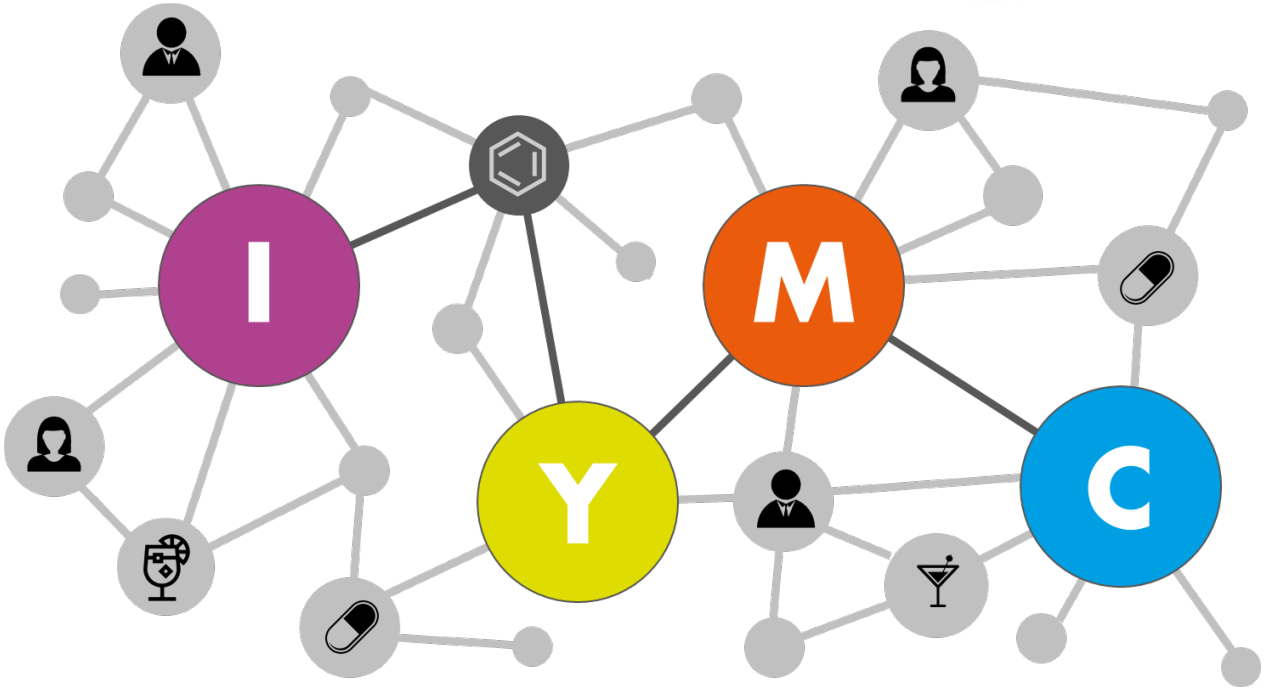
Società Chimica Italiana
Divisione di Chimica Farmaceutica

EFMC
European Federation for Medicinal Chemistry



ITALIAN YOUNG MEDICINAL CHEMISTRY

VIRTUAL MEETING

#IYMCVMEET

ABSTRACT BOOK

22-24 JULY, 2020

WELCOME

The mission of the I-YMC-VMeet is to provide an online conference with keynote lectures and flash communication by the most distinguished Italian young scientists who have produced important, break-through discoveries in the last years in Medicinal Chemistry. This idea is aimed to promote networking, gathering young researchers and encouraging discussions on any scientific field related to drug discovery. We strongly believe in the importance of consolidating the interactions of our Community in science and beyond, for this reason we organized a poster session opened to all the members of scientific societies affiliated to EFMC.

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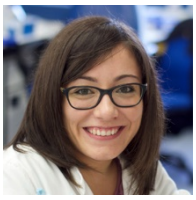
SCIENTIFIC PROGRAM

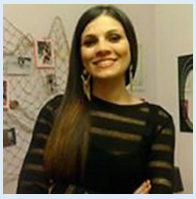
Wednesday, 22nd July

3 pm CET	**Opening** *Gianluca Sbardella - President of the Medicinal Chemistry Division*	
Session 1	Chair: **Alberto Massarotti** *(University of Piemonte Orientale, Italy)*	
3.20 pm CET		**KS, Alfonsina D'Amato** *(University of Milan, Italy)* OMICS profile of SARS-Cov2 *Summer School in Pharmaceutical Analysis SSPA* *Director: Manuela Bartolini*
3.45 pm CET		**Samuele Cazzamalli** *(Philochem, Switzerland)* Small Molecule-Drug Conjugates: getting small to enhance targeting
4.00 pm CET		**Marta Serafini** *(University of Piemonte Orientale, Italy)* *Benzimidazoles as IDO1 inhibitors: from a virtual screening to a potential treatment in pancreatic ductal adenocarcinoma*
4.15 pm CET	**Coffe break**	
Session 2	Chair: **Michele Mari** *(University of Urbino Carlo Bo, Italy)*	
4.30 pm CET		**KS, Tiziano Bandiera** *(IIT, Italy)* Market watch: novel drug approvals for 2019 *European School of Medicinal Chemistry ESMEC* *Director: Marco Macchia*
4.55 pm CET		**Alessandra Altomare** *(University of Milan, Italy)* Analytical strategies for understanding the molecular mechanisms of N-acetyl-cysteine as extracellular antioxidant
5.10 pm CET		**Giulia Palermo** *(University of California Riverside, USA)* Aiding the SARS-CoV2 detection through emerging CRISPR-Cas genome editing technologies
5.25 pm CET	**Social event**	
5.50 pm CET	**Closing remarks**	

Thursday, 23rd July

3 pm CET	**Opening**	
Session 3	Chair: **Isabella Romeo** *(University of Calabria, Italy)*	
3.05 pm CET		**KS, Carmine Talarico** *(Dompé farmaceutici, Italy)* The Exscalate4CoV project: how CADD is supporting the fight against SARS-CoV-2. A real journey from HPC to virtual reality
3.30 pm CET		**Francesca Morreale** *(IMP – Research Institute of Molecular Pathology, Austria)* BacPROTACs: bringing the PROTAC approach to the bacterial world
3.45 pm CET		**Bruno Cerra** *(University of Perugia, Italy)* Size-tuneable flow synthesis of functional nanoparticles for biomedical applications
4.00 pm CET	**Coffe break**	
Session 4	Chair: **Giannamaria Annunziato** *(University of Parma, Italy)*	
4.15 pm CET		**Alessia Romussi** *(IFOM - the FIRC Institute of Molecular Oncolology, Italy)* Potent reversible inhibitors of Histone Lysine Demethylase KDM1A/LSD1 efficacious in Acute Myeloid Leukemia models
4.30 pm CET		**Monica Viviano** *(University of Salerno, Italy)* Identification of novel pyrazole derivatives as inhibitors of lysine acetyltransferase p300
4.45 pm CET	**Medicinal Chemistry Division of the Italian Chemical Society's Awards** *Gianluca Sbardella - President of the Medicinal Chemistry Division*	
4.50 pm CET		**Francesco S. Di Leva** *(University of Naples Federico II, Italy)* Targeting the αvβ6 RGD Integrin: from Computer-Aided Drug Design to Preclinical Applications
5.05 pm CET		**Azzurra Stefanucci** *(University of Chieti-Pescara, Italy)* Potent, Efficacious, and Stable Cyclic Opioid Peptides with Long Lasting Antinociceptive Effect after Peripheral Administration
5.20 pm CET	**Social event**	

Friday, 24th July

3 pm CET	**Opening**	
Session 5	Chair: **Gianluca Sbardella** *(University of Salerno, IT)*	
3.05 pm CET		**KS, Rita Petracca** *(Marie Sklodowska-Curie Imperial College London, UK)* RaPID Selection of Novel Macrocyclic Peptides as Modulators of Rab27a-effector Interactions *13th Young Medicinal Chemist's Symposium* *Chair: Maria Novella Romanelli*
3.30 pm CET	**Flash prize and Poster prizes presentations**	
4.00 pm CET	**Coffe break**	
	Chair: **Gabriele Costantino** *(University of Parma, Italy)*	
4.15 pm CET	   	**Round table** About Medicinal Chemistry Career **Yves Auberson** *(EFMC President) - Novartis, Switzerland* **Kristina Goncharenko** *(EFMC-YSN) - SpiroChem, Switzerland* **Rui Moreira** *(EFMC President Elect) - University of Lisbon, Portugal* **David Peralta** *(Editor-in-Chief, ChemMedChem) - Chemistry Europe, Germany*
5.00 pm CET	**Closing remarks**	

Keynote presentations

OMICS profile of SARS-Cov2

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Network medicine based on OMICS data aims to understand diseases at the systems-level. Pharmacological studies attempt to recognize both the network connectivity and dynamics of signaling systems as components of drug targets.
The study of the proteome, metabolome and transcriptome of the host cell and of the viral proteome translated into the SARS-Cov2 infected cell has been applied in recent studies.[1-5]
OMICS based papers, reviewed in this lecture, show how the host cell responds to the viral infection, which molecular pathways are activated, and which are inhibited as well as the cell pathways promoted for viral replication. These and future results will provide a precise understanding of the molecular mechanism of infection and viral replication in the host cell and it will allow the identification of novel drug targets and the efficacy and mechanism of action of tested drugs. Identification of drug targets will be used for a drug repurposing *in silico* approach.

References

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Market watch: novel drug approvals for 2019

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The approval of new drugs in 2019 remained high although the total number was lower than in 2018. In the United States, the Center for Drug Evaluation and Research (CDER) of the Food and Drug Administration (FDA) approved 48 new drugs[1]. Among them, 38 are new molecular entities while 10 are new therapeutic biologics. In Europe, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicine Agency (EMA) recommended 66 medicines for marketing authorization[2]. Of these, 30 had a new active substance which had never been authorized in the EU before.

Most of the new drugs approved in the US were for orphan diseases: 21 new drugs, representing 44% of all approvals. The breakdown of new approvals for therapeutic areas shows that oncology was the area with most approvals (11 or 23% of new drugs), followed by neurology (9 new drugs), haematology (6 new drugs), and infectious disease (5 new drugs). In Europe, 23% of new medicines (7 drugs) were recommended for approval in haematology, followed by 5 new medicines (17%) for cancer treatment, and 3 new drugs (10%) for infectious diseases and endocrinology[2].

Some of the drugs approved in 2019 add new modalities in the treatment of certain diseases. Among them, the FDA approved the first nanobody, caplacizumab, for the treatment of adult patients with acquired thrombotic thrombocytopenic purpura, a potential life threatening disease. Both the FDA and EMA approved Ervebo, the first vaccine for the prevention of Ebola infection.

Other notable approvals include upadacitinib, the first JAK1-selective inhibitor for the treatment of rheumatoid arthritis, trikafta, a triple combination of modulators of the activity of certain mutant forms of the CFTR protein for the treatment of cystic fibrosis, and entrectinib, a new small molecule for the treatment of *NTRK* gene fusion-positive solid tumors, including CNS tumors due to its ability to cross the blood–brain barrier.

The presentation will give an overview of the new drugs approved in 2019.

References

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[2] EMA Human medicines highlights 2019. https://www.ema.europa.eu/en/news/human-medicines-highlights-2019

The Exscalate4CoV project: how CADD is supporting the fight against SARS-CoV-2. A real journey from HPC to virtual reality

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E4C is a public-private consortium supported by the European Commission's Horizon 2020 tender for projects to counter the Coronavirus pandemic and improve the management and care of patients.
At the core of E4C is Exscalate (EXaSCale smArt pLatform Against paThogEns), at present the most powerful and cost-efficient intelligent supercomputing platform in the world. Exscalate has a "chemical library" of 500 billion molecules and a processing capacity of more than 3 million molecules per second. The E4C consortium, coordinated by Dompé Farmaceutici, is composed by 18 institutions from seven European countries.
First, E4C will select, through the EXSCALATE platform and the virtual screening protocols, the most promising drugs against coronavirus taken from the commercialized and developing drugs safe for humans (> 10000 drugs, SIM). At the same time, the proprietary Tangible Chemical Database (TCDb), comprising > 500 billion molecules, will be screened to identify new potential drugs to be tested against coronavirus, thus enhancing the success rate of the virtual screening step.
The aim of E4C is twofold: to identify molecules capable of targeting the coronavirus (2019-nCoV) and to develop an effective tool for countering future pandemics to be consolidated over time.
More specifically, E4C aims to:

- Establish a sustainable example for a rapid scientific response to any future pandemic scenario. The model leverages a rapid and effective High Performance Computing platform for the generation and analysis of 3D models and experimental 3D structures, X-ray resolution of protein targets from pandemic pathogens.
- Drive a fast virtual identification and repurposing of known drugs or proprietary/commercial candidate molecules to be further experimentally characterized.
- Define a workflow scheme for biochemical and cellular screening tests to validate candidate molecules at previous points and assure through phenotypic and genomic assays.
- Prepare, together with EMA, a development plan for successful candidates for direct "first in human" type studies or for further testing in animals with bridging studies.
- Identification of 2019-nCoV genomic regions involved in host adaptation, pathogenicity and mutations.

