treatment of MCF7 with 100 µM of Me-Cyclocoumarol, results showed a significant decrease in number of living cells and increase in number of dead cells instead.

Clonogenic Assay
Long term effect



$$\% \text{ of cell survival} = \frac{\text{Nb of colonies in treated well}}{\text{Nb of colonies in Ctl well}} \times 100$$

Legend
Ctl: Control
DMEM: Dulbecco's modified eagle medium
DMSO: Dimethyl sulfoxide



A significant dose dependent decrease in the colony formation was observed with increasing the concentrations of Me-Cyclocoumarol.

A significant decrease in the colony formation was observed with increasing the concentrations of OMe-Cyclocoumarol.

Conclusion

These preliminary results suggest that these compounds may have potential as novel anticancer agents. Me-Cyclocoumarol was shown to be the most potent compound. Pharmacomodulation on Me-Cyclocoumarol structure will be considered in order to develop new pyranocoumarin derivatives as selective COX-2 inhibitors.

H. D.M., Perspectives in Biol Med, 62(4), 778-784, 2019; M. R. S.M.I. et al, Expert Opin Ther Pat, 29(6), 407-427, 2019; H. G. N. et al. J Cell Physiol, 234(5), 5683-5699, 2019; Z. L. et al. Eur J Med Chem, 181, 111587, 2019

SCT Young Research Fellows Meeting 2021

Nutritional composition and lipid profile of various spent coffee grounds

BIJLA Laila [(1)] *, IBOURKI Mohamed [(1)], AIT BOUZID Hasna [(1)], GHARBY Said [(1)].

(1) Université Ibn-Zohr, Equipe de recherche physicochimie des milieux naturels, Matériaux et Environnement. Laboratoire de Biotechnologie, Matériaux & Environnement, Faculté Polydisciplinaire de Taroudant, Taroudant 83000, Morocco

Restricted to organizers

Spent coffee grounds (SCG) represent a great pollution hazard if discharged into the environment. Taking this fact into account and to contribute to the outcome of this matter, we have decided to conduct this study whose objective is to evaluate the phytochemistry of spent coffee grounds. Twelve samples of spent coffee grounds have been collected in different neighborhoods in Agadir. Then, several analyses were carried out; namely: oil yield, minerals, proteins content, sterol and fatty acids.

According to the obtained results, spent coffee grounds has a significant amount of oil and proteins. Furthermore, among all minerals studied in SCG, potassium concentration was the highest, followed by calcium and magnesium. On the other hand, spent coffee grounds oil contain also a significant amount of linoleic acid, palmitic acid and oleic acid. In terms of sterol composition, ß-sitosterol was the most abundant sterol, followed by Stigmasterol and compesterol.

In the line with our results and other studies, SCG present good water holding capacity due to its protein content, it has also several applications in environmental solutions, food industry, pharmaceutical fields and agricultural sector.

Keywords: spent coffee grounds, environment, agriculture, photochemistry, fertilizer, oil of spent coffee grounds, proteins.

* Correspondence: bijla.laila@gmail.com

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[1]Equipe de recherche physicochimie des milieux naturels, Matériaux et Environnement. Laboratoire de Biotechnologie, Matériaux et Environnement. Faculté Polydisciplinaire de Taroudant, Université Ibn-Zohr, Taroudante 83000, Morocco

Corresponding Email: bijla.laila@gmail.com

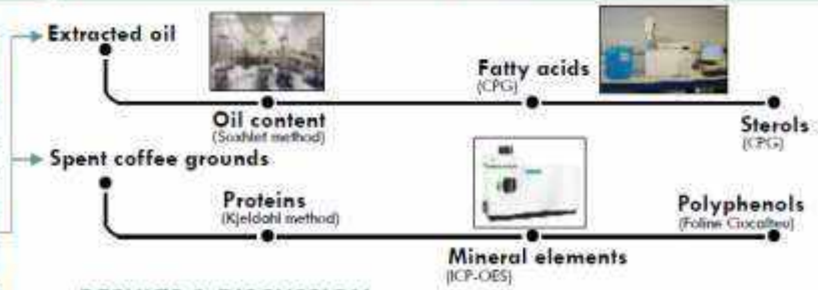
INTRODUCTION

Spent coffee grounds (SCG) represent a great pollution hazard if discharged into the environment. However, its chemistry could be of great interest to provide insights onto different ways of valorization. In this optic, we have decided to conduct this study whose objective is to evaluate the phytochemistry of spent coffee grounds. Twelve samples of spent coffee grounds have been collected in different neighborhoods in Agadir. Then, several analyses were carried out; namely: oil yield, minerals, proteins content, sterols and fatty acids.

MATERIAL & METHODS

Sample collection and preparation

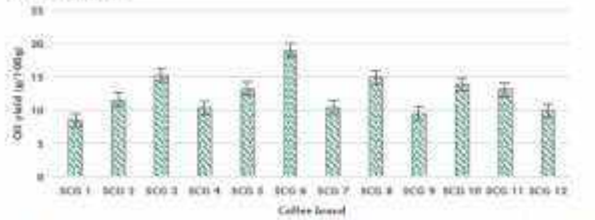
Analytical methods



RESULTS & DISCUSSION

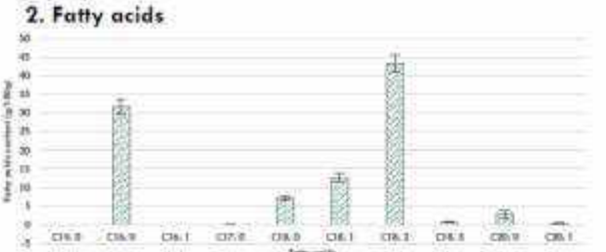
Spent Coffee Grounds Oil

1. Oil yield



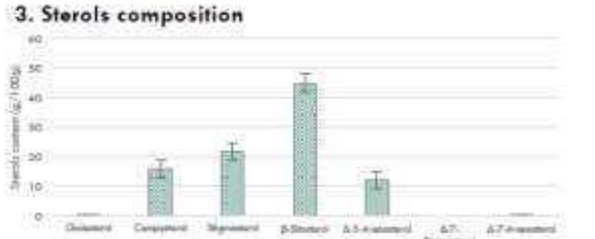
Oil content of SCGs in all brands ranges from 8.58±0.01 and 18.55±0.01 g/100g. This yield is higher than that found in cactus oil, and similar to that observed in olive oil and soybean oil[1].

2. Fatty acids



The main fatty acids in spent coffee grounds oil in ascending order are linoleic acid and palmitic acid. linoleic acid content is higher than that observed in rapeseed oil and coconut oil. While palmitic acid content is comparable to that detected in palm oil[2].

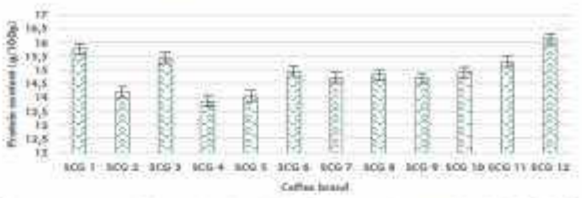
3. Sterols composition



β-sitosterol was the most abundant sterol in spent coffee grounds oil, this content is quite similar to that of coconut oil. The second major sterol is stigmasterol followed by compesterol.

Spent Coffee Grounds

4. Protein content

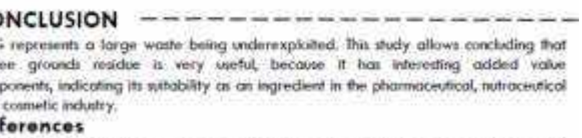


The protein content of the twelve brands of coffee grounds ranges from 13.87±1.12 to 16.12±1.30g/100g. The most important value is higher than that found in wheat bran[3] and similar to that found in onion[4].

5. Mineral elements

Among the studied element, potassium was the most abundant element in all samples (8323,23±974 mg/Kg). The second element was calcium (6181,18±500,67 mg/Kg), followed by magnesium with 2526,51±204,64 mg/Kg.

6. Total polyphenols content



Our results show that the content of total polyphenols in SCGs ranges between 36.5±11.36 and 166.93±71.92 mg (GAE)/g. The highest value is recorded in SCG 1 followed by SCG 10. While the lowest content of total polyphenols is observed in the sample SCG 1.

CONCLUSION

SCG represents a large waste being underexploited. This study allows concluding that coffee grounds residue is very useful, because it has interesting added value components, indicating its suitability as an ingredient in the pharmaceutical, nutraceutical and cosmetic industry.

References

Radiolabeling by aluminum fluoride-18 complexation: method optimization on a NODA-based model for application to sensitive biomolecule as PET tracers for oncology

Carine SAN[(1,2)*], Nicolas VIGNAL[(2,3)], Benoît HOSTEN[(2,4)], Marc PORT[(1)], Laure SARDA-MANTEL[(2,3)], Fabienne DIOURY[(1)*].

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(2) Université de Paris, Institut de Recherche Saint-Louis (IRSL), Unité Claude Kellershohn (UCK), 1 avenue Claude Vellefaux, 75010 Paris
(3) AP-HP, Hôpital Lariboisière, Médecine Nucléaire, 2 rue Ambroise-Paré, 75010 Paris
(4) AP-HP, Hôpital Saint-Louis, Pharmacie, 1 avenue Claude Vellefaux, 75010 Paris

Restricted to organizers

Fluorine-18 (^{18}F) is the most favorable positon emitter for tumor imaging. However, direct ^{18}F-labeling of biomolecules is challenging as it involves multiple steps and stringent conditions that are generally not suitable for biomolecules whose integrity may be altered. Over the past decade, an elegant new approach has been developed by coordination of aluminum fluoride {Al-^{18}F}.[(a)] The objective of this work was to implement such a method and to find the mildest conditions compatible with heat- or acid-sensitive biomolecules. To evaluate {Al-^{18}F} complexation, NODA-MP-C4 was prepared as a model compound from the commercially available NODA-MP-NCS (*Figure*). This model bears a thiourea function to mimic that present in the final conjugates. The corresponding radioactive complex Al^{18}F-NODA-MP-C4 was obtained following the most common reported radiofluorination conditions (*pH* 4, buffer AcO/AcOH, reflux). Based on this preliminary result, several parameters have been varied to obtain the highest radiochemical yield (RCY) under the mildest conditions. To this end and to guide the operational conditions in radiochemistry, a complementary study in cold series was performed in parallel. Al^{18}F-NODA-MP-C4 was obtained with a reproducible satisfactory RCY on comparison with reported results on NODA-derivatives.[(b,c)] Herein, we will present and discuss the optimization study.

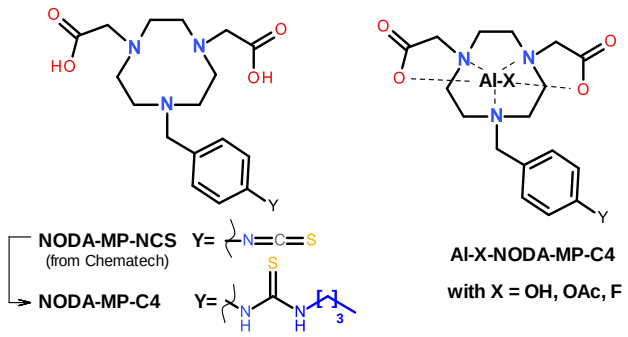

***Figure**. Structures of NODA-MP-NCS, NODA-MP-C4 and its aluminum complexes*

Bibliographic references:

[(a)] McBride, W. J. et al. *J. Nucl. Med.* 2009, 50 (6), 991–998.

[(b)] Huynh, P. T. et al. *New J. Chem.* 2019, 43 (38), 15389–15395.

[(c)] Shetty, D. et al. *Bioorg. Med. Chem.* 2012, 20 (19), 5941–5947.

* Correspondence: carine.san@aphp.fr ; fabienne.dioury@lecnam.net

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Introduction

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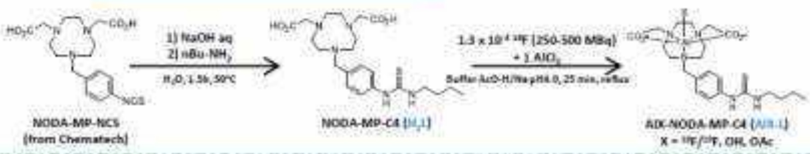
Materials and methods

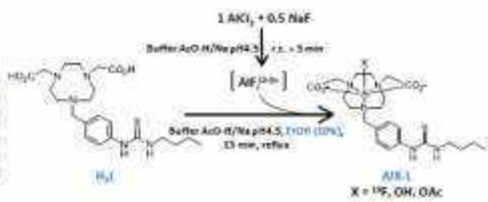
Radiochemistry

1. Production and purification of ^{18}F
2. Radiolabeling of H_2L
3. Radiofluoration yield determination by radio-HPLC



"Cold" chemistry

1. Synthesis of references
2. Identification of references by LC-MS
3. Optimization of the analytic LC method



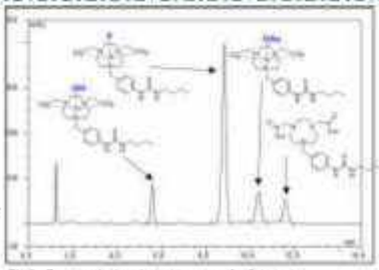


Fig 1. Representative chromatogram of references. Column: Kinetex Evo C18 100 x 4.6mm 5 µm, mobile phase: acetate buffer pH4.5/MeCN 85/15 isocratic, flow rate: 1 mL/min, injection volume: 25 µL, UV detection: 254 nm

Results and discussion

Table: Radiofluoration yield according to ^{18}F concentration, reactor type and number of pots

N°	^{18}F activity (MBq)	^{18}F volume (µL)	Reaction volume (µL)	Reactor type*	1 or 2 pot(s)**	Radiofluoration yield (%)
1	250	50	110	Tube	2	52 %
2	250	50	110	Tube	1	48 %
3	500	50	110	Tube	2	22 %
4	500	50	110	Tube	1	24 %
Fluorine-18 transport tubing replacement						
5	250	50	110	Tube	2	87 %
6	250	50	110	Tube	1	81 %
7	500	50	110	Tube	2	83 %
8	500	50	110	Tube	1	82 %
9	500	50	110	Vial	1	75 %
10	500	25	85	Vial	1	71 %
11	500	25	85	Vial	1	74 %
12	250	12.5	72.5	Vial	1	76 %
13	250	12.5	72.5	Vial	1	79 %

*Tube = hot tube = open reactor ; vial = for HPLC sample = sealed reactor
**one pot = mixture of H_2L, $AlCl_3$ and ^{18}F ; two pot = premixture of $AlCl_3$ and ^{18}F added on H_2L

- Reactor wall thickness influences the inertia to reach the reflux and the real time of warming.
- Transport tubing replacement highlights the impact of fluorine-18 molar activity (ratio ^{18}F/^{19}F) on radiofluoration yield.

On Kinetex C18 column:
- Radio detection: good separation between uncomplexed {Al^{18}F} + ^{18}F and radiolabelled Al^{18}F-L.
- UV detection: no separation of the different AlX-L species.
☞ Change for Kinetex Evo C18 (Fig 1.)

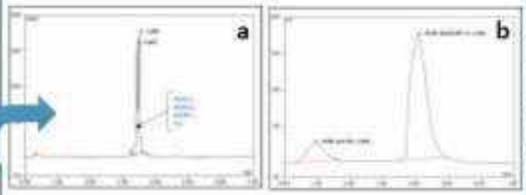


Fig 2. Representative radio-HPLC chromatograms of the radiolabeling reaction mixture (a: UV 254 nm, b: radio). Column: Kinetex C18 50 x 2.1 mm 2.6 µm, mobile phase: H₂O/MeCN, gradient: 95/5 to 5/95, flow rate: 0.7 mL/min, injection volume: 5 µL

Conclusion

Al^{18}F-NODA-MP-C4 was obtained with a reproducible satisfactory radiofluoration yield (79 % ± 8 % (n=9)) on comparison with reported results on NODA-derivatives (60 % to 90 %).[b,c]

Bibliographic references:
[a] McBride, W. J. et al. J. Nucl. Med. 2009, 50 (6), 991–998.
[b] Huynh, P. T. et al. New J. Chem. 2019, 43 (38), 15389–15395.
[c] Shetty, D. et al. Bioorg. Med. Chem. 2012, 20 (19), 5941–5947.

Acknowledgements:
With financial support: ITMO Cancer of Aviesan on funds administered by Inserm, and Comité Paris de la Ligue Nationale contre le Cancer

XXVIIIth Young Research Fellow Meeting – 11-12th February 2021

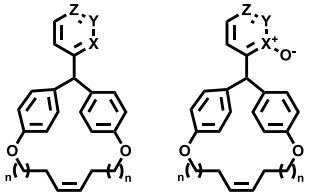
Synthesis of new macrocyclic triarylmethanes using cross metathesis

Corinne Coutant (1)*, Ameni Hadj Mohamed (1) (2), Moncef Masddeck (2), Maité Sylla-Iyarreta Veitía (1)

(1) Equipe de Chimie Moléculaire du Laboratoire Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Conservatoire National des Arts et Métiers (Cnam), 2 rue Conté, 75003, HESAM Université, Paris, France.