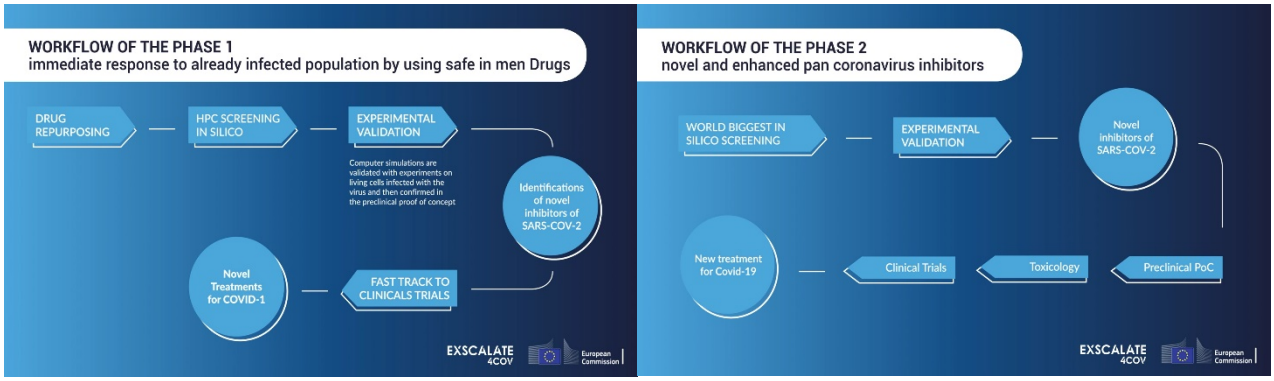

Figure 1. Representation of the two workflows used during the E4C project, helpful for identifying molecules against SARS-CoV-2.

RaPID Selection of Novel Macrocyclic Peptides as Modulators of Rab27a-effector Interactions

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Rab27a belongs to the Rab subfamily of small GTPases, a highly conserved group of GTP/GDP-mediated molecular switch proteins involved in the regulation of intracellular trafficking.[1] Recent studies correlated Rab27a cellular activity with the regulation of tumorigenic signals secretion involved in metastatic breast cancer progression and exosome release.[2] The crystal structure of Rab27a in complex with an effector protein Slp2a[3,4] shows that the protein globular structure is defined by a conserved nucleotide binding site and an extensive Rab-effector interaction surface. To date, a few small molecules have been claimed as putative reversible Rab27 ligands which can inhibit effector interactions, but previous studies in our labs have demonstrated that none of these compounds bind to Rab27a, strongly suggesting that their observed activity in cells is off-target and non-specific. However, discovery and validation of a cell-active stapled peptide probe for Rab25 suggests the feasibility of small GTPase targeting by effector binding disruption.[5] We screened a mRNA display library against Rab27a in order to identify novel macrocylic peptide modulators of the Rab27a-effector interaction (Figure 1). Following successful enrichment, the corresponding cyclic peptide sequences were analysed and several selected for synthesis and further testing. These peptide hits thus constitute a starting point for successive structure- and activity-guided modifications, aiming to determine Rab27a preferred conformations and binding modes, and ultimately to engage the active protein in cells.

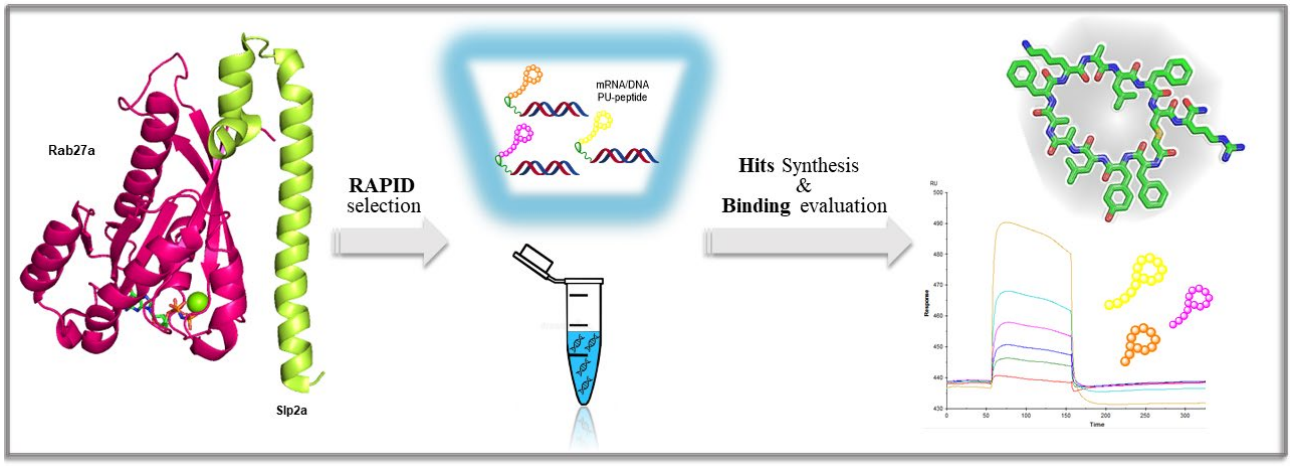

Figure 1. Schematic workflow for RaPID selection of Rab27a macrocyclic peptide derivatives.

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Oral presentations

Small Molecule-Drug Conjugates: getting small to enhance targeting

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Conventional cancer chemotherapeutic agents do not preferentially localize at the tumor site [1]. Monoclonal antibodies and small organic ligands have been proposed as delivery vehicles of cytotoxic compounds, allowing the construction of antibody−drug conjugates (ADCs) [2] and small molecule−drug conjugates (SMDCs) [3].
Clinical efficacy of ADC products is hindered by their slow and inefficient accumulation in solid tumors [4, 5]. Small Molecule-Drug Conjugates have been recently proposed as an alternative approach to effectively deliver potent cytotoxic compounds to the neoplastic site and to solid metastatic lesions [5, 6].
Acetazolamide is a small organic compound that exhibits a high binding affinity to the tumor-associated antigen Carbonic Anhydrase IX (CAIX), a marker of Renal Cell Carcinoma and of hypoxic tumors [6]. We compared acetazolamide-based SMDC products to anti-CAIX ADCs for their ability to reach tumors in vivo and for their anti-tumor performance [5].
Recent clinical trial data have confirmed the efficient accumulation of an acetazolamide-based radiotracer (PHC-102) to solid tumors and metastatic lesions in Renal Cell Carcinoma patients [7] and provide a strong rational for the clinical development of anti-CAIX Small Molecule-Drug Conjugates.

References

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Benzimidazoles as IDO1 inhibitors: from a virtual screening to a potential treatment in pancreatic ductal adenocarcinoma

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Depletion of tryptophan and the consequent increase in kynurenines in the tumour microenvironment have been shown to enhance inflammation and lead to immune tolerance, as well as to promote neo-angiogenesis. For this reason, the key catabolic enzyme for tryptophan to kynurenines, indoleamine 2,3-dioxygenase 1 (IDO1), has taken center-stage as a druggable target in cancer immunotherapy in the last decades. High expression of this enzyme is indeed associated with poor prognosis in various human tumours and aggressive tumor phenotype.[1] It is not surprising, therefore, that 5 different IDO1 inhibitors have entered clinical trials in the past, either alone or in combination with other immunotherapeutic drugs. Yet, the report at the 2018 ASCO Annual Meeting that epacadostat failed to show a clinical benefit in combination to pembrolizumab in unresectable or metastatic melanoma leads to think whether some elements have been neglected in IDO1 landscape.[2] From a medicinal chemistry point of view, the design of novel small molecules with improved biological profile and able to specifically target this enzyme still represents a challenge.

In this context, starting from a virtual screening, a successful medicinal chemistry campaign has led to the identification of a novel class of benzimidazole IDO1 inhibitors in which the progenitor is represented by compound **10** (Figure 1). This family of compounds is endowed with an extensive bonding network in the protein active site, including the interaction with pocket C, a region not commonly exploited by previously reported IDO1 inhibitors. Moreover, benzimidazole derivatives exhibit a strong binding interaction with IDO1, a inhibitory potency at the low nanomolar level in several tumoral settings and high selectivity towards IDO1 over TDO and CYPs. The synthesized compounds are also able to significantly reduce L-Kyn plasma levels in mice and to exert a potent effect on abrogating immunosuppressive properties of MDSC-like cells isolated from patients affected by pancreatic ductal adenocarcinoma. The peculiar biological profile points to this class of molecules as a valuable template for boosting the antitumor immune system.[3]

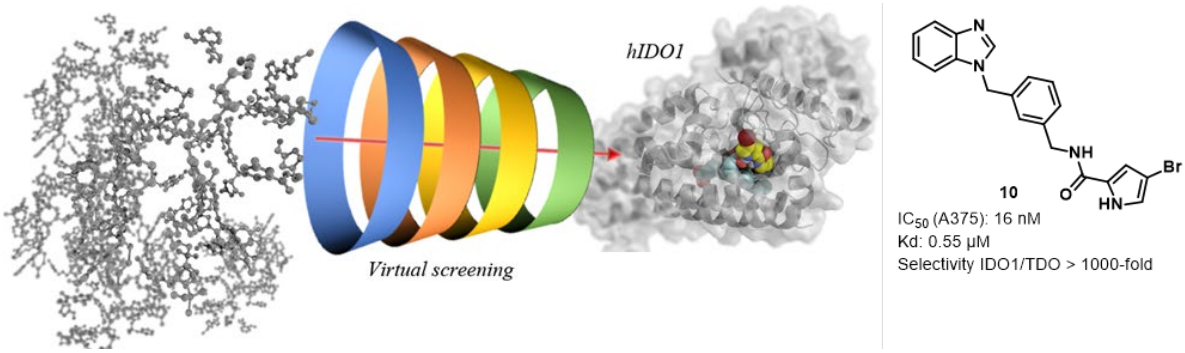

Figure 1

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Analytical strategies for understanding the molecular mechanisms of N-acetyl-cysteine as extracellular antioxidant

Alessandra Altomare,[a] Giovanna Baron,[a] Maura Brioschi,[b] Elena Tremoli,[b] Marina Carini,[a] Piergiuseppe Agostoni,[b] Giulio Vistoli,[a] Cristina Banfi,[b] and Giancarlo Aldini[a]

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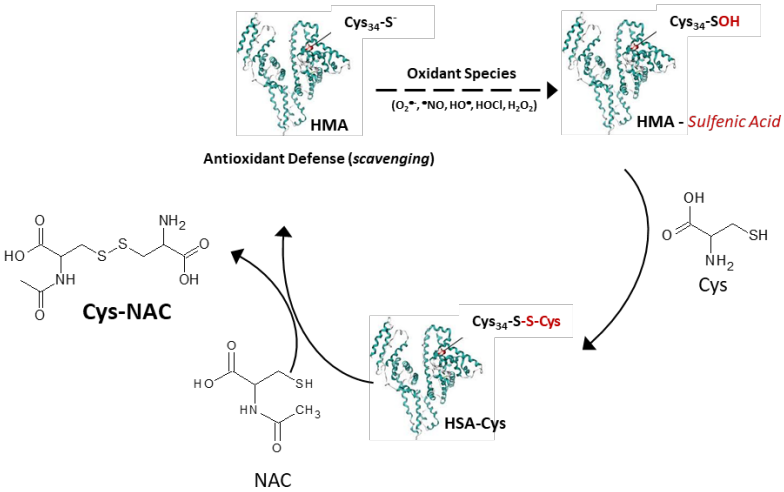
N-Acetylcysteine (NAC) is a well-established antioxidant agent and since the 1980s it has been proposed for the treatment of diseases in which oxidative stress is considered to be involved in the onset and progression of the disease state [1]. The *in vivo* antioxidant activity of NAC was largely demonstrated by different biomarkers of oxidative stress [2,3], but the molecular mechanisms explaining it are far from being fully elucidated. The aim of this work was to understand the mechanism through which N-acetyl-cysteine (NAC) acts as an extracellular antioxidant. We proposed and demonstrated that NAC acts as an antioxidant by regenerating the free form of albumin Cys34 by a disulfide breaking activity of the cysteinylated form (HSA-Cys). The ability of NAC to regenerate albumin Cys34 (HSA-SH) was studied by MS intact protein analysis in human serum and in a concentration range of NAC easily achievable after oral and iv administration (5-50 µg/ml) and then confirmed using human serum from healthy donors. NAC, dose-dependently broke the cysteinylated form of albumin to form the dimer NAC-Cys thus regenerating Cys34 which was maintained for at least 120 min. Cys was faster in restoring the Cys34 according to the reaction constant determined with GSSG reaction, but after almost 60 min of incubation the recovery of HSA-SH ended and HSA-Cys then increased above beyond initial concentration, due to the dimer Cys-Cys which upon formation, rapidly reacts with Cys34 turning back to the mixed disulfide HSA-Cys. The explanation for the different nucleophilic exchanges between Cys-Cys and Cys-NAC with Cys34 was given by molecular modelling studies. Finally, the Cys34 regenerating effect of NAC was related to its ability to improve the total antioxidant capacity of plasma as measured by the total peroxyl radical-trapping potential (TRAP) assay. The results well indicate that NAC greatly increases the plasma antioxidant activity and this effect is not reached by a direct effect but through the regenerating effect of Cys34. Cys, even if more effective as thiol-disulfide breaking agent, was much less effective as antioxidant because it is unable to maintain the Cys34 in a free form due to the reaction of cystine with Cys34 [4]. A scheme reporting the indirect antioxidant mechanism of NAC at extracellular level is depicted in the figure.