(2) Laboratoire de Chimie hétérocyclique, produits naturels et réactivité (LR11ES39). Faculté des sciences de Monastir, Tunisie.

In the last years, a large number of bioactive molecules developed in MedChem concern a macrocycle scaffold. It is described in the literature that macrocycles are able to reach certain hard-to-reach biological targets and have a positive impact on pharmacokinetic and pharmacodynamic properties of the molecule [a, b, c].



X, Y, Z = N or CH
n = 1 or 2

The metathesis reaction using Grubbs II catalyst have been described as ineffective on chemical structure bearing a pyridine moiety. Indeed, the Lewis basicity and the nucleophily of azote leads to the catalyst deactivation. In order to circumvent this problem, it is possible to form imidazolium or pyridinium salts or even to decrease the nucleophilicity or the Lewis basicity of azote by the introduction of an electron-withdrawing group, doublet relocation or even introduction of steric hindrance.

In this work we demonstrate the feasibility of the ring closing metathesis (RCM) on chemical compounds bearing a pyridine moiety without any prior modification. We describe the design, synthesis and characterization of a serie of triarylmethanes macrocycles derivatives. A second serie of *N*-oxydes triarylmethanes macrocycles was also studied. The synthetic approach developed concerns 3 or 4 steps including reactions such as Friedel-Crafts acylation, saponification, *O*-alkylation, RCM and *N*-oxidation. The antibacterial and anticancer activity of all synthesized compounds is being assessed.

Bibliographic references:

[a] Ermert, P. Design, Properties and Recent Application of Macrocycles in Medicinal Chemistry. Chim. Int. J. Chem. 2017, 71 (10), 678–702. Doi: 10.2533/chimia.2017.678.

[b] Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. The Exploration of Macrocycles for Drug Discovery - an Underexploited Structural Class. Nat. Rev. Drug Discov. 2008, 7 (Copyright (C) 2020 American Chemical Society (ACS). All Rights Reserved.), 608–624. Doi: 10.1038/nrd2590.

[c] Mallinson, J.; Collins, I. Macrocycles in New Drug Discovery. Future Med. Chem. 2012, 4 (11), 1409–1438. Doi:10.4155/fmc.12.93.

*** Correspondence**: cori.coutant@gmail.com

Synthesis of new macrocyclic triarylmethanes using cross metathesis

Corinne Coutant* [1], **Ameni Hadj Mohamed** [1,2], **Moncef Msaddeck** [2], **Maïté Sylla-Iyarreta Veitia** [1]

1) Equipe de Chimie Moléculaire du Laboratoire Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Conservatoire National des Arts et Métiers (Cnam), 2 rue Conté, 75003, HESAM Université, Paris, France.
(2) Laboratoire de Chimie hétérocyclique, produits naturels et réactivité (LR11ES39). Faculté des sciences de Monastir, Tunisie.

Introduction

In the last years, a large number of bioactive molecules developed in MedChem concern a macrocycle scaffold . [a, b, c]

In this work we demonstrate the feasibility of the ring closing metathesis (RCM) on chemical compounds bearing a pyridine moiety without any prior modification. We describe the design, synthesis and characterization of a serie of triarylmethanes macrocycles derivatives and their *N*-oxides analogues.

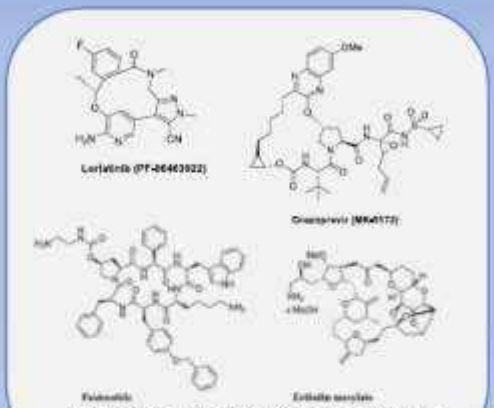


Figure 1 : Examples of macrocycles developed in MedChem

Results and Discussion

3 or 4 steps :
Friedel-Crafts acylation or saponification
O-alkylation
RCM
N-oxidation

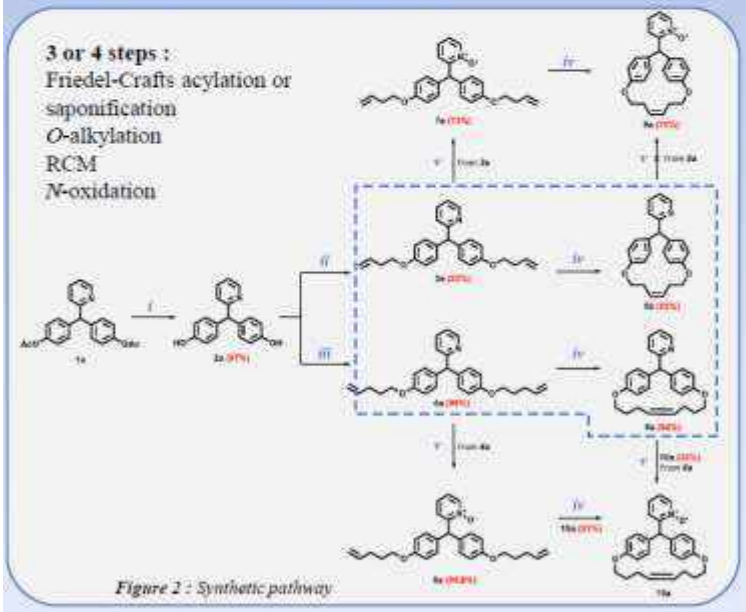


Figure 2 : Synthetic pathway

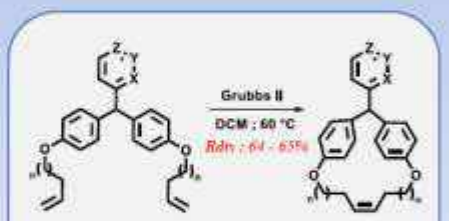


Figure 3 : RCM step

Reactions conditions
i. KOH, 10% EtOH, r.t., 48 h.
ii. Bromobutene, KI, K_2CO_3, acetone, 60 °C.
iii. Bromopentene, KI, K_2CO_3, acetone, 60 °C.
iv. Grubbs II catalyst, DCM, 60 °C.
v. *m*-CPBA, DCM, r.t.

- Any prior modifications at level of azote in order to decrease Lewis's basicity or nucleophilicity was necessary. The RCM products were obtained with yields between 45 and 77% [d].

Conclusion

- 7 macrocycles were synthetized with yield between 15 and 58%
- The macrocycles *N*-oxyde were obtained with yield between 49 and 87%.
- The biological activity of the synthesized macrocycles is currently under study.

(a) Ermert, P. Design, Properties and Recent Application of Macrocycles in Medicinal Chemistry. Chim. Int. J. Chem. 2017, 71 (10), 678–702.
(b) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. The Exploration of Macrocycles for Drug Discovery - an Underexploited Structural Class. Nat. Rev. Drug Discov. 2008, 7 (Copyright (C) 2020 American Chemical Society (ACS). All Rights Reserved.), 608–624.
(c) Mallinson, J.; Collins, I. Macrocycles in New Drug Discovery. Future Med. Chem. 2012, 4 (11), 1409–1438.
(d) A. Hadj Mohamed, C. Coutant, M. Msaddek, M. Sylla-Iyarreta Veitia. Design, synthesis and characterization of new macrocycle triarylmethanes using RCM, *article in preparation*

Correspondance: corinne.coutant@gmail.com, maite.sylla@lecnam.net

SCT Young Research Fellows Meeting

Synthesis and biological evaluation of new fluorinated ligands targeting P2X receptors

Simon Garnier (1) *, Agnieszka Zak (1), Johnny Vercouillie(2), Sylvie Chalon(2), Patrick Emond(2), Nicolas Arlicot(2), Frederic Buron(1) and Sylvain Routier(1).

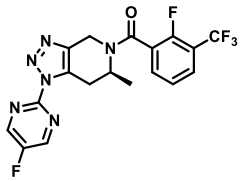
(1) ICOA, Université d'Orleans, CNRS UMR 7311, 45067 Orléans, France.
(2) Inserm U1253, Imagerie et Cerveau (iBrain), Université François Rabelais de Tours, France.

Restricted to organizers

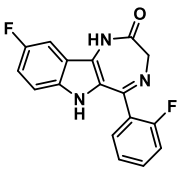
The central nervous system (CNS) is the part of the nervous system consisting of the brain and spinal cord. Neurodegenerative diseases as Parkinson's, Alzheimer's or epilepsy are typically associated with chronic inflammation of the CNS - neuroinflamation. It is a part of the complex biological response of body tissues to pathogens or damaged cells. The function of inflammation is to eliminate the initial cause of cell injury and initiate tissue repair. The initial microglial response that occurs in neuroinflamation is characterized by microglial accumulation in the injured sites of the brain (a).

Inflammatory conditions are associated with the extracellular release of nucleotides, particularly ATP. Extracellular ATP is activated by ionotropic P2X receptors. Among seven members of P2XR family, P2X7 is expressed by variety of cells type in brine as neurons and microglial cells. Although several other P2XRs are functional during inflammation, P2X7R in particular has been shown to affect the outcomes of inflammatory or infectious diseases (b). Similar receptor P2X4 may also act as an initial trigger of neuroinflammation. It forms a large conductance pore on the cell membrane, facilitating ion efflux and subsequent inflammasome activation (c).

In the literature, there are few described references targeting those two receptors. Based on literature research, we designed and synthesized new scaffolds and series of original fluorinated compounds. The activity of all the molecules was evaluated. The most promising ligands will become a potential ^{18}F probes to early diagnosis CNS disorders.



$P2X_7$ IC_{50} = 5,9 nM
Janssen Pharmaceutical NV



$P2X_4$ IC_{50} <100 nM
Sunovion Pharmaceuticals Inc.

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(a) Domercq, M., Vázquez-Villoldo, N., Matute, C. Front. Cell. Neurosci., 2013, (7), 49.
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* Correspondence: simon.garnier@univ-orleans.fr

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW FLUORINATED LIGANDS TARGETING P2X RECEPTORS

Simon Garnier[1], Marion Michel[1], Zuhal Gulhan[2], Johnny Vercouillie[2], Sylvie Chalon[2], Patrick Emond[2], Nicolas Arlicot[2], Fréderic Buron[1] and Sylvain Routier[1]

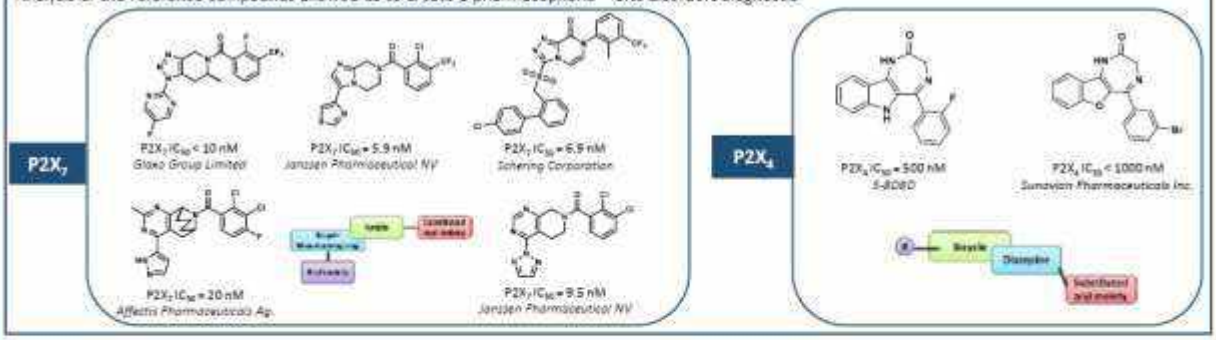
[1] ICOA - CNRS UMR 7311 - Université d'Orléans, Pôle de Chimie - 45100 Orléans, France
[2] Inserm U1253, Imagerie et Cerveau (iBrain), Université de Tours, France.

Introduction

Neurodegenerative diseases as Parkinson's, Alzheimer's or epilepsy are typically associated with chronic inflammation of the Central Nervous System (CNS). The neuroinflammation is a part of the complex biological response of body tissues to pathogens or damaged cells wich is characterized by the accumulation of activated microglia in the injured sites of the brain. Inflammatory conditions are linked with the extracellular release of ATP, activated by ionotropic P2X receptors. Among seven members of P2XR family, $P2X_7$ is expressed by a variety of brain cells type such as neurons and microglia and has been shown to affect the outcome of inflammatory or infectious diseases. Similarly, the receptor $P2X_4$ may also act as an initial trigger of neuroinflammation. It forms a large conductance pore on the cell membrane, facilitating ion efflux and subsequent inflammasome activation. Although, a number of $P2X_7R$ ligands have to date been developed, few compounds are still available for $P2X_4R$. In this context, we initiated the synthesis of new $P2X_4R$ ligands, preferably having a fluorine atom in their structure, in the perspective to focus of new fluor-18-labeled imaging tracers.

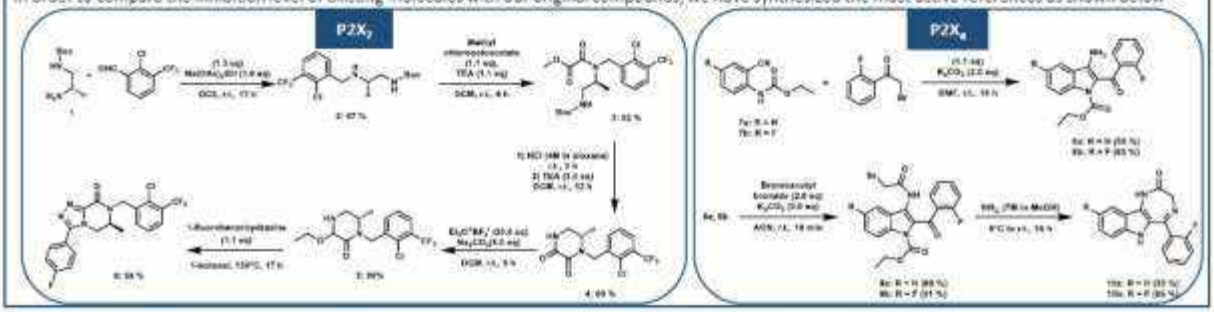
Reference molecules

The great interest of pharmaceutical companies in the $P2X_7R$ has resulted in the creation of many active compounds for the target. Nevertheless, $P2X_4R$ ligands are not well developed. Recently, 5-BDBD has been proposed as a molecule selective to $P2X_4Rs$, however, it exhibits low inhibitory properties.
Analysis of the reference compounds allowed us to create a pharmacophoric model of molecules targeting P2XR's. For our studies, we have selected references containing halogen, especially fluorine. Replacment of halogen atom with radioactive isotope ^{18}F will allow us to use the active molecules as a probe for Positron Emission Tomography (PET) imaging in the aim of early CNS disorders diagnostic



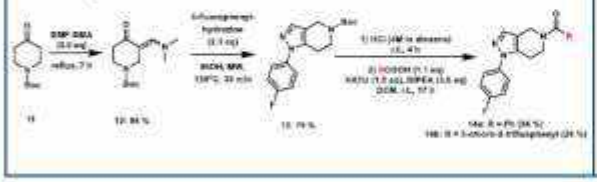
Synthesis of Reference molecules

In order to compare the inhibition level of existing molecules with our original compounds, we have synthesized the most active references as shown below



New scaffold synthesis

Following the pharmacophoric model for the two targets, we have designed and synthesised 4 different core structure, allowing us to obtain 36 compounds containing fluorine atoms. The synthesis of one of the new scaffolds is presented below.



Biological evaluation

Inhibition potency measurement *via* ATP-induced Ca^{2+} release, on culture of HEK293 cells expressing human $P2X_4R$.

With a concentration of 10 µM : % inhibition of 51% (**10a**) and 59% (**10b**) (mean of 5 experiments), in agreement with described properties of $P2X_4R$ ligands (WO 2015088565) : validation of our biological method.

In same condition : around 30.% inhibition was obtained for 3 derivatives which structure was expected to bind und to $P2X_4R$. no inhibition was observed for **14a** and **14b** which structure orientated them to $P2X_7R$ ligands.

These data validate our design of selective series and prompt us to characterize other new compounds.

Conclusion

Based on literature research, we designed and synthesized 4 different families (36 compounds) of molecules targeting $P2X_4R$ and $P2X_7R$. The molecules were tested considering a response to $P2X_4R$ human cells. The primary results show that some of the molecules could antagonize $P2X_4R$-mediated responses, however, the inhibition level is low. Further efforts will aim to increase the activity. Additionally, BBB permeability will be verified. The most promising ligands will become a potential ^{18}F probes to early diagnosis CNS disorders by PET

Computer-Aided design of XIAP inhibitors.

Marc RAGUI*, Charline KIEFFER, Anne-Sophie VOISIN-CHIRET, Jana SOPKOVA - DE OLIVIERA SANTOS.

CERMN, EA 4258, Boulevard Becquerel 14032 Caen Cedex, France.

Restricted to organizers

XIAP (X-linked chromosome) is one of the human inhibitory apoptosis protein family including also other members like cellular IAP1/2 (cIAP 1/2), neuronal apoptosis protein (NAIP), survivin (TIAP), Apollon, melanoma IAP (ML-IAP), and IAP-like protein 2 (ILP2)[1]. They are considered as key regulators of cell death (apoptosis). Specifically, XIAP is known for its higher affinity to caspase enzymes released from mitochondria, through its baculoviral IAP repeats domains (BIR). Also, it contains a ubiquitin-associated domain (UBA) and a really interesting new genes (RING) with a ligase activity. XIAP-binding mechanism results in promoting cell survival regulated by the action of the second mitochondrial activator of caspases (SMAC/DIABLO). The endogenous antagonist, SMAC, binds to XIAP (also cIAPs) BIR domains releasing caspases and reactivating intrinsic signaling pathways leading to apoptosis. Overexpression of XIAP is involved in cancer and autoimmune diseases like Multiple sclerosis. That is why XIAP is considered a potential target especially, for cancer therapeutics[2]**.**

In the market, there is no approved drug acting as XIAP inhibitor. Most of the molecules in clinical trials are either peptide or peptidomimetics[3] prone to peptidases actions. Also, the lack of XIAP- selectivity, even for the only non-peptidomimetic ligand was shown[4], and this could be the cause of adverse effects during patient's treatments[5]. So, the design of small and selective chemical compounds will be of great interest.

This project aims to design selective non-peptide ligands for BIR2 and BIR3 domains of XIAP by optimizing previously synthesized ligands in the laboratory. The main work relies on using molecular dynamics simulations associated with different methods such as Poisson-Boltzmann surface methods (MM-PBSA)[6] and Free energy perturbation (FEP)[7] to predict ligand-binding affinity of the hits *in silico* and compare theoretical results with experimental ones. After, the optimized ligands will be synthesized and then tested using different techniques like Alpha screen and Fluorescence polarization assay (FPA). Promising candidate ligands will be re-evaluated using enhanced sampling techniques like Steered Molecular Dynamics (SMD), Supervised Molecular Dynamics (SuMD), and Meta-dynamics[8].

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Correspondence: marc.ragui@unicaen.fr , charline.kieffer@unicaen.fr, anne-sophie.voisin@unicaen.fr , jana.sopkova@unicaen.fr

Computer-Aided design of XIAP inhibitors

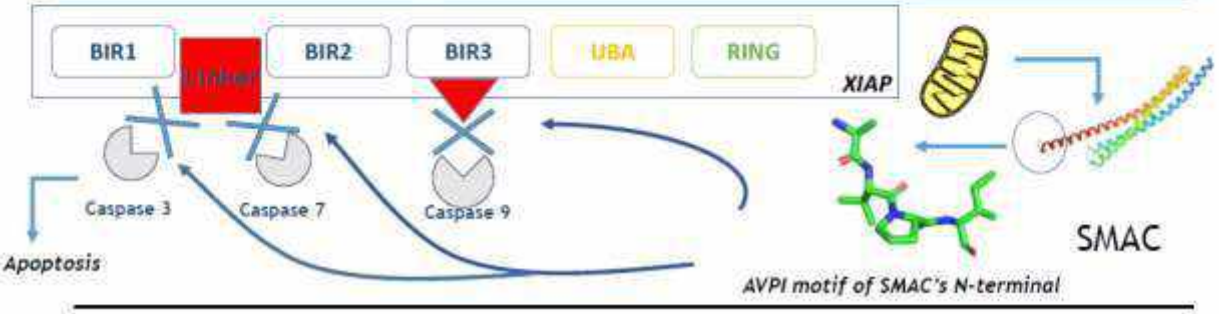
Marc RAGUI[1]*, Charline KIEFFER[1], Anne Sophie VOISIN-CHIRET[1] and Jana SOPKOVA-DE OLIVIERA SANTOS[1]

(1) Centre d'Etudes et de Recherche sur le Médicament de Normandie, Université de Caen Normandie, Caen, France, 14000

* Correspondence: marc.ragui@unicaen.fr

General outlook:

XIAP (X-linked chromosome) is one member of the human inhibitory apoptosis protein family and is considered as key regulators of cell death (apoptosis). It is known for its high binding affinity to caspase enzymes released from mitochondria, through its baculoviral IAP repeats domains (BIR). XIAP-binding mechanism to caspase enzymes results in promoting cell survival, but it is regulated by the action of the second mitochondrial activator of caspases (SMAC/DIABLO). The endogenous antagonist, SMAC, binds to XIAP (also cIAPs) BIR domains via its N-terminal motif (Ala - Val - Pro - Ile) releasing caspases and reactivating intrinsic signaling pathways leading to apoptosis[1].



Over expression of XIAP is involved in:

Objectives:

The aim of this project is to design non-peptide ligands for BIR2/BIR3 domains of XIAP to avoid their sensitivity against peptidases, but also selective to prevent over toxicity observed during patient's treatment[2]. The main work relies on considering different methods for affinity prediction either theoretical ones (MD simulations) or the experimental ones (*in vitro* evaluations) in order to achieve the desired target.

MD methods

1	MM-PBSA	Uses a continuous solvant model to calculate Gibbs free energy[3].
2	FEP	Based on thermodynamics cycles and involve the change of a chemical functional group[4].
3	SMD	The ligand is steered out of his binding pocket by additional force[3].
4	SuMD	Enables a complete recognition of protein-ligand pathway[5].

In vitro evaluations

ALPHA screen test	•Relies on the presence of 2 beads: donnor (bounded to protein) and acceptor (linked to referent ligand). •In case of interaction, a fluorescent signal is produced.
FPA *(Fluorescence polarization assay)*	•The referent ligand is labeled with a fluorescent probe and the non labeled ligand will displace the labeled ligand. •The rupture of interaction will reduce the polarization signal.

References:

1] Chossari, G. *et al. J. Med. Chem.* **58**, 6574-6588 (2015). 2] Fulda, S. *J. Clin. Oncol.* **32**, 3190-3191 (2014). 3] Visvaldas Kairys, Lina Baranauskiene, Migle Kazlauskiene, Daumantas Matulis & Egidijus Kazlauskas. *Expert opinion on drug discovery*, **14**, 755-768, (2019). 4] Michel J, Foloppe N, Essex JW. Rigorous. *Mol Inform.* **29**, 570-578, (2010). 5] Sabbadin D, Salmaso V, Sturlese M, Moro S. *Methods Mol Biol.* **1824**, 287-298, (2018).

Novel arylaminoalcohol-based compounds: promising antimalarial drugs	
Camille Tisnerat*, Jérémy Schneider, Céline Damiani, Patrice Agnamey, Catherine Mullié, Anne Totet, Alexandra Dassonville-Klimpt, Pascal Sonnet *AGIR, UR 4294, UFR de Pharmacie, Université Picardie Jules Verne, Amiens, France.*	***Restricted to organizers***

With 229 million of cases in 2019, malaria still remains as one of the most threatening diseases in the world.[(a)] The causative agents are *Plasmodium* parasites, among which *P. falciparum* (*Pf*) is the most virulent and common for human infection. Fighting resistant *P. falciparum* strains is today one of the main challenges to eradicate malaria. Despite the World Health Organization recommendations for preventing and reducing this risk with artemisinin-based combination therapy[(a)(b)], more and more parasites are spreading with both decreased sensibility to artemisinin derivatives and resistance to the partner drug.[(c)] Genic mutations or overexpression of efflux pumps are, in part, responsible of the decreased efficacy of both mefloquine (MQ) and lumefantrine (LM), two of the most used partners with artemisinin derivatives. Thus, covalent conjugation of their respective pharmacophore with efflux pump inhibitor (EPI) moieties can allow to struggle resistant parasites.[(d)]

Our laboratory has previously developed an asymmetric synthesis to prepare 4-aminoalcohol-quinoline and -fluorene as enantiopure MQ and LM analogs. Some of them were active on nanomolar range against *Pf*3D7 (chloroquine-sensitive) and *Pf*W2 (chloroquine-resistant) with a good selectivity index. Interestingly, eudysmic ratio was often observed between the enantiomers. Following this previous work and inspired from known reversal agent scaffolds, we designed new enantiopure arylaminoalcohol-based hybrids with EPI patterns able to limit the resistance with efflux transporters. The synthesis of these new series will be described. First results concerning their promising antimalarial activities with good selectivity indexes will be debated.

Bibliographic references:

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* Correspondence: camille.tisnerat@etud.u-picardie.fr

Novel arylaminoalcohol-based compounds: promising antimalarial drugs

Camille Tisnerat, Jérémy Schneider, Céline Damiani, Patrice Agnamey, Catherine Mullié, Anne Totet, Fabien Gosselet, Alexandra Dassonville-Klimpt, Pascal Sonnet

AGIR, UR 4294, UFR de Pharmacie, Université de Picardie Jules Verne, Amiens, France

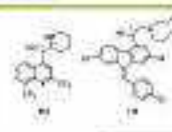
camille.tisnerat@etud.u-picardie.fr

MALARIA[1]

229 millions of cases (2019)
Mostly in Africa (94%)
Deadly infection
Mainly caused by *P. falciparum*
Spread by *Anopheles* mosquitoes

ANTIMALARIALS

Artemisinin-based combination therapy[2,3]
as
artemisinins + mefloquine (MQ) or lumefantrine (LM)



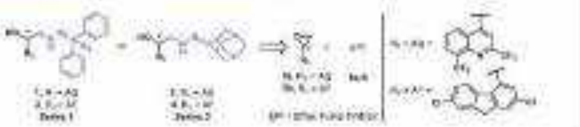
PARASITE RESISTANCE[3]

↓ sensibility to artemisinins
+
genic mutations and efflux pump to MQ / LM

Covalent conjugation of arylaminoalcohol-based compounds with efflux pump inhibitors (EPI) can allow to struggle resistant parasites ?

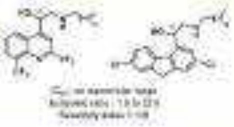
Aim

Inspired from known reversal agents,[9] we designed new enantiopure arylaminoalcohol-based quinolines and fluorenes 1-4 with EPI pattern under two series: benzhydryl/trityl (series 1) and adamantyl (series 2).



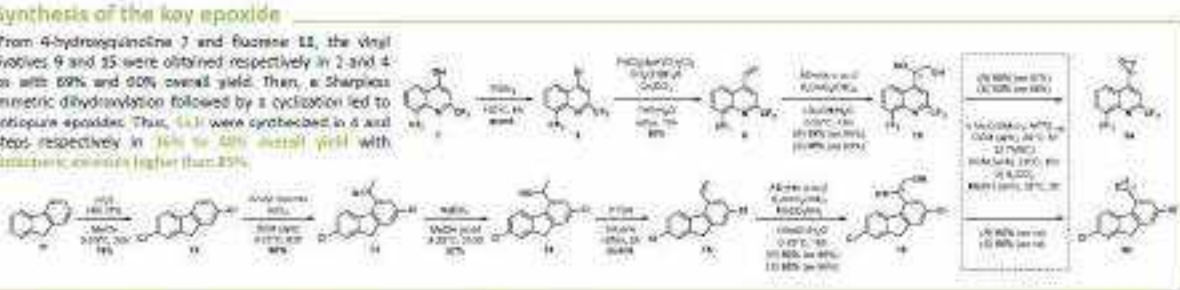
Previous work

Our laboratory has developed an asymmetric synthesis to prepare 4-aminoalcohol-quinolines (4-AQ) and -fluorenes (4-AF) as enantiopure MQ and LM analogs from a key epoxide 5a,b.[4-8]



Synthesis of the key epoxide

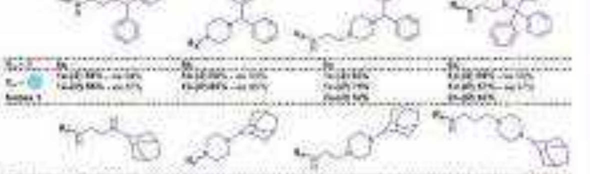
From 4-hydroxyquinoline 7 and fluorene 11, the vinyl derivatives 9 and 15 were obtained respectively in 2 and 4 steps with 69% and 60% overall yield. Then, a Sharpless asymmetric dihydroxylation followed by a cyclization led to enantiopure epoxides. Thus, 5a,b were synthesized in 4 and 6 steps respectively in 36% to 48% overall yield with enantiomeric excesses higher than 85%.



Synthesis of arylaminoalcohol-based compounds

Height amino moieties IPE 5a-h were synthesized in one to five steps, in 35% to quantitative overall yields (not exposed herein). Divided in benzhydryl/trityl and adamantyl series, some of IPE structures contain the piperazine moiety. Arylaminoalcohol-based compounds were then obtained by a regioselective attack on 4-oxirane 5a,b under micro-waves with 1.5 to 3 equivalents of EPI each.

Nowadays, sixteen 4-AQ 1,3 and seven 4-AF 2,4 were obtained with a yield range from 41% to quantitative. First analysed compounds have suitable enantiomeric excesses, higher than 87%.



Biological results

By a SYBR Green I fluorescence method, the *in vitro* antimalarial activity was evaluated against *Pf*3D7 and *Pf*W2 strains. Pleasingly, all IC_{50} were in nanomolar range, except for the compound 4c-(*R*). In series 1, compounds 1a and 1d-(*R*) present the better activity, proving the interest of benzhydryl- and trityl-ethane diamine as EPI, equally for 3a and the adamantyl-ethane diamine in series 2. Interestingly, 3b shows the best eudysmic ratio, whatever the strain, and 3c presents a good activity less than 15 nM. First results lead toward a better activity of 4-AQ moieties than 4-AF. Evaluated against HepG2 cells, cytotoxicity (IC_{50}) was ranged from 2.5 to 28.1 µM, giving selectivity index (HepG2/*Pf*W2) superior to 110, except for 1c-(*R*), and closed to 4 170 for 1d-(*R*).

Compounds			Activity - IC_{50} (nM)		Eudysmic ratio	
Series	R_1		*Pf*3D7	*Pf*W2	*Pf*3D7	*Pf*W2
1	AQ	1a-(S)	5.0 ± 0.61	8.9 ± 1.20	1.4	3.2
		1a-(R)	6.8 ± 1.22	3.1 ± 0.38		
		1b-(S)	26.5 ± 1.61	9.2 ± 1.06	2.11	3.2
		1b-(R)	38.6 ± 3.06	29.2 ± 4.8		
		1c-(S)	75.7 ± 9.52	65.0 ± 3.74	1.7	1.2
		1c-(R)	45.1 ± 5.43	78.5 ± 5.06		
		1d-(S)	< 40	42.3 ± 10.5	nd	36
		1d-(R)	3.26 ± 0.2	1.2 ± 0.1		
	AF	2a-(R)	33.9 ± 4.06	16.2 ± 3.3	nd	nd
2	AQ	3a-(S)	6.8 ± 2.2	13.7 ± 5.8	1.7	1.2
		3a-(R)	11.8 ± 3.9	8.2 ± 0.2		
		3b-(S)	48.8 ± 2.0	18.5 ± 9.2	10.2	14.8
		3b-(R)	499.9 ± 176.7	271.2 ± 3.7		
		3c-(S)	14.2 ± 3.0	7.0 ± 5.9	1.1	1.8
		3c-(R)	8.2 ± 3.7	3.6 ± 4.4		
		3d-(S)	41.5 ± 8.4	19.5 ± 3.5	2.9	3.8
		3d-(R)	12.9 ± 7.1	5.0 ± 2.5		
	AAF	4a-(R)	57.1 ± 10.2	55.3 ± 7.41	nd	nd
		4c-(R)	> 1 000	> 1 000	nd	nd
		4d-(R)	480.0 ± 120.9	> 320	nd	nd
Chloroquine			75.9 ± 3.0	198.8 ± 17.0		
Mefloquine			79.7 ± 8.5	31.8 ± 1.8		

[illegible]

Conclusion

Novel twenty-three arylaminoalcohols were synthesized with good yields following a synthesis in 5 to 7 steps. Most of evaluated hybrids were more active than the references (whatever the strain). Interestingly, 1d-(R) shows the best activity, inferior to 3 nM, and selectivity index (4170). The benzhydryl- and trityl-ethane diamine turned out as the best EPI. Moreover, eudysmic ratio were found, and was even high for 1d and 3b, proving the added value of asymmetric synthesis.

Thanks to these first results, the covalent conjugation of antiplasmodial arylaminoalcohol-based compounds with EPI is promising for next biological studies.

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Antibacterial and antibiofilm activity of non-steroidal anti-inflammatory drugs against *Escherichia coli* and *Staphylococcus aureus*

Anabela Borges [1,2]*, Cláudia Leão [1], Manuel Simões [1,2]

(1) Faculty of Engineering, University of Porto, LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

(2) Faculty of Engineering, University of Porto, DEQ - Department of Chemical Engineering, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal.