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Aiding the SARS-CoV2 detection through emerging CRISPR-Cas genome editing technologies

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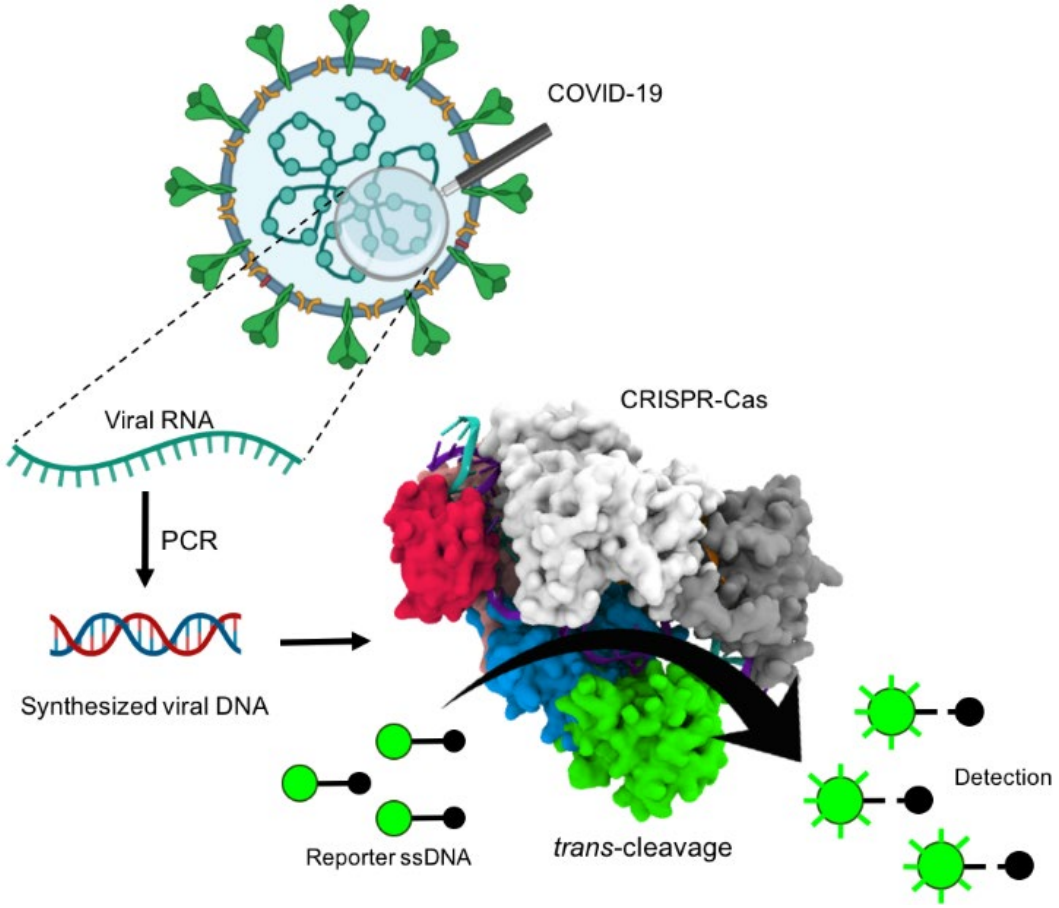
The SARS-CoV-2 coronavirus is rapidly spreading across multiple countries, causing a severe acute respiratory syndrome that threatens the world population. As the number of cases is steadily growing, there is pressing need for rapid testing tools, which could limit the contagion. New versions of the CRISPR gene-editing system are being harnessed as a fast, yet reliable diagnostic tool against SARS-CoV-2 infections. However, the molecular basis of viral nucleic acid detection is largely elusive, demanding improved approaches to expedite detection. Here, we describe how the CRISPR-associated protein Cas12a recognizes viral genetic material through microsecond-long simulations. Molecular simulations of CRISPR-Cas12a reveal that DNA binding induces a switch in the conformational dynamics of the Cas12a protein, which results in the activation of the peripheral REC2 and Nuc domains to enable cleavage of nucleic acids. Accordingly, large-amplitude motions of the Nuc domain favor the conformational activation of the system toward DNA cleavages, while the joint dynamics of REC2 is shown to prime the conformational transition of the DNA target strand toward the catalytic site. Most notably, the highly coupled dynamics of the REC2 region and Nuc domain suggest that REC2 could act as a regulator of the Nuc function, similar to what was observed previously for the HNH domain in the CRISPR-associated nuclease Cas9. Considering that the REC lobe is a key determinant in the system's specificity, our findings provide a rational basis for future biophysical studies aimed at characterizing its function in CRISPR-Cas12a. Overall, the dynamic and mechanistic aspects reported here are at the bottleneck in the process of detecting viral nucleic acids, delivering information that can help in expediting detection. This is the utmost need of the time considering the increasing number of afflicted individuals.



BacPROTACs: bringing the PROTAC approach to the bacterial world

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The possibility of hijacking protein-tagging and degradation machineries offers unique opportunities for drug discovery, as demonstrated by the development of proteolysis-targeting chimeras (PROTACs). PROTACs are bifunctional small molecules able to induce ubiquitination and subsequent degradation of proteins of interest (POIs) in eukaryotic cells, showing many advantages over classic inhibitors.

The aim of our research is to explore the possibility of bringing the PROTAC approach to the bacterial world by reprogramming components of bacterial protein degradation pathways. Among them is the ClpC:ClpP (ClpCP) protease, a central quality-control factor in Gram-positive bacteria. ClpCP is composed of the hexameric AAA unfoldase ClpC, which recognises substrates through the N-terminal domains (NTD) and feeds them into the protease cage formed by ClpP.

In order to induce selective POI degradation in bacteria, we designed bifunctional small molecules, which we call Bacterial PROTACs (BacPROTACs), recruiting model POIs to the NTD substrate receptor domain of ClpC.

Using an *in vitro* reconstituted system, we demonstrated the feasibility of BacPROTAC-mediated POI recruitment, leading to the unfolding, translocation and ultimately proteolysis of a POI by the *B. subtilis* ClpCP complex. Additionally, we succeeded in determining the cryo-EM structure of a ternary complex comprising *B. subtilis* ClpC, BacPROTAC and POI, visualising, for the first time, the structure of the ClpC hexamer in an activated state.

In conclusion, we validate *in vitro* a bacteria-specific targeted protein degradation technology, which could represent an innovative approach to expand the current antibiotic arsenal.

Size-Tuneable Flow Synthesis of Functional Nanoparticles for Biomedical Applications

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The past few decades have witnessed significant advances in the development of functional nanoparticles (NPs) for biomedical applications due to their unique optical, thermal, magnetic and electrical properties.[1] This increasing interest has resulted in a growing demand for efficient and scalable methods that enable the preparation of NPs with a precise control over particle size, distribution and morphology.[2] Recently, flow chemistry systems have shown a promising potential to produce NPs with a high level of reproducibility and with a fine tuning of their properties. Indeed, the accurate regulation of experimental conditions and fluidodynamics parameters ensured by flow devices allows a precise control over the mass transfer, the residence time and the mixing efficiency thus resulting in a spatio-temporal separation between nucleation and growth of NPs.[3] As a continuation of our interest in the use of flow chemistry technology in nanomaterials for biomedical applications,[4] in this communication we describe a flow-based approach for the preparation of size-tuneable gold (Au) NPs by Turkervich method.[5] Au NPs were prepared by reducing Au^{3+} precursor ($HAuCl_4$) to Au^0 using sodium citrate as reducing and stabilizing agent (**Figure 1**). A two level factorial experimental design was used to evaluate the effect of temperature, flow rate and sodium citrate/$HAuCl_4$ molar ratio on Au NPs size and shape. The potential advantages in terms of size control and scalability of the method will be illustrated and discussed.

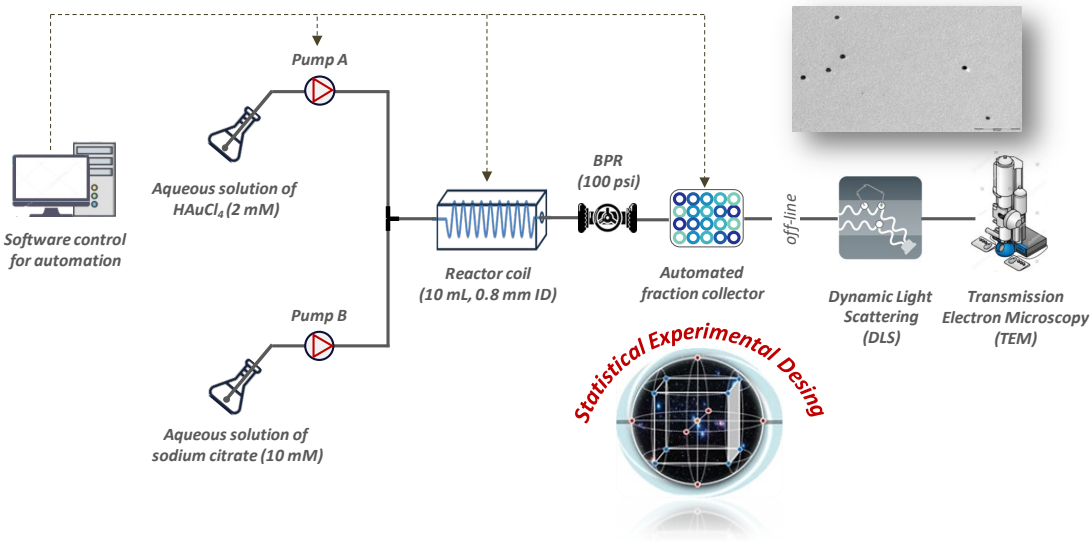

Figure 1. Preparation of gold nanoparticles (Au NPs) by Turkevich method under flow conditions.

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Potent reversible inhibitors of Histone Lysine Demethylase KDM1A/LSD1 efficacious in Acute Myeloid Leukemia models

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Lysine-specific demethylase 1 (LSD1 or KDM1A) is a FAD-dependent monoaminoxidase enzyme that acts as a transcription corepressor or coactivator by regulating the methylation status of histone H3 lysines K4 and K9, respectively.

Several lines of evidences, which include over-expression in solid and hematological malignancies[1],[2] as well as the correlation in certain tumor types between over-expression and poor prognosis,[3] indicate KDM1A as an attractive target for cancer therapy.

While, in the past, the main medicinal chemistry strategy toward KDM1A inhibition was based on the optimization of ligands that irreversibly bind the FAD cofactor within the enzyme catalytic site, the interest in finding reversible KDM1A inhibitors has recently grown and we have also contributed to the field by reporting the identification of thieno[3,2-b]pyrrole-5-carboxamides as reversible inhibitors of KDM1A.[4] The SAR exploration around these derivatives led to the discovery of a series of imidazoles endowed with picomolar inhibitory biochemical activity. [5]

Here we report on the identification of these imidazole KDM1A inhibitors (Figure 1), their preliminary SAR supported by ligand/LSD1-CoRest co-crystal structures, and the characterization of their biological activity up to evidence of *in vivo* efficacy in murine leukemia models.

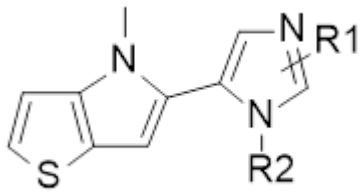

Figure 1

Acknowledgments: this research was supported by Rasna Therapeutics, Inc. and by a generous donation from Ravelli Family.

Authors information: the work by A. R., A. C., P. V., S. B., M.R. C., R. D.Z., G. F., R. F., L. S., P. T., S. V., C. M. and M. V. was performed in part at the European Institute of Oncology IRCCS, Academic Drug Discovery.

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Identification of novel pyrazole derivatives as inhibitors of lysine acetyltransferase p300

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Lysine acetylation is far from being fully understood, even after 50 years from its discovery.[1] Lysine acetyltransferases (KATs) are responsible of this chemical modification, that neutralizes the positive charge of lysine side chains, weakening the interactions between histones and DNA and, as a result, making chromosomal DNA more accessible.[2] Among the nuclear KATs, the acetyltransferase p300 (KAT3B) plays key roles in different cellular processes, implying that the dysregulation of its activity leads to many human diseases, including cancer.[3, 4] These observations suggest that p300 might represents a potential anticancer therapeutic target.

Starting from an Inverse Virtual Screening study, we recently identified the Cannabinoid Receptor 1 (CB1) inverse agonist Rimonabant as a new potential p300 inhibitor and confirmed this outcome in in cell and in vivo studies.[5] Therefore, with the aim to identify new p300 modulators, a computational model was developed and a first series of pyrazole derivatives synthesized. Prompted by our interest in the discovery of small molecule modulators of epigenetic targets, after further structural optimization (Figure 1) here we report the identification of a new series of p300 inhibitors, that might represent new opportunities to investigate the role of this protein in chromatin biology and drug discovery.

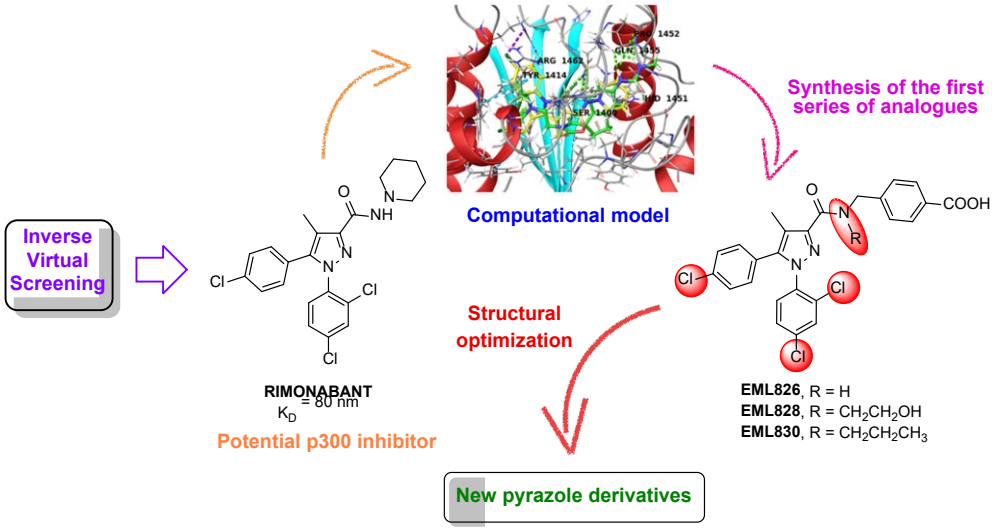

Figure 1.

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Targeting the αvβ6 RGD Integrin: from Computer-Aided Drug Design to Preclinical Applications

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The αvβ6 integrin has recently emerged as a prominent target for the treatment and diagnosis of several tumors and idiopathic pulmonary fibrosis.[1] Therefore, selective, low molecular weight ligands of this receptor are in great demand. Here, a metadynamics[2]-assisted design strategy was successfully applied to transform a linear helical nonapeptide[3] into a cyclic pentapeptide endowed with remarkable αvβ6 potency and specificity. NMR and docking studies elucidated the molecular basis of the affinity and selectivity of this compound, paving the way for the rational design of new αvβ6-specific ligands. Finally, our peptide was developed into a highly efficient PET tracer for specific αvβ6 *in vivo* imaging, prompting future clinical applications.

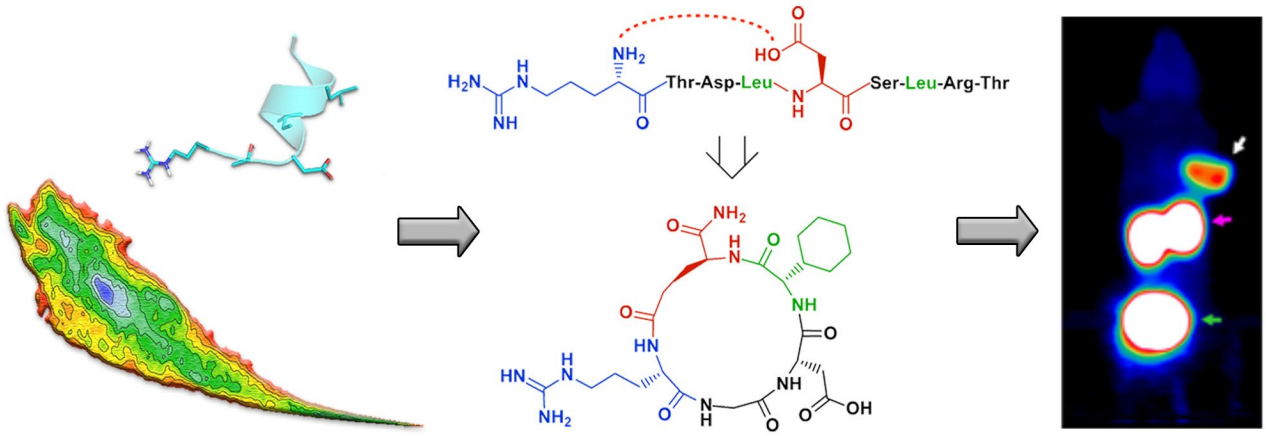

Figure 1. From metadynamics-inspired design to in vivo application of a cyclic pentapeptide as potent and selective αvβ6 integrin ligand.

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Potent, efficacious, and stable cyclic opioid peptides with long lasting antinociceptive effect after peripheral administration

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Four novel fluorinated cyclic analogues of biphalin with excellent to modest binding affinity for μ-, δ-, and κ-receptors were synthesized.[1] The cyclic peptides have a combination of piperazine or hydrazine linker with or without a xylene bridge (Figure 1). Among the ligands, **MACE3** demonstrated a better activity than biphalin after intravenous administration, and its corresponding analogue incorporating the hydrazine linker (**MACE2**) was able to induce longer lasting analgesia following subcutaneous administration. An analogue of **MACE2** containing 2,6dimethyl-L-tyrosine (**MACE4**) showed the best potency and *in vivo* antinociceptive activity of this series.

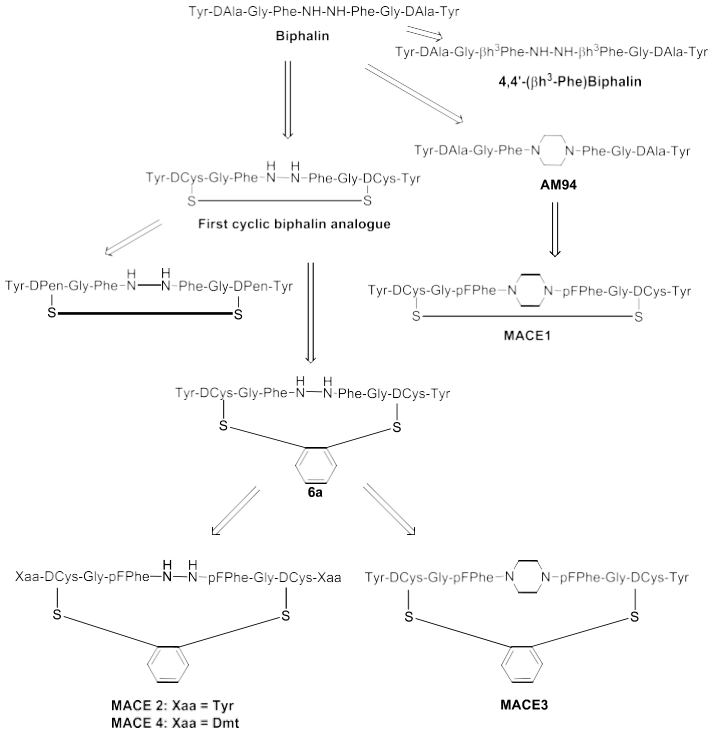

Figure 1. Biphalin structural modifications.

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Poster presentations

Design and synthesis of new constrained GEBR library compounds as potential PDE4D inhibitors

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In the past we developed a panel of compounds (GEBR library, Fig. 1) to obtain selective inhibitors against phosphodiesterase type 4 isoforms (PDE4D) for the treatment of neurodegenerative disorders. [1,2] In those molecules, we identified three essential pharmacophore substructures: 1) a catechol portion (that interact with the Q2 pocket of the catalytic domain); 2) different linker; 3) a basic end. Crystallographic studies evidenced three ligand conformation for the tail portion: twisted, extended and protruding, [3] the latter being the most promising candidates for further investigation. On the basis of our crystallographic information, and also taking into account the recent evidences appeared in the literature, we projected a new series of molecules in which the catechol portion has been replaced by a bulkier 8-methoxy-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine moiety, in order to optimize the interaction with the highly polar groove in the lower part of the catalytic pocket. The new scaffold, never investigated among the pletora of PDE4 inhibitors appeared in the last 25 years, maintains the two oxygen atoms necessary for the interaction with Gln 535 of the Q pocket and, in the meanwhile, the additional NH group could exploit a new type of polar interaction. To maintain the possibility of directing the tail portion out of the catalytic pocket, and therefore to assume the protruding conformation, we synthesized three different series (**1**-**3**, Fig. 1), using the same flexible and polar linkers of previous compounds.
Synthesis of these compounds will be reported in the poster session.

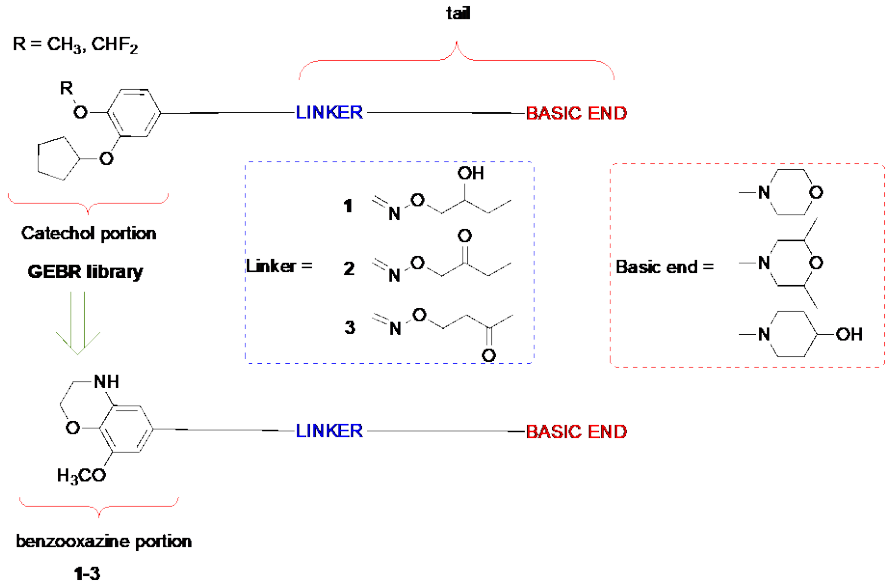

Figure 1. Structure of previous GEBR library and new synthesized compounds **1**-**3**.

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The Antioxidant Capability of Higenamine: Insights from Theory

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Higenamine is a plant-based alkaloid belonging to the structural class of protoberberines. It is present in many plants such as *Aconitum japonicum* and *Aconitum carmichaelii.* It shows a multi-target profile which led researchers to focus on the mechanisms and on the pathways implicated in higenamine action in different diseases. Recent literature indicates that the common denominator could be its antioxidant activity against reactive oxygen species (ROS). For all these reasons and in order to rationalize the existing experimental results,[1] we decided to carefully investigate the antioxidant properties of higenamine by employing a density functional theory (DFT)-based computational protocol previously and successfully used for a series of natural antioxidants. Different reaction mechanisms such as hydrogen atom transfer (HAT), radical adduct formation (RAF), single-electron transfer (SET) were considered for the reaction of higenamine with the $^{\bullet}$OOH radical. The pH values and the molar fraction at physiological pH were determined in aqueous solution. The results show that the preferred reaction mechanism was the hydrogen atom transfer from the catecholic ring. The computed kinetic constants calculated in aqueous and lipid-like environments revealed that, in order to obtain reliable results, it is important to consider all the species present in water solution derived from acid–base equilibria. From the present investigation, it emerges that at physiological pH (7.4), the scavenging activity of higenamine against the $^{\bullet}$OOH radical is higher than that of Trolox, chosen as a reference antioxidant (**Figure 1**). Furthermore, higenamine results to be more efficient for that purpose than melatonin and caffeine, whose protective action against oxidative stress is frequently associated with their reactive oxygen species (ROS) scavenging activity.[2]

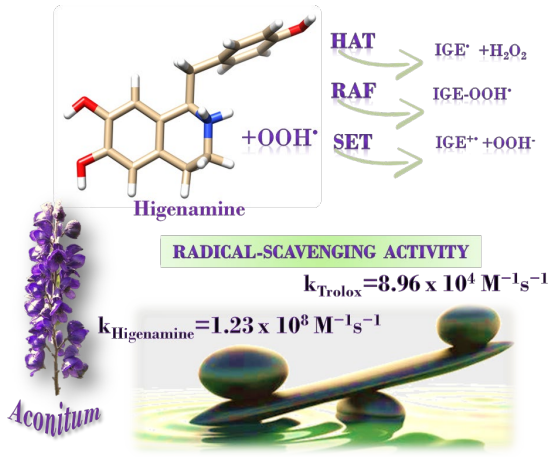

Figure 1. Schematic representation of the considered mechanisms for Higenamine. HAT, hydrogen atom transfer, RAF, radical adduct formation, SET, single-electron transfer.

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Conformational equilibria of N-anilinoethylamides for perspective design of melatonergic agonists

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N-anilinoethylamides are a class of nonselective nanomolar melatonin receptor ligands obtained through the bioisosteric replacement of the melatonin indole ring with an aniline portion [1]. A methyl group was inserted on the methylenes of the ethylamide chain, to investigate the effect on the biological activity and on conformational equilibria. Binding affinity of newly synthesized compounds was evaluated on human MT_1 and MT_2 receptors stably expressed in NIH3T3 cells, using 2-[^{125}I]iodomelatonin as radioligand. While methylation of the α carbon decreased MT_1 and MT_2 binding affinity compared to the unsubstituted precursor, substitution of the β carbon provided a couple of potent enantiomers with ten-fold stereoselectivity. When docked into the MT_2 receptor crystal structure, the two enantiomers were able to accommodate the methyl group in the same region of the binding site, assuming different conformations of the ethylamide side chain.

Molecular dynamics simulations of the two enantiomers in solution evidenced the presence of a set of preferred conformations (Figure 1), in agreement with results obtained from experimental NMR spectroscopy. The bioactive conformation of the eutomer was the most abundant in solution, while the conformation of the distomer able to bind the receptor was characterized by a higher energy content and a lower abundance in solution.

This finding suggests that the β-methyl group is able to induce a conformational enrichment of the bioactive conformation of the eutomer, identified through docking and, possibly, stabilized at the membrane interface, consistent with the stereoselective behavior observed for the two enantiomers.

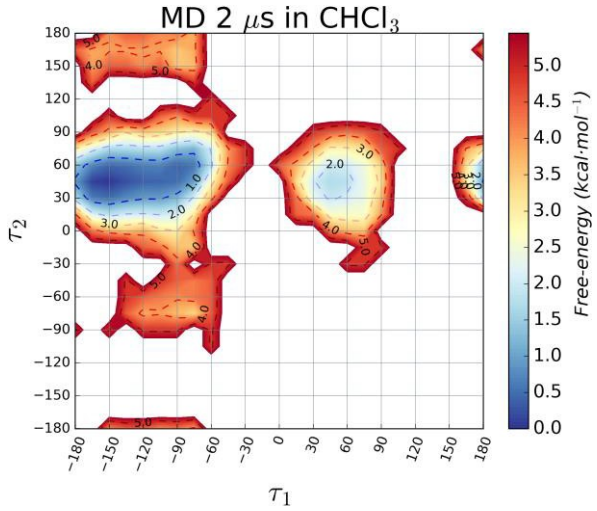

Figure 1. Free-energy surface of the beta-methyl N- anilinoethylamide eutomer calculated from a molecular dynamics simulation.