Restricted to organizers

Persistent infections, usually associated with biofilm-producing bacteria, continue to be a challenge for both medical and scientific communities. The potential interest in drug repurposing for biofilm control is growing due to the disinvestment in antibiotic R&D, reduced efficacy of the available panel of antibiotics and increased risks associated with new development plans[(a)]. In the present study, the antibacterial and antibiofilm activities of four non-steroidal anti-inflammatory drugs (NSAIDs), piroxicam (PXC), diclofenac sodium (DCF), acetylsalicylic acid (ASA) and naproxen sodium (NPX), were evaluated against *Escherichia coli* and *Staphylococcus aureus*. The minimum inhibitory/bactericidal concentrations (MIC and MBC) and the dose response curves from exposure to the selected NSAIDs were determined by the broth microdilution method and culturability, respectively. The potential of NSAIDs to eradicate preformed biofilms was performed using a microtiter plate assay and characterized in terms of biofilm mass removal (crystal violet staining), metabolic activity reduction (alamar blue staining) and biofilm cells culturability. Additionally, the ability of the selected NSAIDs combined with current in use antibiotics (kanamycin - KAN and tetracycline - TET) to eradicate 24-h old biofilms was also tested in order to evaluate their ability to potentiate the putative antibiofilm activity of antibiotics. MIC were found for PXC (800 µg/mL) and ASA (1750 µg/mL) against *E. coli*, and for DCF (2000 µg/mL) and ASA (2000 µg/mL) against *S. aureus*. No MBC were found (> 2000 µg/mL). Regarding biofilm eradication, ASA, DCF and PXC promoted significant reductions in metabolic activity (66.1% to 86.7%) and culturability (2.22 to 6.46 log CFU/cm^2). However, only PXC promoted biofilm mass removal (maximum of 28.6%). Additive interactions were obtained for most of the combinations between NSAIDs and KAN or TET. Overall, the results obtained suggest that NSAIDs appear to be a promising strategy to control biofilms as they demonstrated to be more effective than conventional antibiotics.

Bibliographic references:

(a) Leão, C., Borges, A. and Simões, M., 2020. NSAIDs as a Drug Repurposing Strategy for Biofilm Control. *Antibiotics*, *9*(9), p.591.

Acknowledgements: This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); Projects PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219 and PTDC/ASP-PES/28397/2017 - POCI-01-0145-FEDER-028397, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Anabela Borges thanks the Portuguese Foundation for Science and Technology (FCT) for the financial support of his work contract through the Scientific Employment Stimulus - Individual Call - [CEECIND/01261/2017].

* Correspondence: apborges@fe.up.pt

Antibacterial and antibiofilm activity of non-steroidal anti-inflammatory drugs against *Escherichia coli* and *Staphylococcus aureus*

Anabela Borges[1,2]*, Cláudia Leão[1], Manuel Simões[1,2]

[1] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[2] DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal. *Correspondence: aborges@fe.up.pt

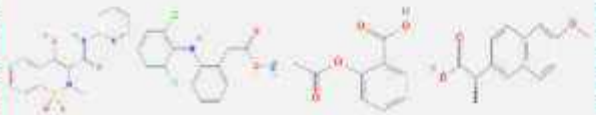
Introduction

Persistent infections, usually associated with biofilm-producing bacteria, continue to be a challenge for both medical and scientific communities. The potential interest in drug repurposing for biofilm control is growing due to the disinvestment in antibiotic R&D, reduced efficacy of the available panel of antibiotics and increased risks associated with new development plans[a]. In the present study, the antibacterial and antibiofilm activities of four non-steroidal anti-inflammatory drugs (NSAIDs), piroxicam (PXC), diclofenac sodium (DCF), acetylsalicylic acid (ASA) and naproxen sodium (NPX), were evaluated against *Escherichia coli* and *Staphylococcus aureus*.

(a) Leão, C., Borges, A. and Simões, M., 2020. NSAIDs as a Drug Repurposing Strategy for Biofilm Control. Antibiotics, 9(9), p.591.

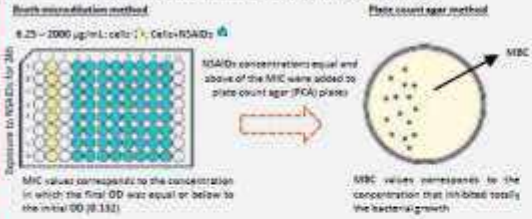
Methods

- **Bacterial strain:** *Escherichia coli* CECT 434 and *Staphylococcus aureus* CECT 976
- **NSAIDS from 4 different families** (Oxicams [1]; Acetic acids [2]; Salicylates [3]; Propionic acids [4]):



[1] Piroxicam (PXC) [2] Diclofenac sodium (DCF) [3] Acetylsalicylic acid (ASA) [4] Naproxen sodium (NPX)

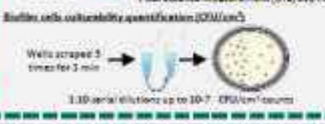
- **Minimum inhibitory and bactericidal concentrations (MIC and MBC):**



- **Dose response curves – culturability:**

- **Biofilm control – antibiotic-NSAID dual combinations:**

3. Analyze in terms of:



4. Interaction between NSAIDs and antibiotics classification:

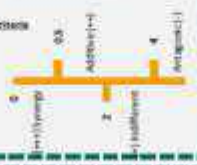
Combinatorial index of NSAIDs (C_{NSAIDs})/ATB (C_{ATB}):

$$C_{NSAIDs} = \frac{B_{NA}/A}{B_{NA}}$$

Total Combinatorial Index (TCI):

$$TCI = C_{NSAIDs} + C_{ATB}$$

Scoring criteria



Results

- **Antibacterial activity of selected NSAIDs and antibiotics**

- MIC were found for PXC (800 μg/mL) and ASA (1750 μg/mL) against *E. coli*, and for DCF (2000 μg/mL) and ASA (2000 μg/mL) against *S. aureus*. No MBC were found with any of the studied NSAIDs for both bacteria, in the range of tested concentrations (~2000 μg/mL) (**Table 1**). For ATB the MIC values were slightly higher than ones of the CLSI guidelines.

Table 1. MIC and MBC values for the selected NSAIDs (PXC, DCF, ASA and NPX) and antibiotics (KAN and TET) against *E. coli* and *S. aureus*.

	MIC (μg/mL)		MBC (μg/mL)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
PXC	800 ± 0	>2000	>2000	>2000
DCF	>2000	2000 ± 0	>2000	>2000
ASA	1750 ± 0	2000 ± 0	>2000	>2000
NPX	>2000	>2000	>2000	>2000
KAN	2.5 ± 0	2 ± 0	2.5	2.5
TET	4 ± 0	2 ± 0	4.4	4.8

- **Effect of different doses of the selected NSAIDs on the culturability of *E. coli* and *S. aureus***

- The maximum of log CFU/mL reduction for PXC, DCF and ASA was 1.54, 0.48 and 1.08, respectively (**Table 2**). It was observed a dose-dependent effect.

Table 2. log CFU/mL reduction values for NSAIDs at ½×MIC, MIC and a concentration above MIC, after 8h.

	E. coli			*S. aureus*	
NSAID	C (μg/mL)	log reduction	NSAID	C (μg/mL)	log reduction
PXC	400	1.20	DCF	1000	2.50
	800	1.27		2000	3.86
	1500	1.34		2500	3.68
ASA	875	0.29	ASA	1000	0.05
	1750	0.83		2000	0.92
	2500	0.90		2500	1.06

- **Effect of the selected NSAIDs and ATB on the *E. coli* and *S. aureus* biofilm eradication**

- ASA, DCF and PXC promoted significant reductions in metabolic activity (66.1% to 88.7%) and culturability (2.22 to 6.48 log CFU/cm2). However, only PXC promoted biofilm mass removal (maximum of 10.6%) (**Figure 1**). Additive interactions were obtained for most of the combinations between NSAIDs and KAN or TET (**Table 3**).

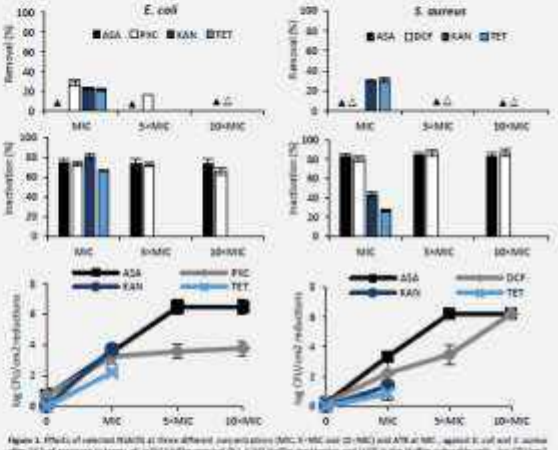


Figure 1. Effect of selected NSAIDs at three different concentrations (MIC, 5×MIC and 10×MIC) and ATB at MIC, against *E. coli* and *S. aureus* after 24 h of exposure in terms of: (a)(b) biofilm removal (b), (c)(d) biofilm inactivation and (e)(f) in the biofilm culturable cells - log CFU/cm2 reduction. Δ (b) represents the biofilm removal. Mean values ± SD of three independent experiments are illustrated.

Table 3. EC scoring of the selected NSAIDs (at MIC, 5×MIC and 10×MIC) with TET and KAN (at MIC) in terms of biofilm removal/inactivation and culturability reduction. (++) - Additive; (+) - Indifferent; (-) - Antagonistic.

	E. coli				*S. aureus*			
Conc.	NSAID+ATB	Removal	Inactivation	Culturability	NSAID+ATB	Removal	Inactivation	Culturability
MIC+MIC	PXC+KAN	+	+	++	DCF+KAN	+	++	+
5×MIC+MIC		+	+	++		++	++	+
10×MIC+MIC		++	+	++		++	++	++
MIC+MIC	PXC+TET	-	++	++	DCF+TET	++	++	++
5×MIC+MIC		+	++	++		++	++	+
10×MIC+MIC		++	++	++		++	++	++
MIC+MIC	ASA+KAN	++	++	+	ASA+KAN	++	++	+
5×MIC+MIC		++	++	++		++	++	++
10×MIC+MIC		++	++	++		++	++	++
MIC+MIC	ASA+TET	++	++	++	ASA+TET	++	++	++
5×MIC+MIC		++	++	++		++	++	++
10×MIC+MIC		++	++	++		++	++	++

Conclusions

Overall, the results obtained suggest that NSAIDs appear to be a promising strategy to control biofilms as they demonstrated to be more effective than conventional antibiotics.

Acknowledgments

This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); Project PTDC/BII-BIT/30113/2017 - POCI-01-0145-FEDER-030113 and PTDC/ASP-PES/30113/2017 - POCI-01-0145-FEDER-030113, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Anabela Borges thanks the Portuguese Foundation for Science and Technology (FCT) for the financial support of his work contract through the Scientific Employment Stimulus - Individual Call - (CEECIND/01282/2017).

Synthesis of new CXCR1/2 receptors antagonists for exudative AMD treatment.	***Restricted to organizers***

Fabre M.[(1)*], Dufies M.[(2)], Pages G.[(2)], Demange L.[(1, 3)], Ronco C.[(1)], Benhida R.[(1)].

(1) Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR7272, 06108 Nice, France
(2) Université Côte d'Azur, CNRS UMR 7284 and INSERM U 1081, Institute for Research on Cancer and Aging (IRCAN), 28 Avenue de Valombrose, 06107 Nice, France
(3) CNRS UMR 8038 CiTCoM, Faculté de Pharmacie de Paris, Université de Paris, 4 avenue de l'observatoire, 75006 Paris, France.

In western countries, exudative age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly. This disease is characterized by an abnormal vascularization of the choroid and a strong intraocular inflammation (Fig. 1). Currently, only symptomatic treatments exist, which rely on humanized monoclonal antibodies (mAbs) and recombinant fusion glycoprotein targeting pro-angiogenic factors. Moreover, only 30% of the patients present a durable response to this treatment.

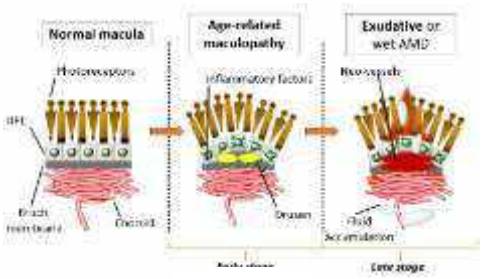


Figure 1

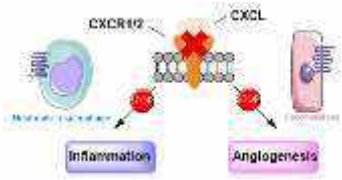


Figure 2

The blockage of the ERL+ CXCL cytokines signalling pathway has been proposed as promising alternative to target simultaneously choroid vascularization and inflammation[1]. Indeed, this sub-family of cytokines is specifically involved both in inflammation and in early stage of the pro-angiogenic signal[1] (Fig. 2).

Two hits were already developed[2] and validated both *in vitro* and *in vivo*, (MCK133 and MCK140). However, they suffer from limited solubility for further eye related applications. We have demonstrated that the drawbacks of these compounds are mainly due to the inherent structure of arylurea. Therefore, a new series of compounds has been designed that futures the same pharmacophore but with new chemical functions to enhance the activity and the physicochemical properties.

A new straightforward synthetic pathway has been developed to prepare two series of molecules aimed at modifying two key parts of the structure. The structure activity relationships of theses series were studied through the preparation of 48 analogues, and their subsequent biological evaluation on two models: 1/ XTT cell viability assays and 2/ migration ability with a Boyden chamber assay. Encouraging results have been obtained, showing that 2 compounds efficiently block the cell migration associated to CXCR-CXCL interaction, without toxicity on retinal epithelium cells. After verification of the pharmacological profile of these lead compounds, the aim is to evaluate them *in vivo* on a retinopathy model.

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(1) a) Chang, L.S. Steelman, J. T. Lee, J. G. Shelton et al., Leukemia, 2003, 17, 1263-1293; b) A. Sparmann, D. Bar-Sagi, Cancer Cell, 2004, 6, 447-458.

(2) Benhida, R., Pages, G., Dufies, M., Ronco, C., Demange, L., Grytsai, O. *European Patent* 2018 EP18306362.7.

*** Correspondence**: Marie.Fabre@univ-cotedazur.fr

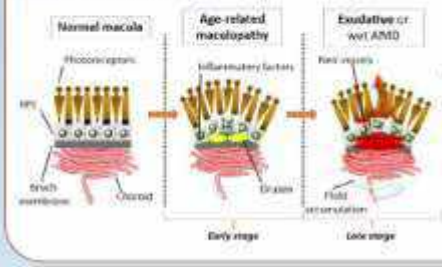
Synthesis of new CXCR1/2 receptors antagonists for exudative AMD treatment

Marie Fabre[1], Maeva Dufies[2], Gilles Pagès[2], Luc Demange[1,3], Cyril Ronco[1] and Rachid Benhida[1]*

1. Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR7272, 06108 Nice, France
2. Université Côte d'Azur, CNRS UMR 7284 and INSERM U 1081, Institute for Research on Cancer and Aging (IRCAN), 28 Avenue de Valombrose, 06107 Nice, France
3. CNRS UMR 8038 CITCoM, Faculté de Pharmacie de Paris, Université de Paris, 4 avenue de l'observatoire, 75006 Paris, France

State of the art

Exudative age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly, characterized by an abnormal **vascularization of the choroid** and by an **inflammatory context**.

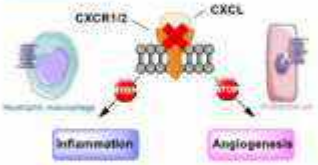


Anti-VEGF therapy

Humanized monoclonal antibodies (mAbs)
Recombinant fusion glycoprotein

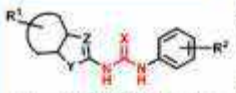
- ☑ **Efficient anti-angiogenesis activity**
- ☒ Only 30% of durable response
- ☒ Only blindness delay, palliative treatment
- ☒ No effect on inflammation axis

Strategy



To target simultaneously **vascularization** and **inflammation**, our team aims at developing new therapeutic strategies to **block ERL* CXCL cytokines signaling pathway**. Indeed, this sub-family of cytokines is specifically involved both in inflammation and in pro-angiogenic signals[1].

Proof of concept



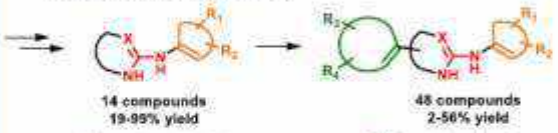
New CXCR1/CXCR2 inhibitors
IC_{50}= 1 - 5 µM

Two series of CXCR1 / CXCR2 inhibitors have been already developed. *In vivo*, two hit molecules[2] exert significant **anti-angiogenic effects in a wet-AMD mice model** (reduction of the choroid vascularization).

Synthesis

Nicotinamide-like family

Development and optimization of new series of small-sized CXCR1/2 antagonists to increase the potency against exudative AMD, the safety index and the pharmacological properties.



14 compounds
19-99% yield

1st functionalization

48 compounds
2-56% yield

2nd functionalization

- ☑ **Stable** in acidic, neutral and basic aqueous buffers (C=1,7x10^{-3} M)
- ☑ **Soluble** in 5% DMSO/aqueous solution

Biological evaluation

Compound	% viability 10 µM
ARPE: retina cells	
Ref 1	43,7
Ref 2	13,6
MAF254	88,1
MAF261	86,4
MAF286	75,7
MAF287	69,1
MAF290	65,8
MAF293	51,5
MAF301	93,8

☑ **No toxicity in retinal cells**

Migration tests by Boyden chamber (10 µM)

☑ **Blocking interaction of CXCL-CXCR**

Conclusion & Perspectives

Following an original therapeutic strategy based on a dual targeting of both inflammation and angiogenesis in ocular diseases, 48 new compounds and 14 intermediates have been synthetized and evaluated in *in vitro* assays. The encouraging results obtained prompt us to further evaluate these series on other anti-inflammatory and anti-angiogenic models, and to evaluate the *in vivo* efficiency of the lead compound on a wet AMD induced mice model.

References

1. J. Vandercappellen, J. Van Damme, S. Struyf, *Cancer Lett.*, 2008, *267*, 226–244
2. R. Benhida, G. Pages, M. Dufies, C. Ronco, L. Demange, O. Grytsai *European Patent* 2018 EP18306362.7.

Acknowledgments

The authors thank the ANR, UCA, CNRS, INSERM, IRCAN, the centre hospitalier universitaire de Nice and IRIS Pharma for their financial and/or collaboration support.

Comparative study of *in vitro* metabolism of nitroxides

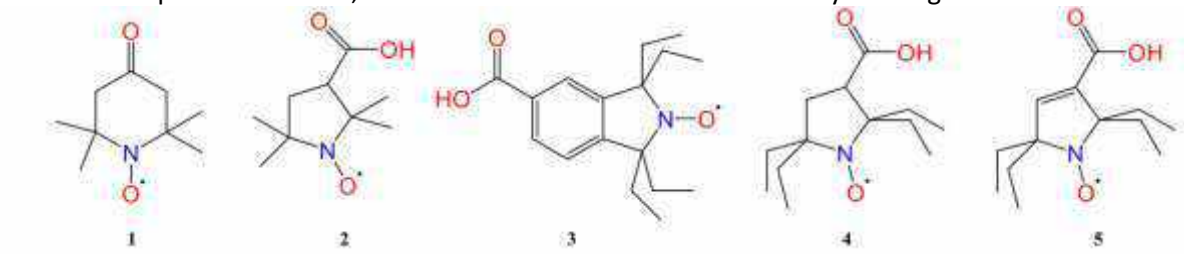
A. Stojanović [(1)], N. Babić [(1)], F. Peyrot [(1,2)]

(1) Université de Paris, UMR8601 CNRS, 75006 Paris, France.

(2) Sorbonne Université, ESPE de Paris, 75016 Paris, France

Restricted to organizers

Evidence suggests that cancer, neurodegenerative, and cardiovascular diseases share a common state known as oxidative stress. The condition of oxidative stress can occur when the disbalance between the production and removal of reactive oxygen species exists. In association with EPR spectroscopy and imaging, aminoxyl radicals (nitroxides) can be used for its *in vivo* evaluation and mapping. [(a)] Before using nitroxides for *in vivo* experiments, it is necessary to investigate their stability and metabolism. Unlike tetramethyl-substituted compounds, the characteristics and behavior of tetraethyl-substituted ones are not entirely known. [(b)] The metabolism of a series of spin probes in rat liver microsomes was investigated. The main components of microsomes are cytochrome P450-a family of redox enzymes containing heme protein as a cofactor - and the associated reductases. An electron donor is required for the functioning of P450 enzymes, and for that purpose, NADPH was used. The experiments were performed under aerobic and anaerobic conditions, due to the fact that oxygen has a fundamental role in the P450-catalytic cycle. [(c)] The signal disappearance kinetic was tracked using EPR. In the present study, the behavior of five different nitroxides (compounds 1-5) was compared. Special attention was drawn to the nitroxide with isoindoline core: 5-carboxy-1,1,3,3-tetraethyl-2-isoindolinyloxyl (3). It showed unexpected behavior, and its metabolism must be additionally investigated.



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* Correspondence: E-mail:
fabienne.peyrot@parisdescartes.fr
aleksandra.stojanovic@parisdescartes.fr

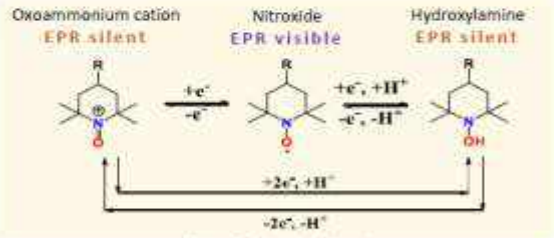
Comparative study of *in vitro* metabolism of nitroxides

A. Stojanović[1], N. Babić[1], F. Peyrot[1,2]
1) Université de Paris, UMR8601 CNRS, 75006 Paris, France
2) Sorbonne Université, INSPE de Paris, 75016 Paris, France

PICS funding 2019-2021

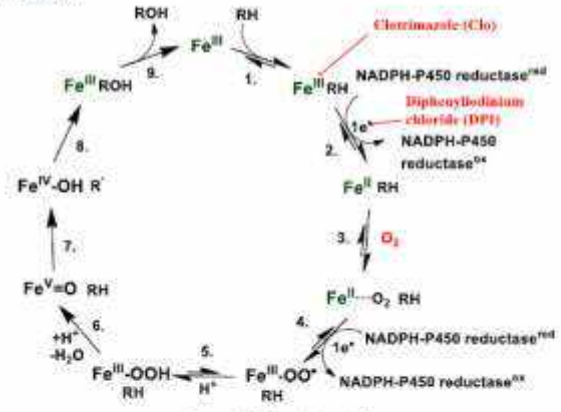
Introduction

Evidence suggests that cancer, neurodegenerative, and cardiovascular diseases share a common state known as oxidative stress. The condition of oxidative stress can occur when the disbalance between the production and removal of reactive oxygen species exists. In association with EPR spectroscopy and imaging, aminoxyl radicals (nitroxides, *Scheme 1.*) can be used for its *in vivo* evaluation and mapping. [a] Before using nitroxides for *in vivo* experiments, it is necessary to investigate their stability and metabolism. Unlike tetramethyl-substituted compounds, the characteristics and behavior of tetraethyl-substituted ones are not entirely known. [b]



Scheme 1. Redox cycle of nitroxide

- **Microsomes** – an *in vitro* system largely used for metabolic studies – contain **Cytochrome-P450** and **associated reductases**;
- For the functioning of P450 enzymes an **electron donor** is required (NADPH was used), *Scheme 2.*
- **Clotrimazole** and **Diphenyliodinium chloride** – used as **inhibitors**



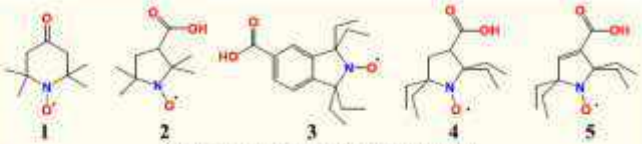
Scheme 2. P450 catalytic cycle [c]

Methodology

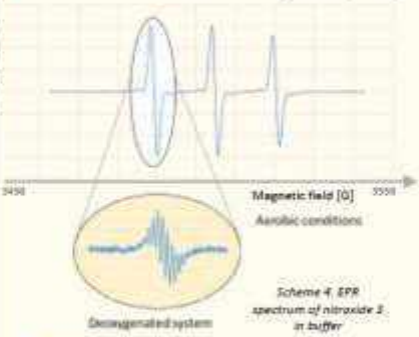
- **The metabolism** of nitroxides, *Scheme 3.*, was investigated in **rat-liver microsomes**, under **aerobic** and **anaerobic** conditions.
- The **disappearance kinetics** of nitroxides was tracked by **EPR**
- 1 μM **potassium-ferricyanide** was used to reoxidize hydroxylamine back to nitroxide after the recording finished

Complete system

- Potassium-phosphate buffer (0.1 M, pH 7.4) containing 1mM DTPA
- Nitroxide (100 μM)
- Microsomes preparation (1 μM of cyt-P450)
- NADPH (1mM)



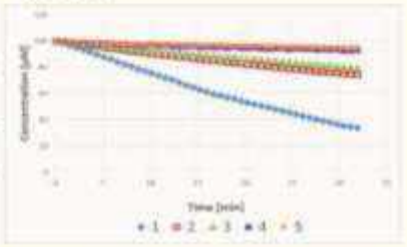
Scheme 3. Structural formulas of investigated nitroxides



Scheme 4. EPR spectrum of nitroxide 3 in buffer

Nitroxide **3** has expressed **super-hyperfine structure** under **anaerobic** conditions, *Scheme 4.* To determine whether the system was deoxygenated, this phenomenon was used.

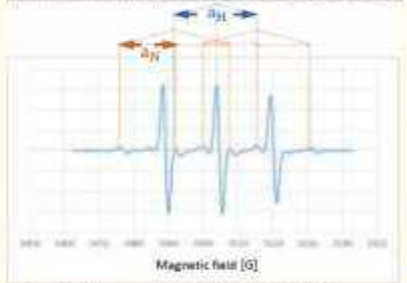
Results



Graph 1. Concentrations of nitroxides incubated with microsomes supplemented with NADPH under aerobic conditions during 30 min.

Table 1. Decay of EPR signal intensity of nitroxides after 30 min

Nitroxide / Conditions	1	2	3	4	5
Microsomes, aerobic	0	0	0	0	0
NADPH, aerobic	0	0	0	0	0
Microsomes+NADPH, aerobic	54%	25%	20%	7%	< 5%
Microsomes+NADPH+Clo, aerobic	7%	8%	0	0	0
Microsomes+NADPH+DPI, aerobic	15%	7%	0	0	0
Microsomes+NADPH, anaerobic	76%	0	20%	< 5%	< 5%
Microsomes+NADPH+Clo, anaerobic	10%	0	0	0	0
Microsomes+NADPH+DPI, anaerobic	26%	0	0	0	0



Graph 2. EPR spectrum of nitroxide 3 incubated with microsomes supplemented with NADPH, aerobic conditions; after 30 min

- For **complete system** under **aerobic** conditions, the decay was observed for nitroxides **1-3**, while **4** and **5** are much more stable, *Graph 1.*
- The shape of EPR spectra of nitroxides **1, 2, 4,** and **5** was unchanged
- New paramagnetic species ($a_H = 23.6\ G$, $a_N = 15.6\ G$) appeared when nitroxide **3** was incubated in microsomes supplemented with NADPH under aerobic conditions, *Graph 2.*
- The reoxidation was possible for nitroxide **1** (15% recovered) and **2** (42% recovered) under aerobic conditions, **pointing that hydroxylamine was formed**
- 37% of nitroxide **1** was recovered after reoxidation under anaerobic conditions
- **Reoxidation of nitroxide 3 was not possible!**

Discussion

- **Tetraethyl-substituted nitroxides are more stable than tetramethyl-substituted ones**
- Nitroxide 1: partially reduced to hydroxylamine by heme-Fe(II) and partially degraded; increased conversion under degassed conditions was observed
- Nitroxide 2: oxidized to oxoammonium cation and then reduced to hydroxylamine by taking two electrons from NADPH [d]
- Nitroxide 3: **degradation of the core**; This probe has different mechanisms under aerobic and anaerobic conditions; **Further investigation by mass spectrometry is required!**

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(a) Babić, N., Peyrot, F.; Magnetochemistry, 2019; 5(1):13;
(b) Babić, N., Orio, M.; Peyrot, F.; Free Radic. Biol. Med., 2020; 156:144-156;
(c) Guengerich, F. P.; Chem. Res. Toxicol., 2001; 14(6):611-650.
(d) Murali C Krishna, David A. Grahame, Amram Samuni, James B. Mitchell, Angelo Russo; Proc. Natl. Acad. Sci. USA, 1992; 89: 5537-5541;

correspondence email: aleksandra.stojanovic@parisdescartes.fr

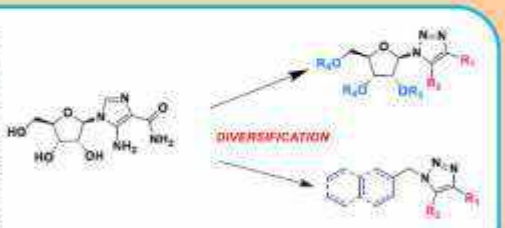
Development of a new approach of targeted covalent therapy for the treatment of Myelodysplastic syndromes and acute myeloid leukemia

Sarah Bailly (1), Anthony Martin (1), Guillaume Robert (2), Patrick Auberger (2), Rachid Benhida (1).

(1) Institut de Chimie de Nice UMR7272, Université Côte d'Azur, CNRS, 06108 Nice, France
Contact: sarah.bailly@univ-cotedazur.fr
(2) Centre Méditerranéen de Médecine Moléculaire, Université Côte d'Azur, UMR INSERM U1065, 06204 Nice, France

Introduction

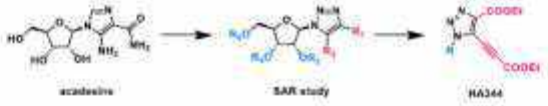
In drug discovery, **covalent drugs** were barely considered due to their toxicity and poor selectivity, since the turn of the century, the access to more specific inhibitors coupled to a better understanding of cellular biology permitted the rehabilitation of covalently acting drugs[a]. The goal of this project is to develop a new therapeutic approach belonging to the **Targeted Covalent Therapy** (TCT) strategy and based on small bioactive molecules able to overcome resistance mechanisms in **Myelodysplastic Syndromes** (MDS) and **Acute Myeloid Leukemia** (AML)[b]. Recently, we reported that **acadesine** acts as an antineoplastic agent that inhibits Chronic Myeloid Leukemia (CML) progression of naive and resistant cells[c]. The synthesis of more potent analogues of acadesine were done using the scaffold hopping strategy, in particular the imidazole ring was replaced by a **triazole** core, then 2 new derivative families were designed: nucleosidic analogues *vs* aryl analogues *(scheme 1)*.



Scheme 1: Structure of acadesine and the diversification study

Goals

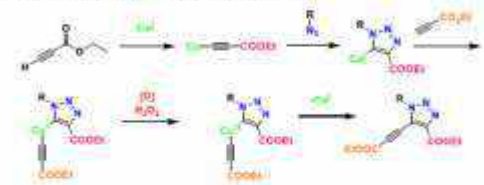
We identified, in collaboration with the C3M (*Centre Méditerranéen de Médecine Moléculaire*), a lead compound 'HA344' as a specific covalent inhibitor able to target key proteins involved in cancer cell metabolism[d]. Fundamental aspects of this project are now devoted to the confirmation and validation of HA344 targets, and to the elucidation of its site and mode of binding. For this purpose, relevant chemical biology tools were synthesized. Notably, HA344 derivatives harbouring a terminal alkyne function were synthesized to enable the ***in situ*** **click-chemistry** with biotin tag, hence allowing the trapping of the target proteins and their identification.



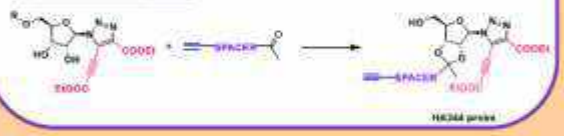
Scheme 2: from the SAR study effected from acadesine structure to the design of lead compound HA344

ORGANIC CHEMISTRY

Synthesis of lead compound HA344: Mechanism of copper catalyzed azide-alkyne cycloaddition reaction (CuAAC)

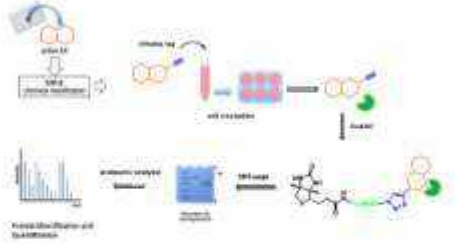


Synthesis of HA344 probe:



BIOCHEMISTRY

Affinity Based Probe Profiling: After covalent trapping of targets using the in-situ click approach, proteomic analyses will be carried out using mass spectrometry methods.



Scheme 3 : Synthesis of molecular probes with a biotin-streptavidin system using an in situ click chemistry pathway

CONCLUSION

We herein reported nucleoside analogues bearing a 1,4,5-trisubstituted-1,2,3-triazole synthesized using a straightforward click/oxidative coupling procedure. Afterwards, chemical probes using a biotin-tagged version of HA344 were synthesized in order to confirm the molecular target. The next step will focus on the characterization of the interactions between HA344 and targeted proteins using in house calorimetric methods. Moreover, analogs of alkynyl-HA344 will be prepared, where the spacer and its anchoring site will be varied in order to rule out any contribution of the spacer in the mode of binding.

References

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Acknowledgment

The authors thank UCA, CNRS, INSERM AND ITMO CANCER for their financial support.