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Transfer learning for protein-ligand binding kinetics prediction

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Predicting binding kinetics of a protein-ligand complex is a challenging interdisciplinary task that has potential in lead optimization. Hypothesis that longer protein-ligand residence times increase in vivo drug efficacy [1], motivated the kinetics-based lead optimization and search for the key determinants that could affect binding mechanisms by applying various experimental and computational approaches including machine learning. Currently there is a plenty of factors that bottleneck the application of high-throughput approaches for this task: lack of high-quality database, poor generalizability of the prediction models on external data and poor performance while training on widely available descriptors.

Previous exploratory works in this problem domain trained machine learning models such as support vector machines or partial least squares regression on molecular dynamics-based descriptors [1, 2, 3], we tried to overcome the challenge of low amount of data by applying a transfer learning approach utilizing 3D deep convolutional neural network architecture (3D-CNNs), using two datasets, namely PDBbind v.2016 core set [4] for initial learning of the network weights and our own curated binding kinetics dataset for rate constants prediction. We show that correlation between dissociation constant, pK_D and dissociation rate constant, pk_{off} values for the protein-ligand complexes in the dataset justifies the use of binding affinity data for knowledge transfer. To adequately evaluate the performance of our model, we conducted 5-fold cross-validation and test on 20% of the database.

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A bicyclic α-iminophosphonate improves cognitive decline in 5xFAD murine model

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Imidazoline I_2 receptors (I_2-IR) are widely distributed in the CNS and appear increased in the patients that suffer from Alzheimer's disease (AD). Since structural data for I_2-IR remains unknown, the discovery of selective I_2-IR ligands is necessary for their pharmacological characterization. In this framework, we have recently contributed with a new structural family of I_2-IR ligands and reported their beneficial biological activities improving the cognition of a murine model of neurodegeneration in senescence-accelerated prone mouses (SAMP8) [1,2]. Herein, we present the synthesis and pharmacological profile of a new family of bicyclic α-iminophosphonates endowed with high affinities for human brain I_2-IR (Figure 1). 37 Bicyclic α-iminophosphonates were prepared via diastereoselective silver catalyzed [3+2] cycloaddition reaction by using α-substituted PhosMic derivatives and diversely *N*-substituted maleimides. 3D-QSAR, DMPK and physicochemical parameters were calculated in order to rule out warnings of the family. Then, we performed preliminary *in vitro* drug metabolism and safety studies, and *in vivo* pharmacokinetics for a selected compound, B06. *In vivo* studies in a murine model of familial AD (5xFAD) revealed beneficial effects in behavior and cognition. These results are supported by changes in AD biomarkers [3,4]. To sum up, modulation of I_2-IR by bicyclic α-iminophosphonates may open a new therapeutic avenue for unmet neurodegenerative conditions.

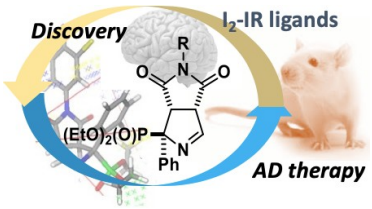

Figure 1. Bicyclic α-iminophosphonates as high affinity I_2-IR ligands

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Quinolinonyl non-diketo acid derivatives as inhibitors of the HIV-1 ribonuclease H function of reverse transcriptase

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The AIDS is a complex of pathological manifestations characterized by progressive degeneration of the immune system caused by the HIV virus. An essential enzyme for the retroviral life cycle is reverse transcriptase (RT), an heterodimeric enzyme with two associated activities: the DNA polymerase and the ribonuclease H (RNase H) activity that selectively degrades the RNA strand of the hybrid RNA/DNA formed during the reverse transcription.[1] Despite such armamentarium, both acute and chronic toxicities limit the prolonged use of several antiretroviral agents. In addition, the selection of drug-resistant strains and the spreading of such strains in newly infected patients is also an increasing concern, underscoring the pressing demand of novel anti-HIV agents, possibly targeting viral functions not yet explored.[2] In such a scenario, an attractive target turns out to be the RNase H function, which has been little explored although it could be potentially vulnerable to a specific inhibition.[3] Although RT is a multifunctional enzyme, all RT inhibitors currently approved for the treatment of HIV infection target only the RT-associated polymerase function, while none of them block the RNase H activity. Nevertheless, several studies have demonstrated that the abolition of the HIV-1 RNase H function stops the virus replication, proving to be, therefore, a validated and attractive target for the development of new anti-retroviral agents, in order to enhance the anti-HIV-1 drug armamentarium effectiveness. Despite this, it has been little explored and it needs to be further developed through the support of new HIV/AIDS drug discovery programs, in order to identify more efficient anti-HIV drugs that could be used for therapy. To date, only few compounds have been described to inhibit the HIV-1 RNase H function. Among them, aryldiketo acid derivatives proven to inhibit both integrase enzyme and RNase H function of the RT. Pursuing our studies on DKA derivatives as dual inhibitors of IN and RNase H,[4-6] we decided to apply an isosteric approach to our previously reported anti-HIV-1 quinolonyl DKA derivatives to obtain a new class of non-DKA compounds as RNase H inhibitors endowed with antiviral activity. The data coming from the biological assays will be shown and discussed.

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Multimatrix therapeutic drug monitoring of patients under antidepressant treatment

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Patients with eating disorders (ED) are affected by comorbidities involving the psychic and physical sphere of individuals. To treat ED-related anxiety and depression, reduce binge-eating behaviours and vomiting, new-generation antidepressants are often prescribed. Moreover, especially in case of polypharmacy regimens, to overcome suspected non-compliance and adverse effects, a frequent therapeutic drug monitoring (TDM) is necessary to evaluate drug and main metabolite concentrations in different biological fluids.[1] In order to have an overall assessment of patient clinical outcomes and being able to personalise pharmacological therapies, in this research work whole blood and oral fluid were investigated as complementary matrices. To make biological fluid collection less invasive and more frequent and feasible, an innovative and promising dried microsampling approach, i.e. volumetric absorptive microsampling (VAMS), was proposed to patients under treatment with fluvoxamine, an antidepressant drug belonging to selective serotonin reuptake inhibitors (SSRIs). VAMS strategy allows to collect an accurate and high reproducible micro-volume of biological fluid, regardless of its density in a minimally invasive way directly from a fingerprick. Moreover, the drying step takes place at room temperature, as well as transport and storage. Finally, stability profiles of dried microsamples are usually comparable with respect to biological fluids stored at controlled temperature.[2] An original analytical workflow based on capillary whole blood and oral fluid VAMS sampling, followed by miniaturised pretreatment procedure by means of microextraction by packed sorbent (MEPS) and a sensitive LC-UV-MS/MS method, was developed and optimised with respect to all the main parameters involved from sampling to instrumental analysis, granting satisfactory validation results (extraction yield > 84%, precision RSD < 8.9%, stability > 85.0% after 3 months). The validated miniaturised approaches were successfully applied to the analysis of blood and oral fluid collected from patients undergoing fluvoxamine treatment. Then, quali-quantitative evaluations were made between the results obtained from both matrices and between drug dosage and concentrations in whole blood and oral fluid, as different but complementary biological matrices with the aim of having an overall assessment of the drug. In order to demonstrate the reliability of the proposed strategies, not commonly used for TDM purposes, comparisons were also made with respect to fluid matrices subjected to standard reference procedures, showing good agreement. The developed methodology has proved to be suitable for the TDM of patients affected by ED and under antidepressant drug therapy and it could be proposed as self-sampling procedure aimed to reach a better personalisation of pharmacological therapies, with the perspective of a patient-centric precision medicine.

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Identification of selective TERRA-RNA G-quadruplex binders through computational techniques.

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The telomeric repeat-containing RNA, known as TERRA, is a long non-coding RNA transcribed from telomeres in mammalian cells. TERRA is a G-rich RNA sequence, characterized by a non-canonical structure such as G-quadruplexes (G4s) and it can interact with telomerase, resulting in the inhibition of this enzyme *in vitro* (1-2). In light of this, TERRA binders can be an innovative strategy for cancer treatment. A previous Structure-based Virtual Screening (SBVS) on bimolecular DNA/RNA G4 leads to the discovery of three best candidates (3). Currently, these promising *hits* have also been studied through molecular docking simulations on the G4 Tel monomolecular DNA (Tel) and TERRA (TERRA). The computational protocol, which included homology modeling, molecular dynamics and docking studies, provided the selectivity of two compounds towards TERRA with respect to the DNA counterpart, while the third *hit* acts as dual Tel/TERRA G4-ligand (Figure 1). Biophysical and biological assayes are in progress with the aim to confirm computation predictions and to characterized their potential anti-cancer profile.

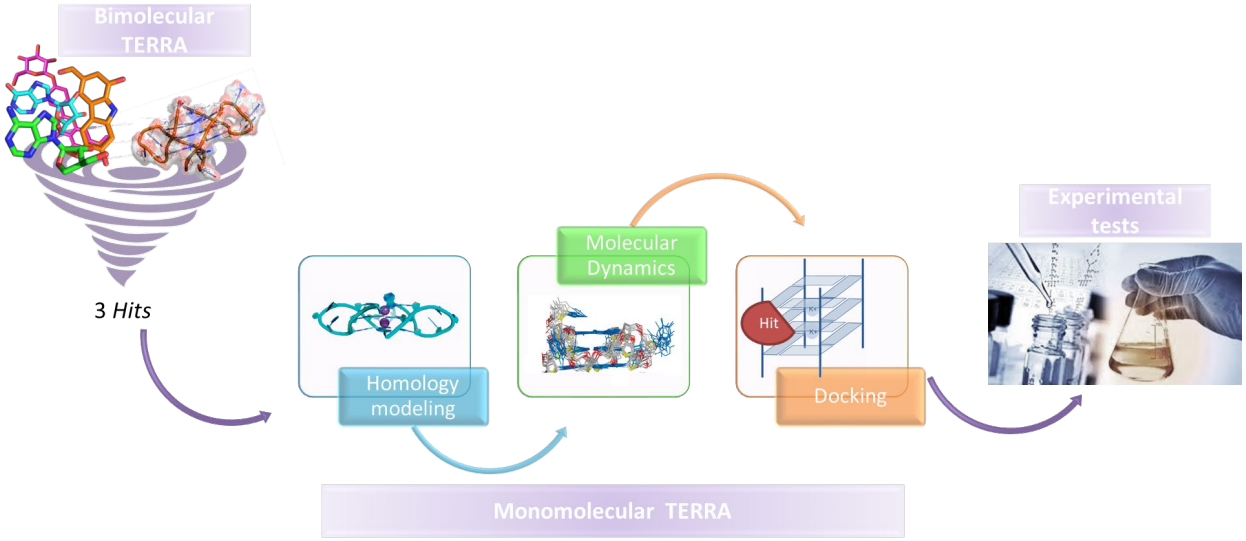

Figure 1. Workflow for the discovery of selective monomolecular TERRA binders.

Acknowledgment: This work was supported by Italian Association for Cancer Research (AIRC) research project "Small molecule-based targeting of lncRNAs 3D structure: a translational platform for the treatment of multiple myeloma" (code 21588).

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Efficient and sustainable synthesis of a potent and selective 5-HT$_7$ receptor antagonist using a mechanochemical approach

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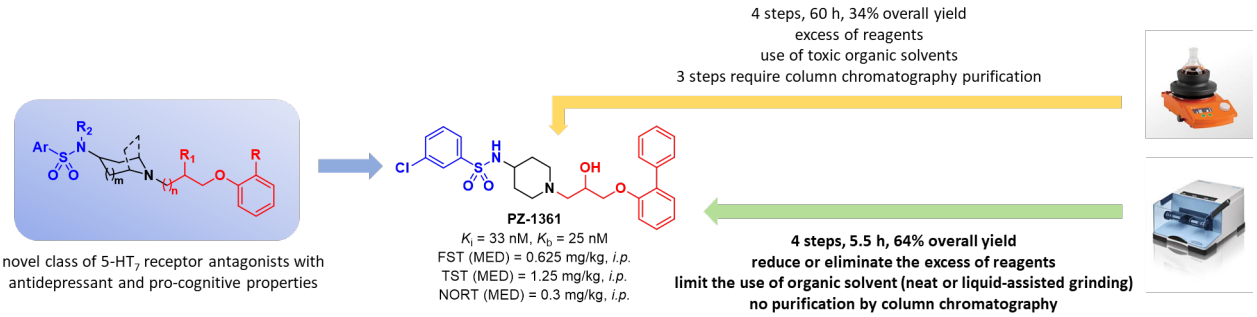
Recently, mechanochemical synthesis has been recognized as an innovative and efficient methodology [1], and has a hot topic of study in both academic and industrial research. The primary driving force underlying the rediscovery of mechanochemistry is green chemistry,[2] in particular the need of chemical and pharmaceutical industries for the development of more sustainable synthetic protocols characterized by energy efficiency of chemical transformations and reduction of solvent use. The use of such approaches offers additional advantages of mechanosynthesis over classical organic chemistry, in terms of excellent selectivity and the possibility to perform previously unknown reactions. Interestingly, an increasing number of mechanochemical procedures for generating pharmaceutically relevant fragments and functionalities led to coining the term “medicinal mechanochemistry”[3].