MAKING GOOD'S BUFFERS GOOD FOR FREEZING: THE ACIDITY CHANGES AND THEIR ELIMINATION VIA MIXING WITH SODIUM PHOSPHATE [1]

*Lukáš Veselý [1], Behera Susrisweta [1], Dominik Heger **

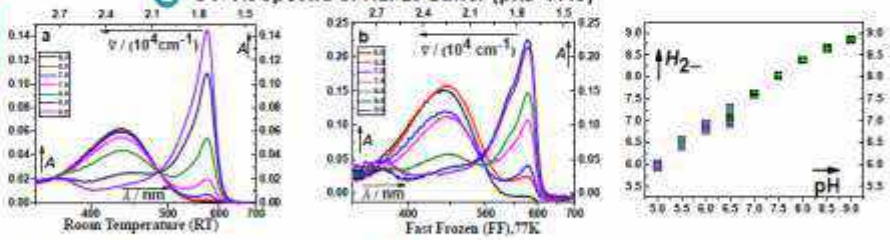
[1]Department of Chemistry , Masaryk University, Kamenice 5, 625 00, Brno Czech Republic

e-mail:susrisweta@gmail.com ,502102@muni.cz,*hegerd@chemi.muni.cz

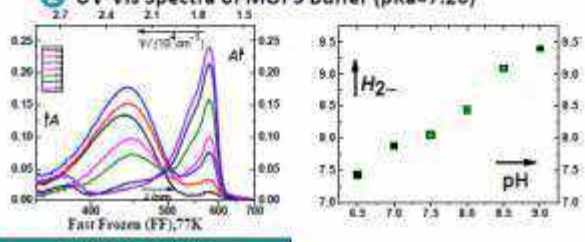
Introduction: Freezing of (bio)chemicals often leads to deviations from the optimum pH which cause compound's degradation, e.g., protein aggregation[2] .So, we would like to present a straightforward and efficient approaches to reduce the freezing-induced acidity changes by blending two different buffers which are commonly used for biological samples . This approach can be highly helpful in both the pharmaceutical domain and those branches of science where freezing is the preferred method for long-term preservation.

A. Freezing-induced acidity changes in Good's Buffers

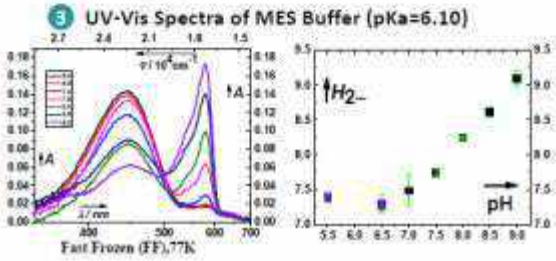
1 UV-Vis Spectra of HEPES Buffer (pKa=7.45)



2 UV-Vis Spectra of MOPS Buffer (pKa=7.20)



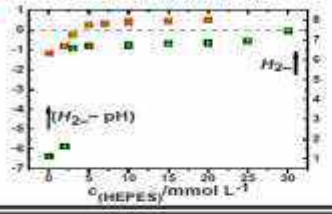
3 UV-Vis Spectra of MES Buffer (pKa=6.10)



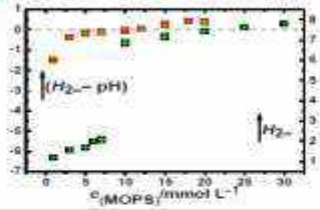
B. Sodium Phosphate (Na-P) buffer acidifies upon freezing [3]

What Happens when we Mix Good's Buffers with Sodium Phosphate Buffer ?

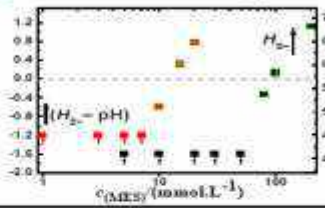
1 HEPES and Na-P Mixture, pH=7.5



2 MOPS and Na-P Mixture, pH=7.5



3 MES and Na-P mixture , pH=5.5

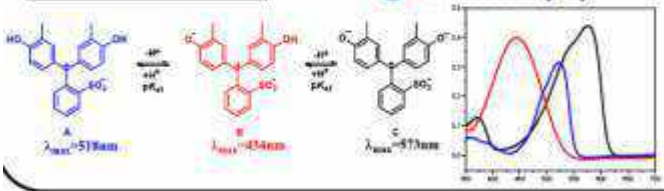


How we measure the pH Change?

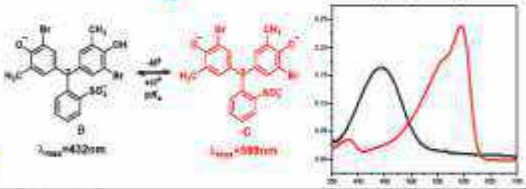
$$H_{2-} = pK_{a,Ind} + log\frac{c_{Ind^x}}{c_{HInd^{x+1}}}$$

Molecular Probes for H0

1 Cresol Red (CR)



2 Bromocresol Purple (BCP)



Conclusion

- Good's buffers basify upon freezing and, more intensively at lower pHs.
- The acidity varies most prominently in MES buffer.
- The relevant values are 3mM HEPES,10mM MOPS and 80mM MES can cancel out the strong , freezing-induced drop in 50mM phosphate (Na-P) buffer.

References

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DISCOVERY OF hERAP2 MODULATORS *VIA* HIGH-THROUGHPUT SCREENING

Rebecca.deprez@univ-lille2.fr
www.deprezlab.fr

Sieroçki Pierre[1]; Vatinel Rodolphe; Medve, L[1]; Gealageas, R[1]; Guillaume, V[1]; Rosell, M[1]; Warenghem, S[1]; Leroux, F[1]; Piveteau, C[1]; Deprez-Poulain, R[1]* and Deprez, B[1],

[1] Univ. Lille, Inserm, Institut Pasteur de Lille, U1177 - Drugs and Molecules for Living Systems, F-59000 Lille, France

1 ERAP2 AS THERAPEUTIC TARGET

Endoplasmic reticulum aminopeptidase 2 (ERAP2) is a M1 family zinc metalloprotease playing a key role in antigen presentation pathway. This intracellular aminopeptidase trims peptide precursors resulting from protein degradation by the proteasome and thereby generates mature antigenic epitopes of appropriate length for presentation on the cell surface by major histocompatibility complex class I (MHC-I) molecules. The recognition of the extracellular peptide by cytotoxic T-cell triggers immune response against infected or diseased cells through biological cascades that lead to apoptosis of the target cell (Fig. 1). Thereby ERAP2 is a major regulator of adaptive immune responses in humans and plays a crucial role in autoimmunity, infections, and cancers[1]. Thus, it is of paramount importance to find biological tools that can either inhibit or enhance their action, whether for the better understanding of biological mechanisms implying ERAP2 or for the discovery of potential therapeutic compounds. One main challenge of targeting ERAP2 is the development of selective modulators (ERAP1 displays highly homology about ERAP2 with 50% sequence identity).

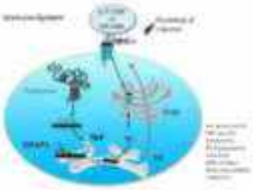


Fig. 1 : Role of ERAPs in the immune system

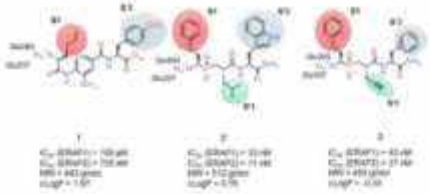


Fig. 2 : Example of hERAP2 inhibitors reported in the literature

In the literature, only a few ERAP2 inhibitors have been identified: series of diaminobenzoic acid2 1 (DABA) derivatives with moderate potency, series of pseudopeptides 2 containing a phosphinic group3 and derivatives 3 with comparable potency and moderate selectivity resulting of the side chains optimization4 of 2 (Fig. 2).

2 HIGH-THROUGHPUT SCREENING OF A 1920-MEMBER LIBRARY

Alternative approaches to discover new structures that could serve as starting points for optimization towards ERAP2 inhibitors is still challenging and highly needed. To fulfill the need of new chemical templates able to inhibit ERAP2, a fast, enzyme-efficient 384-well plate HTS assay was developed, and applied to a focused *in-house* library (Fig. 3). This screening assay allowed us to discover structurally original inhibitors, but also, surprisingly, activators of small peptide hydrolysis by ERAP2.

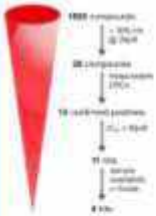
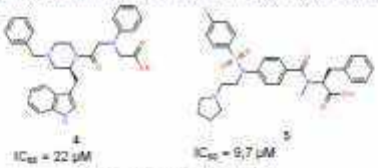


Fig. 3 : Screening cascade



4 IC_{50} = 22 µM

5 IC_{50} = 9,7 µM

Fig. 4 : Structures and IC_{50} of hits 4-5

28 compounds displayed an inhibition above 30% at 30 µM, half were confirmed (dose-dependent inhibition of ERAP2). Among these hits 3 displayed a carboxylic acid as putative zinc binding group and a singleton bore a tetrazole. We thus prioritized the study of the best inhibitors 4 and 5 which displayed a better activity (Fig. 4).

So far, published inhibitors of ERAP2 retain activity on the homologous ERAP1 and/or IRAP. Compound 5 is selective of ERAP2 over these two other enzymes. It is indeed completely inactive on ERAP1 and shows a 1-log selectivity towards IRAP. 5 is thus a good starting point for pharmacomodulation.

3 DESIGN OF ANALOGUES TO EXPLORE BINDING

Analogues 17 to 33 were designed and evaluated to validate interactions identified in the docking study and key structural elements like configuration (17), nature of amino-acid (18-19,31-33), importance of tertiary amine and linker length (22-26, 28-33), importance of the methylation of amide (27), importance of aryl group (20-21) (Fig. 5).

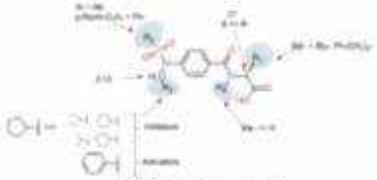
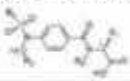


Fig. 5 : SAR of analogues 17 to 33



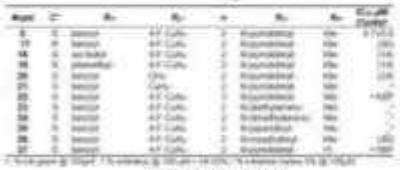


Table 1 : Analogues of hit 5

All analogues 17-27 were less active than reference compound 5. All these activities are in line with the docking results were multiple stackings were found between aryl groups and aromatic amino-acids, interactions between hydrogen bonds acceptors CO of the amide or SO_2 of the sulfonamide, and ERAP2 (Table 1).

As dockings studies and SAR described in Table 1 emphasize the importance of the basic side chain in R_3 position, we synthesized analogues bearing a phenyl ring, instead of the pyrrolidine ring 28-33 (Table 2). Surprisingly, for these compounds, activity shifted from inhibition to activation[5] in 143% to 184%. All compounds are selective of ERAP2 over ERAP1.

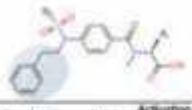


Table 2 : Phenethyl derivatives 28-33

4 DOCKING STUDIES OF INHIBITOR 5 AND ACTIVATOR 29 IN ERAP2

The docking pose shows that inhibitor 5 interacts with ERAP2 S1, S1' and S2' pockets while having the carboxylic acid moiety binded to the Zn^{2+} catalytic site (Fig. 7).

Fig. 7 : Docking pose of 5 in PDF 5IE5

The docking study has also revealed that activator 29[5] lies in the catalytic site (Fig. 8) but does not interact with the Zn^{2+} ion.

Fig. 8 : Docking pose of 29 in PDF 5IE5

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The Microtubule Bench (MTBench): a novel technology to identify modulators of Protein-Protein Interaction (PPI) and Protein-Nucleic Acid Interaction (PNAI) in a cellular environment.	***Restricted to organizers***

Nicolas Babault(1)*, Hélène Henrie(1),Pierrick Craveur(1), Laurie Askenatzis(2), Jérôme Bignon(2), Widad Ghedamsi(1), Vandana Joshi(3), David Pastré(3), Guillaume Bollot(1)*

(1) SYNSIGHT, Genopole Entreprises, Evry France.
(2) Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, Gif-sur-Yvette, France.
(3) SABNP, Université d'Evry, INSERM U1204, Université Paris-Saclay, Evry, France

Drug discovery is in constant evolution to provide the most efficient and specific drugs for new therapies. To identify/optimize New Chemical Entities, the development of robust and sensitive bioassays is mandatory to complement the few gold standard techniques, notably those working in a cellular context. Here we present an innovative cellular bioassay to screen modulators of Protein-Protein Interaction (PPI) and Protein-Nucleic Acid Interaction (PNAI). This new technology, Microtubule Bench (MTBench), is based on the use of microtubules as intracellular nanoplatforms to visualize and quantify PPI or PNAI at the single cell level. We implemented the patented MTBench technology to quantify whether a prey is attracted onto microtubules by a bait, which is the readout of the assay, on High Content Screening (HCS) system at high resolution. We confirmed the robustness and the sensitivity of the MTBench to identify and qualify small molecule inhibitors on the well-known p53/MDM2 PPI system. As for PNAI, we detected and quantify the interaction of different RNA Binding Proteins (RBPs) with RNA. A PNAI system on MTBench to screen modulators of such interaction has already been implemented. This novel cell-based assay, which is suitable for HCS, will facilitate the drug discovery in the fields of PPI and PNAI.

* Correspondence: nicolas.babault@synsight.net, guillaume.bollot@synsight.net

MASTERING SIZE FOR THE DESIGN OF INNOVATIVE THERANOSTIC IRON OXIDE BASED NANOPARTICLES ENSURING MULTIMODAL THERAPY

Barbara Freis[1], Geoffrey Cotin[1], Francis Perton[1], Celine Kiefer[1], Damien Mertz[1], Sophie Laurent[2*], Sylvie Bégin-Colin[1*]

(1) University of Strasbourg, CNRS, Institut de Physique et Chimie des Matériaux, UMR CNRS-UdS 7504, 23 Rue du Loess, BP 43, 67034 Strasbourg, France.
(2 Laboratory of NMR and Molecular Imaging, University of Mons, Avenue Maistriau 19, B-7000 Mons, Belgium).

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In nanomedicine, the goal is to develop multimodal nanoparticles (NPs) to speed up targeted diagnosis, to increase its sensitivity, reliability and specificity for a better management of the disease and to treat it in a specific personalized manner in feedback mode. Combination of therapies to target individual cancer-specific vulnerabilities is a way to increase the efficacy of anticancer treatment. Therefore, besides precision diagnosis, other challenges for personalized nanomedicine are to develop tools to be able to test quickly different treatments and to follow-up the effect(s) of the treatments by imaging.

Besides to be good T2 contrast agents for MRI, iron oxide NPs are promising as therapeutic agents by magnetic hyperthermia when correctly designed[a]. To be a good heating agent, iron oxide NPs have to display a high magneto-cristalline anisotropy and ways to increase it are to tune the NPs size and shape [(a) (b) (c)].Iron oxide nanoparticles have also an interest for photothermal treatment as they express a good photothermal response to laser irradiation[d] [e].

The goal of this project is to develop iron oxides NPs with different sizes by the thermal decomposition method by tuning synthesis parameters such as the reaction temperature, the heating rate, the nature of surfactant and the molar ratio between the precursor and the surfactant [(a)(b)]. Main difficulties were the reproducible synthesis of NPs with mean size higher than 12 nm, a homogeneous spinel composition and the ability to be easily dendronized. NPs with different sizes in the range 5-20 nm were thus synthesized and coated with dendron molecules. Their magnetic properties as well as their MRI properties were determined. Then, the effect of the NPs size on magnetic hyperthermia and photothermia has been investigated allowing to establish the optimal NPs design to combine both therapies.

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*** Correspondence:** barbara.freis@ipcms.unistra.fr, sylvie.begin@unistra.fr

Design and Synthesis of Novel Benzothiazole-Piperazine Propanamide Derivatives for Multi-Targeted Approach in Alzheimer's Disease Treatment

Bengisu Turgutalp[(1)]*, Enise Ece Gurdal[(1)], Chiara Luise[(2)], Tugba Ercetin[(3)], Anna Junker[(4)], Hayrettin Ozan Gulcan[(3)], Dirk Schepmann[(4)], Bernhard Wunsch[(4)], Wolfgang Sippl[(2)], Mine Yarim Yuksel[(1)]

(1) Yeditepe University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul, TURKEY. (2) Martin-Luther-Universität Halle-Wittenberg, Deartment of Medicinal Chemistry, Institute of Pharmacy, Halle (Saale), GERMANY. (3) Eastern Mediterranean University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Famagusta, CYPRUS . (4) Westfälischen Wilhelms-Universität Münster, Department of Medicinal Chemistry, Institute of Pharmacy, Münster, GERMANY.