Here, an efficient and sustainable mechanochemical procedure was developed to obtain **PZ-1361**, a potent and selective 5-HT$_7$ receptor antagonist, with significant antidepressant properties in rodents [4]. The elaborated protocol offered several advantages over classical thermal methods in solution, including improvement of the overall yield (from 34% to 64%), reduction of reaction time (from 60 h to 5.5 h), limitation of the use of toxic solvents and the formation of by-products. The versatility of the protocol was additionally confirmed by introducing diversification at the aryloxy fragment, central amine core, and the benzenesulfonamide moiety. This approach represents a rare example of organic synthesis of biologically active compounds exclusively performed using mechanochemical and solid/gas reactions [5].

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Precision targeting of a mitochondrial channel in intruder cells within the brain. A new approach to brain tumors

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The voltage-gated potassium channels $K_v1.3$ located in the inner mitochondrial membrane plays important roles in cancer. High expression of this channel has been found in several cancers such as melanoma, pancreatic cancer (PDAC), glioblastoma and neuroblastoma. Inhibition of mito$K_v1.3$ leads to pro-apoptotic processes, exploited through the design of selective inhibitors. [1] PAPTP is one of these novel inhibitors of mito$K_v1.3$. It drastically reduces tumor growth in mouse models of melanoma and PDAC, without severe side effects. [2] Unfortunately, while effective against glioma cells in vitro, it turned out to be inactive in vivo upon systemic administration, because it is not able to cross the blood brain barrier (BBB). [3]

One of the approaches to overcome the BBB reported in the literature relies on cell-penetrating peptides (CPPs), short sequences of amino acids capable of crossing biological membranes and of delivering active drugs. Angiopep2 (TFFYGGSRGKRNNFKTEEY) and TAT (GRKKRRQRRRPPQG) are among the most studied for brain delivery. [4-6]

The aim of this study is to synthesize Angiopep2-PAPTP and TAT-PAPTP and to evaluate their absorption into the brain *in vivo*. The conjugates were designed using the pro-drug approach. To obtain this, the TPP^+ moiety of PAPTP was modified adding a linker to one of the phenyl groups (PAPTPL) and then conjugated to the CPPs through a bio-reversible carbamate bond. TAT-PAPTP and Angiopep2-PAPTP were both tested in vivo. TAT-PAPTP was abandoned because of evident toxicity signs. Angiopep2-PAPTP was administered to the mice (5 µmol/kg BW) and the mice were sacrificed after 15 (n=5), 30 (n=4) and 60 (n=6) minutes.

The results show that Angiopep2-PAPTP is able to cross the BBB, showing its presence in the brain at 15 and 30 minutes after the injection. The analysis of the liver showed that Angiopep2-PAPTP is mainly metabolized through the cleavage of the peptide chain. In particular, PAPTPL plus the first one, two or three amino acids of the chain were identified via HPLC/MS analysis.

Summarizing, conjugation to Angiopep2 represents a viable strategy to deliver PAPTP to the brain, overcoming the barrier which completely prevents its access when administered as such.

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Synthesis and preliminary docking evaluation of novel 1-benzyl-N-(4-(4-arylpiperazin-1-yl)phenyl)piperidin-4-amines, as potential acetylcholinesterase inhibitors

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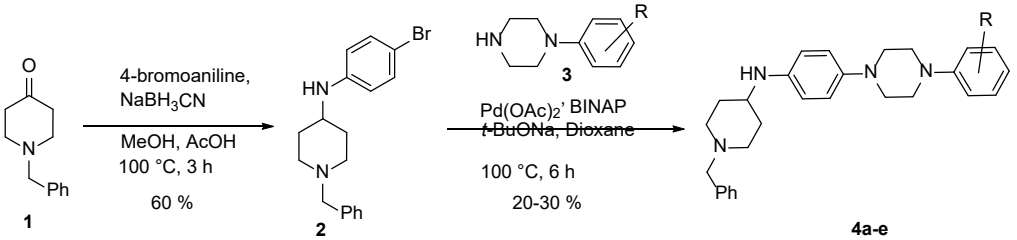
Acetylcholinesterase inhibitors (AChEIs) represent an important class of compounds used for the treatment of Alzheimer's disease symptoms. [1]
As a part of our ongoing research on functionalized piperidines, we designed and synthesized five novel compounds (**4a-e**) that contain *N*-benzyl piperidine and *N*-aryl piperazine moieties, as potential AChEIs.
The synthetic route encompasses two steps (Scheme 1) starting from *N*-benzyl piperidone **1**. Conversion of **1** to anilinopiperidine **2** by reductive amination, followed by the Buchwald-Hartwig coupling of **2** and substituted arylpiperazines **3** afforded final products (**4a-e**).



4a, R=H; **4b**, R=2-OMe; **4c**, R=2,3-Cl; **4d**, R=3-CF_3; **4e**, R=2,3-Me

Scheme 1. Synthesis of novel 1-benzyl-N-(4-(4-arylpiperazin-1-yl)phenyl)piperidin-4-amines **4a-e**

Preliminary docking evaluation revealed that all compounds bind to the active site of acetylcholinesterase (AChE) in similar manner as donepezil, a drug used clinically to alleviate symptoms of Alzheimer's disease. The crystaline structure of donepezil-AChE complex has been published. [2] As evident from Fig. 1, the compound **4a**, a representative of the group, fits well in the AChE active site, forming key stacking interactions against Trp86, Trp286 and Tyr341.[2]

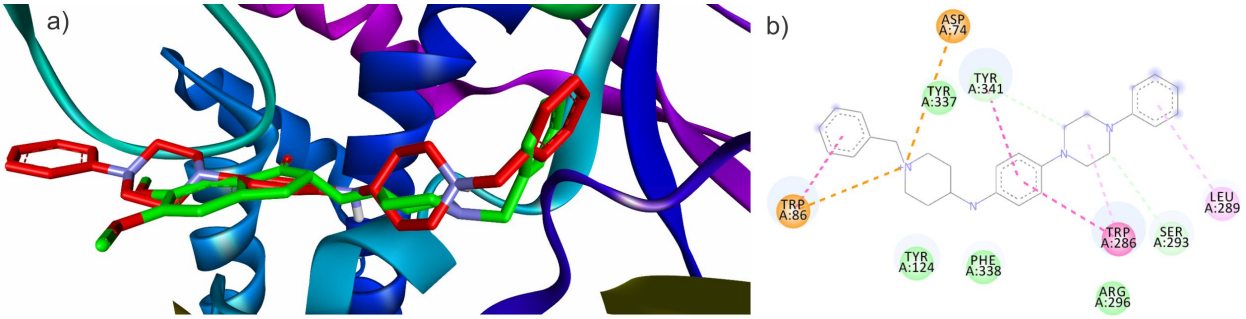


Figure 1. a) Orientation of **4a** in the active site of AchE (red), overlapped with donepezil (green, PDB: 4ey7); b) interactions of **4a** with the active site of AChE (PDB: 4ey7)

AChE inhibitory activity of **4a**-**e** will be assessed as a part of our ongoing work. Depending on the AChE inhibitory activities for the first series, synthesis of more new analogues of **4** will be implemented.

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Interaction of Zinc Metal Ion With Drug Molecules and the Binding Effect on a Transport Protein

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Using UV–Vis, FT-IR, fluorescence spectroscopy and protein-ligand docking, the interactions between the zinc complexes with drug analogues and bovine serum albumin were investigated. In addition, considering the ubiquitous presence of zinc ions in the human system, we studied the interactions between this ion with hymecromone, dihydropyridine analogue, and acetamide (Figure 1), as well as the pH influence on these systems. The complexes were synthesized by interaction between the ligands and the Zn (II) ion in a 2:1 M ratio. Elemental analysis, FT-IR, and UV–Vis spectroscopy studies investigated the structure of the synthesized complexes. Fluorescence spectroscopy, UV–Vis, molecular docking and molecular dynamics were used to study the interactions of the Zn complexes with the BSA. The drug-Zn (II) system's pH effect was investigated using UV–Vis spectroscopy. After the complexation with the zinc, the drug molecules exhibited higher apparent binding affinity to BSA[1]. BSA's fluorescence efficiency by the drug analogues was enhanced. In addition, molecular modelling was used to classify the residue of amino acids in the BSA playing key roles in this binding interaction. An increase in pH appears to contribute to alkaline hydrolysis of the Zn (II) molecules.

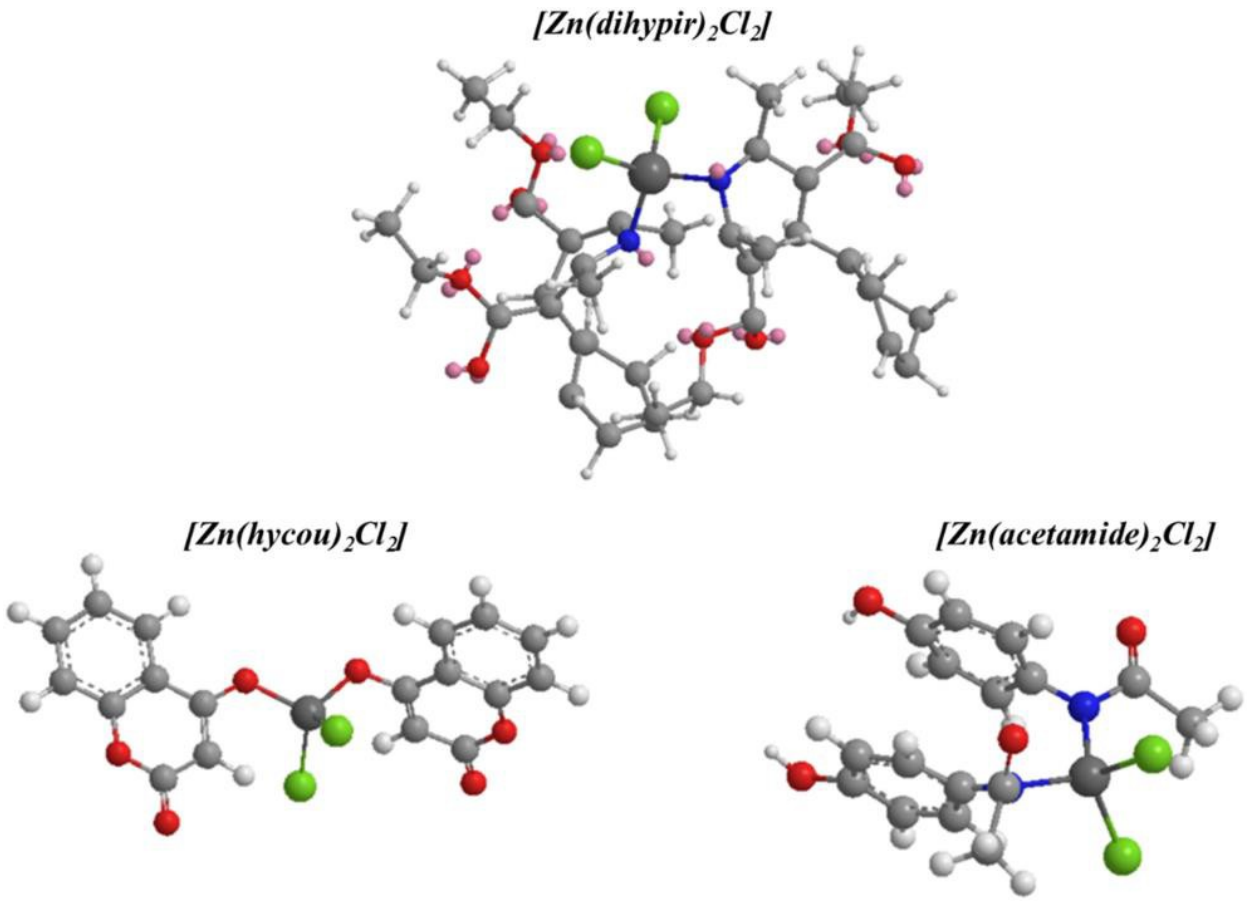

Figure 1. Formation of the complex Zn molecules with the drug analogues as ligands.

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Discovery of novel peptidomimetics containing a benzodiazepine scaffold as inhibitors of rhodesain of *Trypanosoma brucei rhodesiense*

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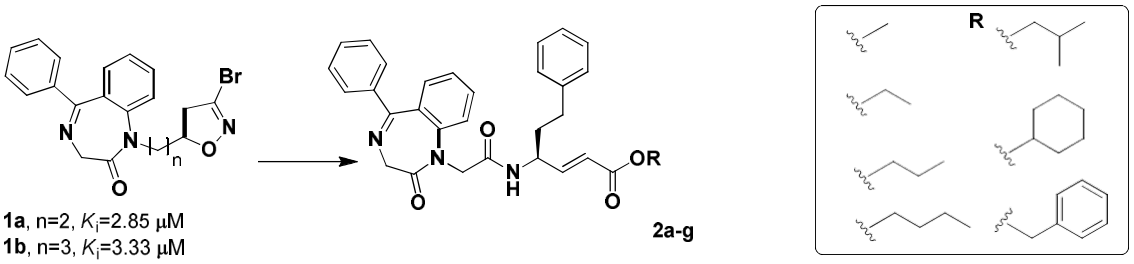
Human African Trypanosomiasis (HAT), well known also as sleeping sickness, is an infection caused by protozoa of Trypanosoma genus. There are two main forms of HAT: the chronic form caused by *Trypanosoma brucei gambiense*, particularly widespread in central and western Africa, and the acute form of HAT induced by *Trypanosoma brucei rhodesiense* occurring in eastern and southern Africa.[1] Due to problems related to the drugs currently available for HAT therapy, like toxicity, route of administration and limited spectrum of action, there is an urgent need to identify new targets for the development of novel drugs for HAT treatment. In this context rhodesain, the main cysteine protease of *Trypanosoma brucei rhodesiense*, due to its key functions, is currently considered an interesting target for the new drug discovery process.[2]

Our research team has been involved in the last decade into the development of potent rhodesain inhibitors. In particular, starting from the structure of the reversible rhodesain inhibitors **1a-c**, endowed with K_i values in the low micromolar range[3] (**Figure 1**), we designed a new series of peptidomimetics **2a-g** maintaining the benzodiazepine scaffold as a β-turn mimetic. We introduced a characteristic peptide sequence for rhodesain inhibition, i.e. Phe-HomoPhe P2-P1, finally the 3-bromoisoxazoline warhead was replaced with a vinyl ester moiety, able to react as Michael acceptor, while at the P1' site we selected a panel of aliphatic or aromatic nuclei to evaluate the size of the corresponding S1' pocket.[4] All the synthesized molecules were tested against rhodesain to evaluate their inhibitory properties, at the same time the peptidomimetics were docked into rhodesain binding site to study their binding mode. The results of this investigation will be reported and discussed.



1a, n=2, K_i=2.85 μM
1b, n=3, K_i=3.33 μM
1c, n=4, K_i=2.44 μM

Figure 1. Design of novel pepditomimetics **2a-g**.

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Development of a Microscale Thermophoresis-based Method for Screening and Characterizing Inhibitors of the Methyl Lysine Reader Protein MRG15.

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Among all the proteins containing methylation reader domains, MRG15 is a transcription factor with a crucial role in embryonic development, cell proliferation and cellular senescence (Figure 1).[1, 2] Despite the involvement of this protein in different physiological and pathological states, to date its role has not been fully elucidated due to the lack of specific and potent chemical probe. Therefore, as for other reader proteins, the interest in developing small-molecule inhibitors is very high.[3]

Here we report the development of a Microscale Thermophoresis (MST) assay for the reader protein MRG15. First, the labeling procedure of the protein was optimized; subsequently the attention was focused on assay development, testing different buffers and the effect of stabilizing agents on the signal. Thus, the assay was validated using the reference compound UNC1215, and the screening of a small library of compounds was performed yielding to the identification of 10 compounds able to interact with the protein with affinities ranging from 37.8 nM to 59.1 µM.

Hence, MST turned out as a robust and fast method for the identification of new ligands of MRG15 and it could be applied for the identification of new chemical probes for other methylation reader domains.

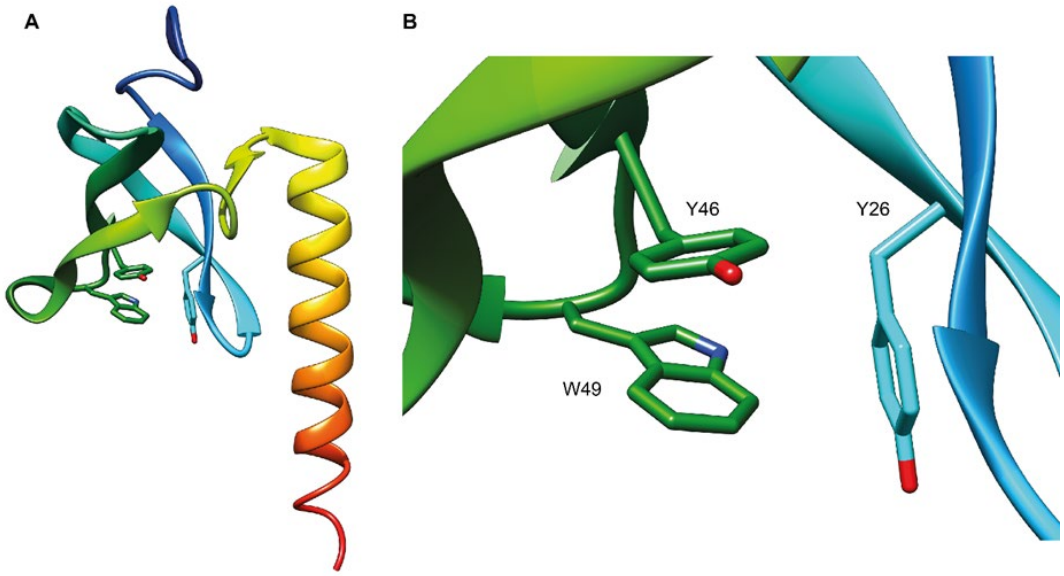

Figure 1. A) Crystal Structure of the human MRG15 chromodomain (PDB: 2F5K) with the detail (B) of the aromatic cage. The picture was prepared using UCSF Chimera (coordinates from Protein Data Bank, PDB ID code 2F5K).[4]

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Identification and full chemical characterization of novel phytocannabinoids isolated from a medical *Cannabis sativa* variety

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Cannabis sativa has been for a long time a neglected plant as it is the most widely spread illicit drug worldwide, especially among young adults. However, in the last decade, a renewed interest on Cannabis has arose in the scientific community, especially for its implication in the treatment of several pathologies including glaucoma and epilepsy, definitely becoming one of the most studied plants. Cannabis is the only plant able to produce a peculiar class of organic molecules called phytocannabinoids. The two most important and studied phytocannabinoids are undoubtedly cannabidiol (CBD), a non-psychotropic compound, but with other pharmacological properties including anti-inflammatory, anti-oxidant and anti-convulsant activity, and Δ^9-tetrahydrocannabinol (Δ^9-THC) which instead possesses psychotropic activity and it is responsible for the recreative use of hemp. Beside CBD and THC, at present, almost 150 phytocannabinoids have been detected in the cannabis plant, although few have been isolated and studied. However, the analysis performed by our research group on a medicinal cannabis variety, the Italian FM2, provided by the Military Chemical Pharmaceutical Institute in Florence by means of UHPLC-HESI-Orbitrap, allowed us to identify some still unknown phytocannabinoids. In particular, the butyl homologues of CBD (called CBDB) and Δ^9-THC (called Δ^9-THCB) were isolated by semipreparative HPLC purification. For the first time, their absolute configurations were assigned, and their chemical and spectroscopic properties were thoroughly characterized and compared to those of authentic standards obtained via stereoselective synthesis.[1–3] Extensive pharmaceutic studies were performed on the two new identified THCs. The biological results obtained in the *in vitro* binding assay indicated an affinity for CB1 receptor comparable (K_i=15 nM, for Δ^9-THCB)[3] to the one reported for Δ^9-THC in the literature. Docking studies confirmed the importance of the length of the alkyl chain on the resorcinyl moiety for CB1 binding affinity.
In vivo formalin test was performed on Δ^9-THCB revealing potential analgesic and anti-inflammatory properties.[3] The tetrad test in mice showed a partial agonistic activity of Δ^9-THCB toward the CB1 receptor. Ongoing studies are devoted to the investigation of the pharmacological activity of CBDB and to expand that of Δ^9-THCB. These new identified phytocannabinoids are present in traces and therefore they do not represent a threat for the human health. However, these remarkable works highlight how much there is to still learn about the properties of cannabis paving the way to pharmacologists, toxicologists, and clinicians to new therapeutic substances and to correlate the observed biological effects with the chemical composition of the different cannabis varieties employed.

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Efflux Pump Inhibitors revert Drug Resistance in MDR-*Mycobacterium Tuberculosis* strains

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Mycobacterium Tuberculosis (MTB) is nowadays the first cause of death from a single agent, according to the World Health Organization (WHO). It can affect the lungs causing pulmonary tuberculosis (TB), or other organs, causing extrapulmonary TB. In recent years, about 10 million people fell ill with TB every year and in 2018 about 1.2 million TB deaths were registered worldwide. [1] Drug-resistant MTBs continue threating global health with an increasing number of MTB strains which become drug resistant, multi drug-resistant or extensively drug-resistant (DR, MDR and XDR respectively). [1] MTB strains develop spontaneous gene mutations that provide the bacteria resistance to the anti-TB drugs. Reduction of drug affinity for the target is caused by mutations in the gene coding for the drug target. [2] Continued exposure of MTB cells to anti-TB drugs also results in upregulation of the efflux pump system genes, leading to an increased number of efflux pumps (EPs) on the cell membrane. [3] Efflux pump inhibition represents a valid strategy in anti-TB research: partial suppression of the drug extrusion results in intracellular therapeutic concentration restored or even increased.

A new series of 3-phenoxymethyl-quinoxaline derivatives (PMQs) has been designed, synthesised and investigated as extrusion pump inhibitors (EPIs) against MDR-MTB strains. Nine clinical strains of MTB, two non-tubercular mycobacteria (NTM) clinical strains, and *M*. *Tuberculosis* H37Rv as reference, were used in REMA assays, testing first and second line antituberculosis drugs both in presence and absence of our PMQs, evaluating how EPIs can impact the drug MICs (minimal inhibitory concentrations) 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 MTB and NTM, and that the inhibition of efflux pumps by the PMQs can enhance the clinical effect of antibiotics.

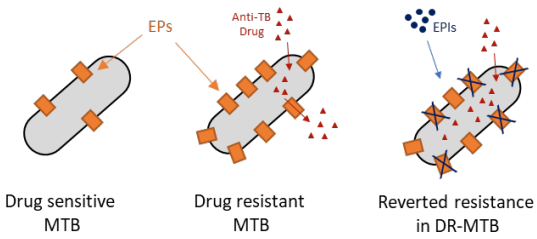

Figure 1. EPIs mechanism of action.

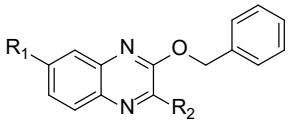

Figure 2. 3-Phenoxymethyl-quinoxaline derivatives (PMQs) general structure.

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Discovery of new potential anti-tyrosinase agents through ligand-based pharmacophore strategy focused on competitive inhibitors.

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Melanogenesis represents the biosynthetic pathway of melanin pigments, which play a pivotal role in the protection of skin against the harmful effects of UV radiation. However, melanin overproduction is related to various hyperpigmentary disorders such as melasma, freckles and melanoma.[1]
The first two steps of melanin biosynthesis are the oxidation of L-tyrosine in L-DOPA and its subsequent oxidation in dopaquinone.[1] These steps are the rate-limiting ones of the whole process of melanin synthesis and they are both catalyzed by tyrosinase, a type-3 copper enzyme whose active site is characterized by the presence of two copper ions each coordinated by three histidine residues.[2] Therefore, the inhibition of tyrosinase activity represents an efficient way for the development of depigmenting agents.
According to kinetic studies, several types of tyrosinase inhibitors have been identified and classified into: competitive, non-competive, uncompetitive and irreversible inhibitors.[3] Nevertheless, the ability of some of them to occupy the catalytic site of the enzyme is still unclear, due to the lack of sufficient crystallographic information.
In this research, 14 competitive mushroom tyrosinase inhibitors of diphenolase activity were selected from literature and employed for the creation of different ligand-based pharmacophores by using LigandScout software. The generated models were ranked and refined basing on the validation made through ROC curves creation.
The selected merged feature pharmacophore model (Figure 1) has been used for the virtual screening of libraries for the discovery of new tyrosinase inhibitors.
To get more insights about the ability of the retrieved hits to occupy tyrosinase active site, docking studies were carried out resulting in the identification of promising tyrosinase inhibitors that were purchased and for which biological evaluation is ongoing.

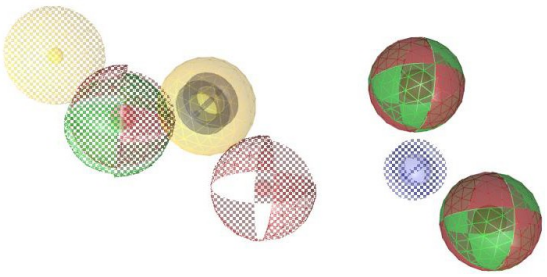

Figure 1 Representation of the selected merged feature pharmacophore used for the virtual screening procedure.

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Structure-based identification of P-glycoprotein inhibitors from natural sources

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ATP-binding cassette (ABC) transporters are a large family of proteins involved in membrane transport of a wide variety of substrates. Among them, ABCB1, also known as MDR-1 or P-glycoprotein (P-gp), is the most characterized. By exporting xenobiotics out of the cell, P-gp activity can affect the ADME properties of several drugs. Moreover, P-gp has been found to mediate multidrug resistance in cancer cells. Thus, the inhibition of P-gp activity may lead to increased absorption and/or intracellular accumulation of co-administered drugs, enhancing their effectiveness.[1]

While some attempts to perform structure-based discovery of P-gp inhibitors have been carried out, many of the currently known P-gp inhibitors were characterized exploiting ligand-based approaches, due to the lack of well-refined human crystal structures.[2]

However, recently human cryoEM structures, with and without co-crystallized ligands, have been resolved, giving more structural insights into the different conformations of the protein.[3]

Using the human cryoEM 3D structure of the P-gp in the inhibitor-bound intermediate form (PDBID: 6qee), we virtually screened approximately 200'000 commercially available natural compounds from the ZINC database.[4] Raccoon2 and AutoDock Vina were used for performing the molecular docking simulations.[5]

To build a model able to discriminate between substrate and inhibitors and to filter the virtual screening results, we also docked a dataset of 3250 compounds with known activity, including P-gp inhibitors and substrates, as well as inactive molecules. The Autodock Vina scoring function was shown to be able to enrich known binders over not binders, with more than 80% of the top quarter of docking results containing known binders. Then, 6 molecular descriptors available in DataWarrior were used to perform structural similarity clustering of known inhibitors and substrates independently.[6] Then, the most representative molecule for each cluster was used to generate 3D common pharmacophores, using their best docked pose in the P-gp binding site.[7] The pharmacophores were then used to re-score virtual screening results, and molecules matching all the inhibitors pharmacophores and none of the substrates pharmacophore were chosen for visual inspection.