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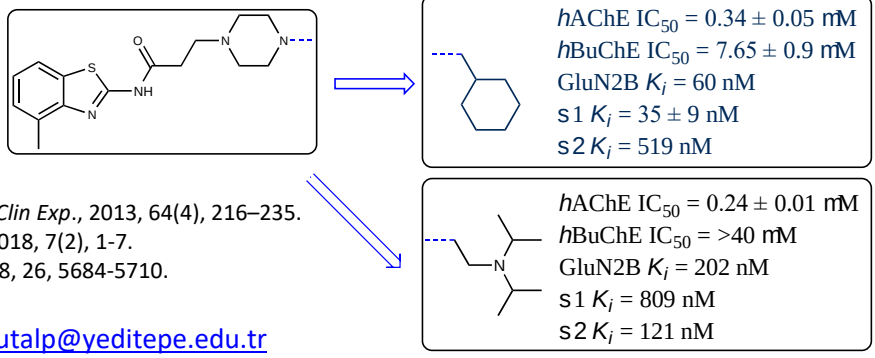
Alzheimer disease (AD) is a progressive neurodegenerative disorder being both the most common and fatal type of dementia[a]. Drug discovery studies aiming to develop a disease modifying treatment which are based on individual targets ended up failure so far during clinical trials[b]. Nowadays, it has been accepted that the multifactorial nature of AD, requires multi-effective treatment[c]. In this regard, **70** novel compounds with benzothiazole ring attached to various piperazines with a propanamide linker were designed for the development of novel multi-targeted ligands that possess symptomatologic effect along with neuroprotective and disease modifying effect by binding to the key receptors involved for the treatment of AD. Firstly, **70** compounds were tested for their cholinesterase inhibitory activities. **25** Compounds found to display potent inhibitory activity (IC_{50} < 10 μM) were then tested for binding affinities over $\sigma_{1/2}$ receptors and NMDA GluN2B domain. **8** Compounds that possessed potent activity in all selected targets were further subjected to cytotoxicity assay for safety evaluations and their predicted drug likeliness properties were evaluated. Lastly, molecular docking studies were performed for each target. Overall, **3** compounds were designated as lead compounds demonstrating nanomolar scale activity against all tested targets (Figure).

Furthermore, **11** compounds were tested for their P2X7 receptor inhibitory activities. Derivatives with nitrogen bearing aromatic ring displayed good P2X7R inhibitory activity whereas lead compounds did not show any significant activity.



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* Correspondence: bengisu.turgutalp@yeditepe.edu.tr

Synthesis and *in vitro* and *in vivo* evaluation of potential bitopic ligands of cannabinoid CB2 receptor.	***Restricted to organizers***

Francesca Gado(1)*, Rebecca Ferrisi (1), Beatrice Polini (1), Sara Carpi (1), Elena Lucarini(2), Maria Digiacomo (1), Simone Bertini (1), Lorenzo di Cesare Mannelli (2), Carla Ghelardini (2), Paola Nieri (1), Clementina Manera (1).

(1) Department of Pharmacy, University of Pisa, Pisa, Italy.
(2) Department of Neuroscience, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy.

The CB2 receptor (CB2R), has emerged as an attractive target for managing microglial-derived neuroinflammation which generally characterizes neurodegenerative diseases. Indeed, CB2R modulation might have beneficial outcomes for specific symptoms and for the slowdown of disease progression. Our group already reported that the co-treatment of the dual target CB1R/CB2R orthosteric agonist **FM-6b**[a] (Figure 1) with the CB2R positive allosteric modulator (PAM) **EC-21a**[b] (Figure 1) improves the anti-inflammatory effect in the modulation of the release of pro- and anti-inflammatory cytokines in lipopolysaccharides (LPS)-activated mouse BV2 microglial cells, compared to the treatment of **FM-6b** alone.[c] On the basis of these results, we synthesized a new class of compounds, **A1**-**A4**, potentially able to bind to both allosteric and orthosteric sites simultaneously linking the pharmacophoric portion of **EC-21a** with that of **FM-6b** (Figure 1). Among all the compounds of the series, **A1** (**FD-22a**) (Figure 1) showed to be the most promising. Indeed, its effect in the modulation of the release of pro- and anti-inflammatory cytokines in lipopolysaccharides (LPS)-activated mouse BV2 microglial cells, resulted potentiated, if compared to **FM-6b** alone, and comparable to the effect due to the co-administration of **EC-21a** and **FM-6b**. **A1** (**FD-22a**) was also tested *in vivo* in a murine model of neuropathic pain giving important results at 5 mg/kg. Binding and functional studies are still ongoing in order to demonstrate the behavior of **A1** (**FD-22a**) as CB2R bitopic compound.

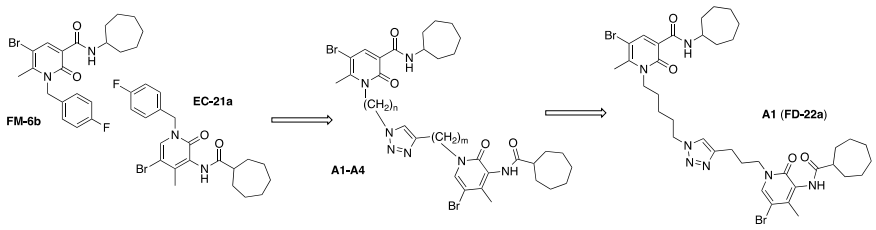

Figure 1 : Structure of compounds **FM6b**, **EC-21a** and derivatives **A1-A4**

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* Correspondence: francesca.gado@for.unipi.it

Vectorization of therapeutic siRNAs

B. Broc[1, 2], G. Jacquot[1], K. Varini[1], R. Sonnette[1], B. Pecqueux[1], J. Temsamani[1] P. Lécorché[1] and M. Khrestchatisky[2].

[1]Vect-Horus, CNS drug delivery, Marseille, France
[2]Institut de Neurophysiopathologie - INP, UMR7051, CNRS - Aix Marseille Université, Marseille, France

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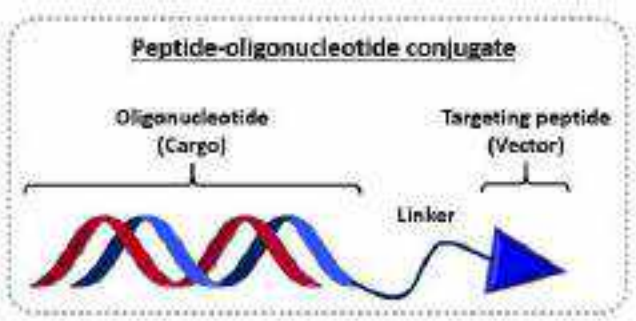
Because they modulate gene expression, oligonucleotide (ON) therapeutics arised as a new class of drugs with the potential to treat a large panel of diseases. Although they are gaining more and more clinical approvals, their development remains limited by several obstacles such as : off-target effects, low cellular uptake, immunogenic potential and instability in physiological environements. In order to overcome these limitations, there is a strong need for the development of appropriate vectors that effictively transport therapeutic ONs to the tissues of interest and to their intracellular target.

We have recently developed a low molecular weight cyclic peptide-vector family (MW~1kDa) endowed with both high affinity for its target, the Low-Density Lipoprotein Receptor (LDLR) and optimized stabilities towards proteases. This receptor has been chosen for its high endocytic potential and its expression at the Blood Brain Barrier (BBB), in some peripheral tissues (liver, adrenal glands) and very interestingly in some tumors.



Here we describe the design, synthesis and *in vitro* evaluation of a set of siRNA-peptide conjugates that comprise the VH4127 peptide-vector that targets the LDLR and that allows liver targeting. The VH4127 peptide was conjugated to a siRNA that targets a gene predominantly expressed in the liver.

First, we developed the chemical strategy to covalently conjugate the siRNA to the VH4127 peptide as well as the quality controls required to ensure the identity and the purity of the conjugates. A stabilized siRNA encompassing modified nucleosides was used to reduce sensitivity of the siRNA toward exo- and endonucleases. Once the conjugation protocol was optimized, we prepared a set of siRNA-VH4127 conjugates with different designs. We assessed that both the siRNA and the VH4127 peptide retain their biological properties post conjugation by: i) binding affinity experiments to the LDLR; and ii) transfection experiments in primary mouse hepatocytes (mHeps). With the best conjugates identified from their LDLR-binding affinities and their intrinsic silencing activities by transfection we now plan to evaluate their *in vitro* free uptake silencing efficacies and to study their *in vivo* efficacy in mice.

In-vivo evaluation of a piperidine-based chemical series identified from a phenotypic screening for the treatment of tuberculosis

Théo Hattabi[1], Claire Hamela[2], Sushovan Dam[3], Marion Michel[1], Céline Lenglart[1], Catherine Piveteau[1], Benoît Déprez[1], Marion Flipo[1], Laurent Kremer[2], Nicolas Willand[1], Ruben C. Hartkoorn[3], Baptiste Villemagne[1]

(1) Univ. Lille, Inserm, Institut Pasteur de Lille, U1177—Drugs and Molecules for Living Systems, F-59000 Lille, France
(2) Institut de Recherche en Infectiologie de Montpellier (IRIM), Université de Montpellier, CNRS UMR 9004, Montpellier, France
(3) Institut Pasteur de Lille, Univ. Lille, CNRS, Inserm, CHU Lille, U1019-UMR8204-CIIL-Centre d'Infection et d'Immunité de Lille, F-59000 Lille, France

Tackling tuberculosis (TB) is still currently a global challenge, despite ever-increasing efforts from the WHO in the last three decades. The first-line treatment for TB is long and causes serious side effects; this results in observance issues among patients, leading to the emergence of drug-resistant strains of *Mycobacterium tuberculosis* (Mtb), the TB-causing bacterium. For instance, there was an estimated 440,000 new cases of multi-drug resistant (MDR) in 2019[a]. New antibiotics are thus required to help treating resistant infections and to reduce the TB treatment time. One of the most commonly used methods to discover new antibiotics is the phenotypic screening of new molecules. With the aim of identifying new antitubercular compounds, we tested over 9,000 original compounds of our in-house library on Mtb, leading to a hit bearing an N-benzylated piperidine moiety, and displaying an interesting MIC of 1.5 µM but poor microsomal stability. Structure-activity relationship exploration around our hit molecule led to optimized compounds with a fiftyfold activity enhancement, as well as a noticeable microsomal stability improvement. These results encouraged us to perform *in vivo* evaluations of our analogs in a zebrafish model of *Mycobacterium marinum* infection. Herein are presented the optimization of the compounds and the results obtained *in vivo* for our most optimized compounds.

Bibliographic references:
(a) *WHO Global Tuberculosis Report 2020*

* Correspondence: theo.abdesselam@univ-lille.fr

Synthesis, conformational studies and preliminary biophysical evaluations of fluorinated foldamers as inhibitors of hIAPP aggregation

José Laxio Arenas*, Thierry Milcent, Julia Kaffy, Tap Ha-Duong, Benoit Crousse, Sandrine Ongeri.

FLUOPEPIT, BioCIS CNRS, Université Paris Saclay, 5 rue Jean-Baptiste Clément, 92290 Châtenay-Malabry Cedex, France

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Today around half a billion people are suffering from Type 2 diabetes (T2D).[1] T2D is an amyloid disease like Alzheimer's, and Parkinson's diseases which are characterized by the aggregation of a protein (amyloid β peptide, α-synuclein, …). In its random or α-helix conformation, the human islet amyloid polypeptide (hIAPP, or amylin) plays a role in glucose homeostasis. But by an unknown mechanism it can change to β-sheet rich structures and aggregate to form amyloid deposits in the pancreas of more than 95% of type 2 diabetic patients. Amyloid aggregates of hIAPP contribute to β-cells dysfunction and death leading to type 2 diabetes and cardiovascular complications.[2] No causal treatment of TD2 exists. Therefore, it is a major international issue to find new drugs to treat this fatal disease.

Since few years foldamers have emerged as useful secondary structure mimetics of proteins.[3] However, the field of fluorinated foldamers is still under-explored. Peptidomimetic foldamers could be particularly interesting in trying to meet the challenge of finding new classes of drugs targeting protein-protein interactions involved in untreated diseases such as Type 2 diabetes or Alzheimer's disease.

On the other hand, introducing fluorine atom(s) into bioactive organic compounds has become a leading strategy for drug design. Due to its unique properties, fluorine is frequently employed to modify biologically relevant properties such as metabolic stability, basicity, lipophilicity, and bioavailability. Nevertheless, in the literature, there is still no fluorinated peptide used as a drug.[4]

In this communication, the *N*-difluoromethyltriazolyl peptidomimetic synthesis and the conformational preferences of this new class of fluorinated foldamers will be presented.[5]

Preliminary results about their ability to modulate the aggregation of hIAPP, using biophysical evaluations will be also provided.

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Correspondence: jose.laxio-arenas@universite-paris-saclay.fr

Development of new water-soluble antimitotic agents with high selectivity towards tumor cells

L. Gallego-Yerga *, **M.González, A. Vicente, M. Marín , C. Sanz, S. Ramos(1), R. Fuentes(1), S. del Mazo(1), R. Álvarez(1), M. Medarde(1), R. Peláez(1).**

(1) Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Salamanca, Miguel de Unamuno Campus, 37007, Salamanca (Spain).

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Microtubule-targeting agents include an heterogeneous group of compounds having the capacity to bind to the main constituent of the microtubules, the αβ-tubulin dimers, thus interrupting the polimerization-depolimerization equilibrium, the formation of the mitotic spindle and finally the cell division. Although αβ-tubulin has different binding sites, the colchicine binding site ligands such as Combretastatin A-4 (CA4, Figure 1) have received special attention since the recent discovery that some of those compounds are not only anti-cancer drugs but are also able to shut down tumor neovasculature. However, four main drawbacks have been reported for CA4 analogues: the double bond isomerization, the low aqueous solubility due to the high hydrophobic nature of the binding site, the metabolic transformations associated to B ring, leading to resistance, and their low selectivity towards tumor cells. We have combined different strategies of structural modification to overcome these limitations: the incorporation of configurationally stable bridges (1,1-ethenylene, carbonil, ketoxime bridges), the replacement of A ring of CA4 by water-soluble substituted pyridines and the modifications of B ring that remove the troublesome groups while allowing additional polarity enhancements. On the pyridine A ring we have explored the methylsulfanyl groups to compensate for the size reduction associated with the removal of the central methoxy group of CA4.

The resulting molecules showed substantial solubility improvements and high antiproliferative activity against several cancer cell lines (HeLa cervix epithelioid carcinoma, HT-29 colon adenocarcinoma, MCF-7 breast adenocarcinoma and HL-60 human acute myeloid leukemia) whereas the toxicity towards non-tumorigenic cells was negligible. Proliferation assays in presence of verapamil, a known MDR protein inhibitor, demostrated that those compounds lack multidrug resistance. The mechanism of action was assessed by tubulin inhibition polymerization experiments and immunofluorescence confocal microscopy. The observed microtubule disruption was accompanied by a cell cycle arrest at G2/M phase and subsequently apoptotic cell death was confirmed by cells gathered at the subG0/G1 population and Annexin V/Propidium Iodide double-positive cells observed after 72 h of treatment. Conformational analysis combined with docking studies suggest binding at the colchicine site with association energies dependent on the conformational preferences of the pyridine substituents. These results validate the proposed design to obtain water soluble and configurationally stable tubulin polimerization inhibitors having high selectivity towards tumor cells.

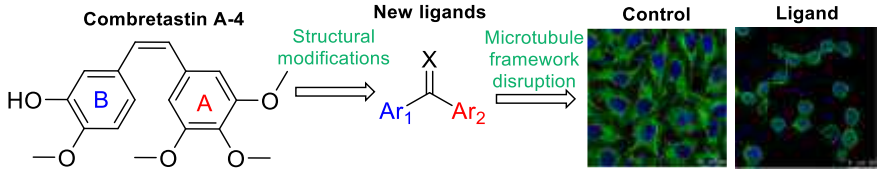

Figure 1. Left: CA4 structure. Right: effects of the ligands on the microtubule network in HeLa cells.

Bibliographic references: [a] **M. González, Y. Ellahioui, R. Álvarez, L. Gallego-Yerga, E. Caballero, A. Vicente, L. Ramudo, M. Marín, C. Sanz, M. Medarde, R. Pelaéz.** *Molecules* **2019**, *24*, 4319. [b] R. Alvarez, L. Aramburu, C. Gajate, A. Vicente-Blázquez, F. Mollinedo, M. Medarde, R. Pelaez. Eur. J. Med. Chem. **2021**, *209*, 112933.

* Correspondence: gallego@usal.es

Quinoxaline-based Efflux Pump Inhibitors restore drug susceptibility in Drug Resistant Non-Tuberculous Mycobacteria

Roberta Ibba [(1)], Paola Corona [(1)], Paola Molicotti [(2)], Antonio Carta[(1)].

(1) Department of Chemistry and Pharmacy, University of Sassari, via Vienna 2, via Muroni 23a, 07100, Sassari, Italy
(2) Department of Biological and Medicinal Sciences, University of Sassari, Viale San Pietro, 07100 Sassari, Italy

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Non-tuberculous mycobacteria (NTM) comprise several ubiquitous organisms that cause infections in different human body sites. NTM-associated pulmonary infections mostly occur and individuals with underlying respiratory disease or chronic disease and immunosuppressed patients are often affected. [1] *Mycobacterium simiae* is a NMT that can infect both humans and animals causing pulmonary disorders. It is a slow-growing, rust-colored photochromogenic mycobacterium. [2] *Mycobacterium abscessus* is a rapid-growing NTM that is responsible for a variety of human infections, mostly cutaneous and pulmonary infections. These are difficult to eradicate due to the natural and acquired multidrug resistance of *M. abscessus*. [3] *M. simiae* and *M. abscessus* infections have been revealed in different states worldwide. Drug susceptibility to antitubercular drugs is performed when NMTs are isolated from patients, and they showed drug-resistance for different tested drugs. [2,3] In this study we isolated one *M. simiae* and one *M. abscessus* strains from patients in one Italian hospital and they were tested for drug susceptibility against azithromycin, amikacin, ciprofloxacin, levofloxacin, moxifloxacin and linezolid.

A new series of 2-Aryl-3-phenoxymethyl-quinoxaline derivatives (QXs) has been designed, synthesised and investigated as extrusion pump inhibitors (EPIs) against two NTM clinical isolates. REMA assays were selected to investigate MICs values of 6 antibiotics both in presence and absence of our QXs, evaluating how EPIs can impact the drug MICs values, and therefore the activity. The different resistance levels tracked in the clinical strains have been reduced by EPIs and in several cases the susceptibility was completely restored. The results obtained in this study indicated that the intrinsic cell-efflux activity significantly contributes to the overall resistance in resistant clinical isolates of NTM, and that the inhibition of efflux pumps by the QXs can enhance the clinical effect of antibiotics.

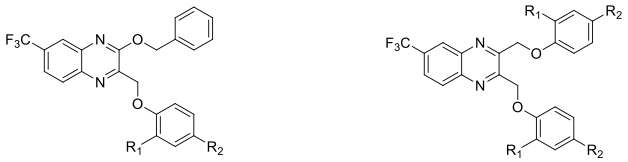

Figure 1. 2,3-Aryl-6-trifluoromethyl-quinoxaline derivatives (QXs) general structure.

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* Correspondence: ribba@uniss.it

Fragment-based kinase inhibitor design using *in silico* tools

Peyrat G.*, Bournez C., Krezel P., Gally J.-M., Bourg S., Aci-Sèche S., Bonnet P.*

Institute of Organic and Analytical Chemistry (ICOA), UMR7311, Université d'Orléans, Pôle de chimie rue de Chartres - BP 6759 - 45067 Orléans Cedex 2 France

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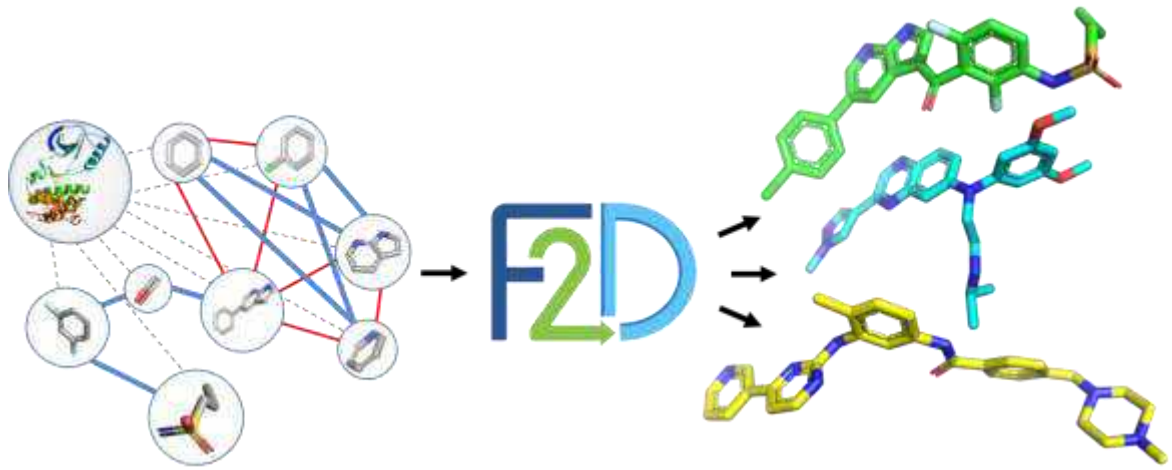
In recent decades, Fragment-Based Drug Design (FBDD) has been widely developed in both academic laboratories and pharmaceutical companies[(a)]. Several drugs approved today by the FDA or in advanced clinical trials have been discovered from FBDD. For instance, vemurafenib and erdafitinib were identified through FBDD and approved by the FDA in 2011 and 2019 respectively.

Fragments are low molecular weight molecules and can cover a larger chemical space than drug-like molecules[(b)]. Fragments can be optimized (growing, merging and linking) in the active site of the target to generate potent and selective novel inhibitors. Frags2Drugs (F2D) is an *in silico* FBDD tool recently developed at the ICOA. It relies on an in-house 3D fragment library obtained from co-crystallized ligands or by molecular docking. This library is stored in a graph-oriented database containing required information to link fragments together. Frags2Drugs builds rapidly every possible molecule fitting in a given ATP site of a protein kinase.



Using additional *in silico* tools, molecules are then filtered to keep those presenting the best potential affinity. Several specific molecular filters are applied, including a specific in-house kinase inhibitor-like filter[(c)]. F2D was validated by reconstructing almost all co-crystallized protein kinase inhibitors. F2D has already shown promising results by finding potent, selective and patentable chemical series developed on five different kinase projects.

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* Correspondence: gautier.peyrat@univ-orleans.fr, pascal.bonnet@univ-orleans.fr

Selection and Characterization of Nanoporous Activated Carbon Derived from palm shell, sugar cane, coconut husk materials for the removal of metal ions	***Restricted to organizers***

J. Michael Ahitha Jose[1] and J.Joseph[2*]

[1]Research Scholar, Department of Chemistry, Noorul islam Centre for Higher Education, Kumaracoil-629180, Tamil Nadu, India.
[2]Department of Chemistry, Noorul islam Centre for Higher Education, Kumaracoil-629180, Tamil Nadu, India.

Innovative new carbon materials that have been used successfully in wastewater treatment and environmental protection technologies [1] have resulted in extensive research carried out worldwide. Carbon nanomaterials are unique due to their nontoxic, higher surface area, easier biodegradation, and useful environmental remediation. Heavy metal contamination in water is a major issue and poses a great risk to human health. The present investigation encompassed with preparation of an activated carbon (derived from agricultural waste materials like palm shell, sugar cane, coconut husk) in nano size through chemical and thermal activation of material. The different parameters affecting in the chemical and thermal activation processes such as chemical types used for activation process, activation time and temperature and carbonization time and temperature for the thermal activation process were optimized to produce nano-size activated carbon. All prepared materials were evaluated for copper decontamination from industrial wastewater. The prepared nano-activated carbon was characterized using Fourier transform infrared spectroscopy (FTIR), Thermal Analysis (TGA), BET, SEM, TEM and X-ray diffraction (XRD). The prepared nano-activated carbon was subjected for the removal of copper, lead, titanium ions sorption from aqueous solution using batch technique. Among the prepared nano-activated carbon derived from palm shell exhibited higher removal of copper, iron, chromium ions are 92, 88 and 90% within 6 hrs (operating time), pH 6. This innovative material opens up new prospects for the productive use of chemical, petrochemical and wastewater treatment plants by nano-sized adsorbents at low cost.

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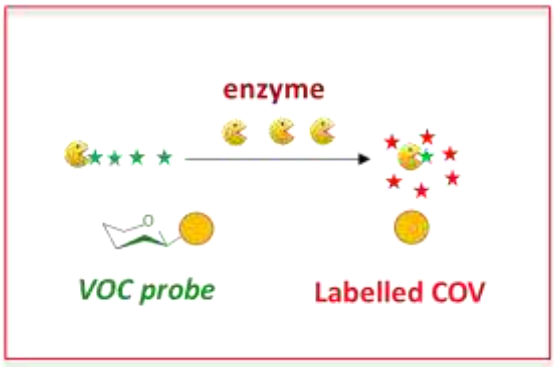
Correspondence: josephniche@gmail.com

Development of Volatile Organic Compound (VOC) probes for cancer biomarker targeting	***Restricted to organizers***

Fabiola DJAGO*, Pauline POINOT

(1) E-BiCoM, Institut des Milieux et des Matériaux, 4 rue Michel Brunet 86000 Poitiers.

Cancer is the second cause of death worldwide. However, if cancers were diagnosed earlier, the mortality rate could be reduced of about 30% according to WHO. Numerous techniques were developed to detect tumors, but none has been used in clinic as a noninvasive, simple, and accurate test so far. In this context, the E-BiCoM team proposed a new diagnosis strategy that relies on a cocktail of isotopic and nontoxic VOC-based probes targeting glycosidases previously reported as markers of tumors.



Diverse glycosidases have been either over-expressed or co-localized with cancer cells, and could also be involved in tumor metastasis[a] . In a previous study[b], we have shown the efficiency of a VOC-based probe, i.e., the ethylglucuronide-D_5, targeting the β-glucuronidase present in high concentration in tumors microenvironment, to diagnose cancers and monitor tumor growth during chemotherapy. Next, we have transposed the strategy to directly detect the enzyme ex vivo, in human plasma samples. A clinical study was thus conducted in collaboration with the Clinical Investigation Centre in the CHU of Poitiers, to demonstrate the efficacy of the probe for diagnosing cancer patients just from a blood sampling. Protocol optimization and first results will be presented here. To go further, and in a clinical research context, targeting only one biomarker can lead to a significant number of false positives and false negatives. In this context, we plan to measure several enzyme activities with a cocktail of multiplexed VOC-based probes to provide the much-needed specificity for (1) disease detection, (2) stratification of patients for personalized therapy, and (3) understanding the cellular processes that led to enzymes overexpression in diseased tissues. First in vitro tests with dual probes will be presented here.

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* Correspondence: fabiola.djago@univ-poitiers.fr

REMOVAL OF METAL IONS USING MICROPOROUS ADSORBENTS FROM NATURAL WASTE MATERIAL (LEMON PEEL)	
D. Arthi[1] and J.Joseph[2*] *[1]Research Scholar, Department of Chemistry, Noorul islam Centre for Higher Education, Kumaracoil-629180, Tamil Nadu, India.* *[2]Department of Chemistry, Noorul islam Centre for Higher Education, Kumaracoil-629180, Tamil Nadu, India.*	***Restricted to organizers***

Abstract

Sustainable methods to produce adsorbent materials are needed to remove a variety of pollutants found in water including organic compounds, heavy metals, and other harmful inorganic and biological contaminants. The present investigations focuses on the removal of Cu(II), Ni(II), Pb(II) from polluted water using activated adsorbent derived from natural waste material (lemon peel, as agro-industrial waste). The effects of solution pH, adsorption time, metal ion concentration and dose of adsorbent on sorption were studied in batch experiments. The maximum Langmuir adsorption capacity was evaluated to be 10.8 mg/g at optimum contact time of 20 min. The maximum removal of copper ions from mining-wastewater at natural pH (pH3) was 90%, indicating that lemon peel could be employed as an effective low-cost adsorbent for removal of copper, nickel, lead ions from wastewater at acidic conditions. The experimental observation indicated that lemon peel, as agro-industrial waste, was used to investigate removal efficiency of metal ions from mining wastewater.

* Correspondence: **josephniche@gmail.com**

DEVELOPMENT OF ENCODED COMBINATORIAL CHEMISTRY FOR THE IDENTIFICATION OF SPECIFIC LIGANDS OF G-QUADRUPLEX DNA STRUCTURES	***Restricted to organizers***

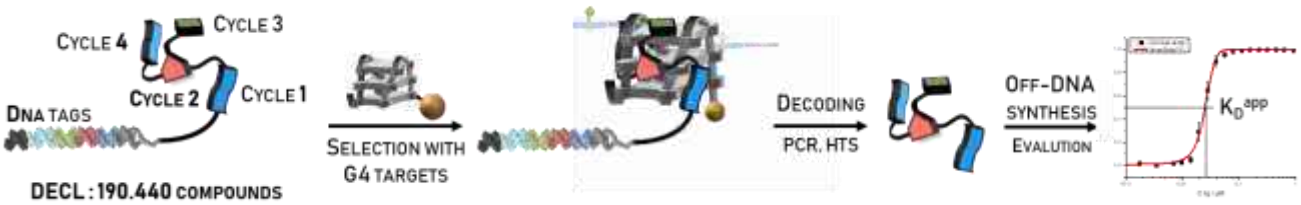
P. RIEBER* ; Y. SI ; C. VAN WESEMAEL ; T. LAVERGNE*.

Université Grenoble-Alpes, Département de Chimie Moléculaire (DCM), CNRS UMR 5250, 570 rue de la Chimie, Grenoble, France.

G-quadruplexes (G4s) are four-stranded nucleic acid structures formed by the stacking of two or more co-planar G-quartets stabilized via Hoogsteen hydrogen-bonds of guanine bases. These thermodynamically stable non-canonical DNA and RNA structures have been found in key regulatory regions of genomes and transcriptomes suggesting their implication in important biological processes such as replication, transcription and translation, but also in the molecular mechanisms of several important diseases[(a)].

Organic molecules specifically targeting and stabilizing G4 structures can provide a better understanding of their functions and amongst them, some have shown significant therapeutic potentials. Typical G4 interacting/stabilizing compounds are synthetic small molecules, combining rigid aromatic heterocyclic architectures, able to act with external G-quartets, and cationic or protonable side chains, able to form additional interaction with negatively charged grooves and/or loops. Herein, we report on the assembly and screening of a DNA encoded combinatorial library (DECL) of potential ligands of G4 structures composed of heteroaromatic hydrophobic cores and polypeptide appendages.

DECL is a cost efficient drug discovery technology bridging the fields of combinatorial chemistry and molecular biology. In DECL, numerically large combinations of building blocks can be efficiently synthesized and connected to individual DNA sequences carrying readable information about the compound structure[(b)]. Over the past few years, DNA encoded libraries targeting proteins involved in diseases had yielded highly promising hits, making DECL a new pillar in the drug/probe discovery pipeline. Our unpublished library, to the best of our knowledge the first DECL dedicated to nucleic acid recognition, was selected against various G4 structures of biological/therapeutic interest. High-throughput sequencing (HTS) of the selected DNA encoded G4 ligands provided remarkable structure affinity relationships along with unambiguously enriched molecular motifs. The most enriched ligands were resynthesized and their affinity toward G4 and stabilization capacities were confirmed by fluorescence based binding assay and thermal denaturation studies. Those ligands showed affinities comparable to the most advanced G4 ligands reported to date and fully endorse the use of DECL to seek novel ligands of structured nucleic acids.



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* Correspondence: pierre.rieber@univ-grenoble-alpes.fr, thomas.lavergne@univ-grenoble-alpes.fr

Tracking G-quadruplex ligands in cells by Immunofluorescence staining

Thibaut Masson*, Corinne Landras Guetta, Eugénie Laigre, Anne Cucchiarini, Patricia Duchambon, Marie-Paule Teulade-Fichou, Daniela Verga

Institut Curie, Chemistry and Modelling for the Biology of Cancer (CMBC), Université Paris-Saclay , CNRS UMR9187, INSERM U1196, Orsay; 91400, France

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G-quadruplex (G4s) are non-canonical DNA or RNA secondary structures that can be generated in repetitive guanine (G)-rich nucleic acid sequences containing tracks of three or four guanines. These structures are formed by the stacking of at least two G-tetrads which are square planar arrays in which four guanines interact through Hoogsteen hydrogen bonding (Figure **1A**). These non-canonical structures are likely to form in G-rich regions throughout the genome suggesting possible functional roles in key biological processes such as transcription, replication, and translation[1]. Therefore, G4s have become the object of intense study with the aim of defining their potential role as regulatory elements and/or therapeutic targets[2]. G4s can interact and be stabilized by small molecules, and as a result, are considered as therapeutic targets to control gene expression at the level of DNA or RNA. Although the high number of small molecules known to interact with these structures, there is still a strong need to perform in vivo studies to understand the biological relevance of G-quadruplex structures. In this context, we developed new functionalized G4 ligands that not only are able to selectively bind these structures but the added function also allows the development and application of new visualization techniques. For this purpose, we synthesized some derivatives of the well known G4 ligand PDC[3], functionalized with different linkers. These ligands are either modified with an alkyne or an azide in order to further functionalize them with a hapten (5-BrdU) by a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC). The molecules before and after functionalization with 5-BrdU are tested for their affinity and selectivity towards G4 structures by employing different biophysical techniques. Cell distribution of 5-BrdU functionalized ligands is next determined by immunofluorescence staining after recognition of the 5-BrdU tag by a specific antibody (Figure **1B**).

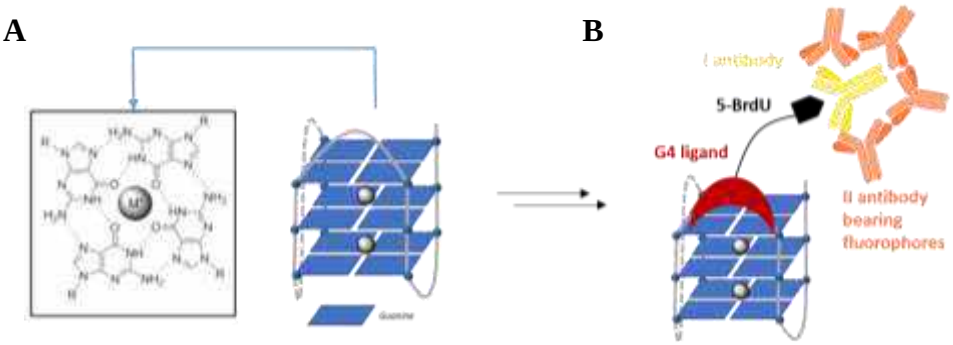

Figure 4: Representation of **A)** a G-quartet (left) and a G-quadruplex formed by the stacking of 3 G-quartet (right) and **B)** the immunodetection of a 5-BrdU modified G4 ligand

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*Correspondence: thibaut.masson@curie.fr