With this consensus approach, we were able to identify 10 potential candidates which will be tested for their ability to inhibit P-gp.

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Computational and synthetic approaches for the development of α-synuclein aggregation inhibitors for the treatment of Parkinson's disease

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Parkinson's disease (PD) is one of the most common neurological disorders. It is characterized by the degeneration of dopaminergic neurons with the subsequent reduction of dopamine levels, leading to bradykinesia, muscular rigidity and postural instability. The hallmark of PD is represented by the presence of Lewy bodies, neuronal deposits mainly composed by aggragates of the presynaptic protein α-synuclein (α-syn). Currently, the therapy of PD includes the administration of drugs able to restore the level of dopamine thus reducing the motor symptoms. However these drugs are unable to slow or block the neurodegenerative process, therefore there is an increasing interest in the search for new and more effective therapeutic tools. In the last decade, the inhibition of α-syn aggregation turned out to be a promising disease-modifying strategy for reducing the neurodegeneration.[1,2] Herein, we described a rational approach applied for the identification of new α-syn aggregation inhibitors. In particular, we generated a ligand-based pharmacophore model (Figure1) which was used to screen two 3D libraries. The hits selected from the virtual screening were tested *in vitro* in order to probe their ability to disrupt α-syn aggregation, resulting in the identification of compound 3-(cinnamylsulfanyl)-5-(4-pyridyl)-1,2,4-triazol-4-amine as promising hit for the development of a new series. The designed compounds were synthesized and their capability to inhibit α-syn aggregation was studied *in vitro* leading to the identification of four interesting derivatives. Finally, the binding mode of this class of inhibitors was deciphered by means of molecular docking simulation.[3]

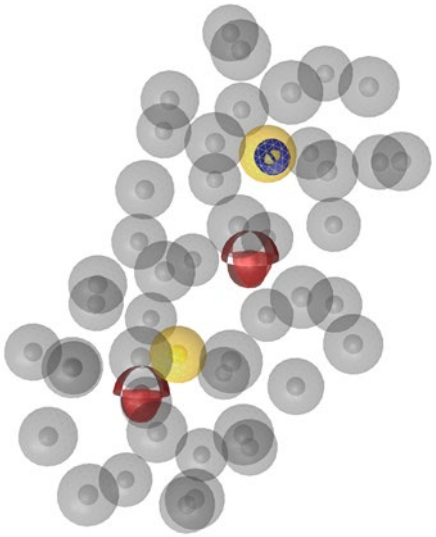

Figura 1 Ligand-based pharmacophore model

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Multi-Targeting Bioactive Compounds Extracted from Essential Oils as Kinase Inhibitors

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Traditionally, Essential Oils (EOs) have been used for their biological activities including antibacterial, antifungal, sedative, antioxidant, spasmolytic, carminative, hepatoprotective, and analgesic effects. Moreover, several studies reported EOs as potential anti-cancer agents by inducing apoptosis in different cancer cell models. In this study, we have considered EOs as a potential resource of new kinase inhibitors with a polypharmacological profile. In particular, we applied computational methods, which offer the possibility to predict the theoretical activity profile of ligands, discovering dangerous *off-targets* and/or synergistic effects due to the potential multi-target action. Therefore, we performed a Structure-Based Virtual Screening (SBVS) of EOs against X-ray models of several protein kinases selected from the Protein Data Bank (PDB) by using a chemoinformatics database. The evaluation of the theoretical binding affinity leads to select 13 molecules as new potential kinase inhibitors with a multi-target profile. The 2 *hits* with higher percentages in the EOs were studied more in depth by means Induced Fit Docking (IFD) protocol, in order to exhaustively consider possible binding modes and the associated conformational changes within receptor active sites. Finally, given its good binding affinity towards five different kinases, cinnamyl cinnamate was biologically tested on different cell lines with the aim to verify its antiproliferative activity (Figure 1). Thus, the next step will be the optimization of the most promising EOs structure as kinase inhibitors with multi-target features.[1]

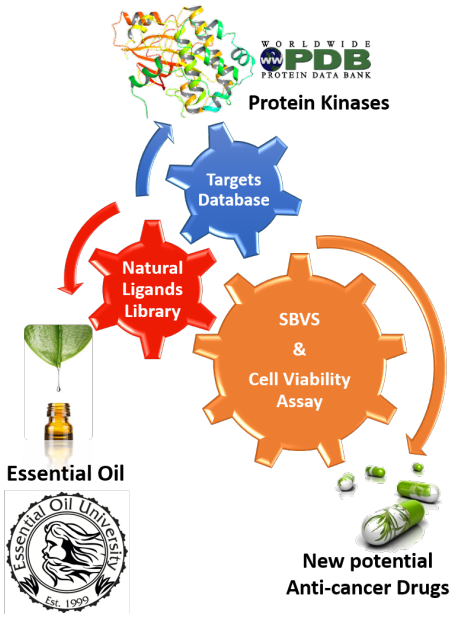

Figure 1. The principal steps for the discovery of multi-targeting bioactive compounds extracted from essential oils as kinase inhibitors.

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Molecular modeling of EmrE, multidrug transporter protein - studies by molecular dynamics simulation

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EmrE is a small multidrug resistance transporter found in *Escherichia coli* that confers resistance to toxic polyaromatic cations by proton coupling of these substrates. One of the mechanisms by which cells neutralize the action of toxic compounds is the action of membrane transporters [1]. Secondary transporters, such as the EmrE protein, combine the outflow of drugs with the internal movement of protons across the cell membrane [2]. EmrE is a prototype member of the small multidrug resistance (SMR) transporter family and is one of the smallest known transporters in nature, consisting of only 110 amino acid residues. Studies have shown that the basic functional unit of EmrE is an oligomer, as could be expected from a small size membrane protein. It seems that the basic functional unit of EmrE is the anti-parallel homodimer, as shown by oligomerization tests, substrate binding experiments, negative domination studies and cross-linking analysis [3].

The long term aim of this work is to find basic knowledge about molecular mechanism of action of this transporter in order to design in future potential inhibitors. In particular, the aim of current part of our work is to present and verify hypothesis regarding the mechanism of transport and the mechanism of recognizing toxic compounds by the EmrE protein using molecular dynamics methods. Furthermore because only a structure consisting purely of alpha carbons with poor resolution is available, this work also focuses on creating a reliable structure needed for simulation. The obtained results present different aspects of thermodynamic and structural properties of studied transporter with regard to their mechanism of action.

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Synthesis and biological evaluation of a new series of 5,6-difluorobenzotriazole-acrilonitrile derivatives as microtubule destabilising agents (MDAs)

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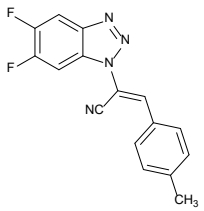
Cancer is one of the noncommunicable diseases (NCDs) and one of the main characteristics of a tumour cell is its uncontrolled division. A crucial element involved during the cell replication is the mitotic spindle, that is mainly composed by microtubules (MTs). An attractive approach to be considered during therapeutic agents designing is the interference with MT dynamic equilibrium. [1] Nowadays, microtubule targeting agents (MTAs) are some of the most effective drugs used in solid and haematological tumours treatments. Colchicine binding site inhibitors (CBSIs) are microtubule destabilising agents (MDAs): the main representatives are colchicine and combretastatins A-1 (CA-1) and A-4 (CA-4), which bind MT in the colchicine site (CBS) located on β-tubulin at its interface with α-tubulin. [2]
In the proposed study, we further explored structure-activity relationship on the previously synthesised 3-aryl-2-(1*H*(2*H*)-benzotriazol-1(2)-yl)acrylonitrile derivatives, already proved to be MDAs. The original structure was properly modified in order to increase the anticancer effect. [3] In order to elucidate the effect of a substituent on the phenyl portion of the main scaffold, in this paper we present a new series of (*E*)(*Z*)-2-(5,6-difluoro-(1*H*)2*H*-benzo[d][1,2,3]triazol-1(2)-yl)-3-(*R*)acrylonitrile derivatives which were synthesised, characterised and submitted to different biological assessments.
The first anti-cancer screening was performed *in vitro* through the NCI60 test (NCI, Bethesda, USA, https://dtp.cancer.gov). At the first stage of the assay, at 10 μM of tested compounds, four derivatives showed interesting scores fulfilling the pre-determined inhibition criteria. The compounds progressed to the second step, consisting in five serial dilution-doses assay. Compound **1a** (depicted in Scheme 1) was selected as lead compound of this series since it gave the best scores: it showed an average growth inhibition (GI) close to 100% against all leukemia cell lines. Generally, derivative **1a** exhibited GI ranging from 60% to 100% in all the sixty solid and haematological cell lines. Compound **1a** also showed a cytotoxic effect against seventeen solid and haematological tumor cell lines. At 1 μM, **1a** presented GI ranging from 70% to 100% against seven cell lines.
In order to evaluate if this class of compounds could be extruded by the efflux pumps, channel proteins often overexpressed in drug-resistant cancer cells, we tested the lead compound **1a** in a cell-based assay. Four cancer cell lines were selected with efflux pumps overexpression. [4] They were treated with compound **1a** together with one efflux pump inhibitor (EPI) from our library and it was beneficial in terms of antiproliferative activity of compound **1a**.



Scheme 1. Structure of lead compound **1a**.

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Chemical characterization of non-psychoactive *Cannabis sativa* L. extracts and evaluation of their antiproliferative activity

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Cannabis sativa L. is an annual cycle herbaceous plant belonging to the Cannabinaceae family. The main classes of compounds present in this plant are cannabinoids, terpenes and flavonoids [1]. Among cannabinoids, the most representative ones are Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA). These native acidic cannabinoids undergo a spontaneous decarboxylation under the action of light and heat, leading to the formation of their neutral counterparts, including Δ^9-tetrahydrocannabinolol (Δ^9-THC), cannabidiol (CBD), and cannabigerol (CBG). Non-psychoactive *C. sativa* (also known as hemp) is characterized by a high content of CBD and CBG, and a level of psychoactive Δ^9-THC lower than 0.2%. CBD displays several biological activities related to the action on different targets. Recently, the interest in non-psychoactive *C. sativa* extracts is increased due to many biological activities related to both its cannabinoids and terpenes [2-4].

The aim of the project is to study the possible role and application of non-psychoactive *C. sativa* extracts as antiproliferative agents, in particular in combination with conventional chemotherapy, and to identify the compounds responsible for this activity. To this aim, it was necessary to fully characterize the bioactive compounds present in different *C. sativa* extracts of different non-psychoactive varieties through high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) and to evaluate their antiproliferative activity on different human cancer cell lines. In particular, HPLC-HRMS was used for the analysis of the decarboxylated ethanolic extracts from three hemp varieties having a different phytochemical composition (CBD-type, CBG-type and a hybrid one). The antiproliferative activity of the extracts was assessed on different human cancer cells of mesodermal origin. The results indicated that the K562 leukemia cell line was the one that responded better to the treatment and the CBD-type extract was the one that provided the lowest IC_{50} value. Then, dose-response curves were built for the associations of the CBD-type extract at 5 µg/mL with anticancer drugs currently used in therapy, including vincristine, imatinib and doxorubicin at 48 h of treatment. The same was done for the association between these therapeutic agents and pure CBD at 5 µM for 48 h.

The dose-response curves did not show a significant decrease of the IC_{50} value for imatinib and doxorubicin in association with the extract or with pure CBD. Differently, vincristine associated with the CBD-type extract showed a 10 times higher efficacy then the vincristine alone. This effect was found to be specific of the CBD-type extract. Since, the pharmacological activity of vincristine is related to its micrutubule-destabilizing properties, some bioactive compounds present in the CBD-type extract might act on the same target, thus enhancing the antiproliferative activity of this anticancer drug. The identification of the compound/s responsible for this antiproliferative acitivty as well as the elucidation of the mechanism/s of action are currently on going.

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More efficient synthesis, cytotoxicity and *in silico* ADME properties of 3-hydroxy-17-oxa-17a-homoestra-1,3,5(10)-trien-16-one

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Large number of cancers are hormone-dependent. This means that their growth is induced by hormones (estrogens or androgens). For example, some breast cancers contain estrogen receptors and they are called estrogen receptor positive cancers (ER+). Antiestrogens and aromatase inhibitors are used for treatment of these cancers. With this in mind, synthesis of 3-hydroxy-17-oxa-17a-homoestra-1,3,5(10)-trien-16-one was perfomed and published.[1, 2] This compound has shown significant antiestrogenic activity.[2] In order to obtain it in more efficient way we have synthesised it in three synthetic steps starting from estrone as opposed to seven previously reported. Furthermore, *in silico* ADME profile was evaluated by comparing phisichemical propreties calculated by SwissADME web tool with five different sets of criteria, as well as using the BOILED-Egg model to analyse possibility of the gastrointestinal absorption and brain penetration. Last but not least, cytotoxicity was tested on six tumor and one normal cell line.

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Discovery of Orexant and Anorexant Agents with Indazole Scaffold Endowed with Peripheral Antiedema Activity

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Metabolic syndrome is a group of multifactorial conditions, characterized by the loss of balance between energy income and caloric needs, that interests a large part of population. In this field, the endocannabinoid system plays a crucial role, representing a neuronal network involved in the control of several functions, such as feeding behavior.[1]

Rimonabant is an inverse agonist of type-1 cannabinoid receptor (CB1) used as antiobesity drug, suspended in 2008 due to its serious side effects on central nervous system.[2]

AB-Fubinaca, ADB-Fubinaca and AMB-Fubinaca are synthetic CB1 and CB2 agonists identified in illicit drugs in the Japanese market, their administration is associated with serious side effects, neurotoxicity and cardiotoxicity.[3]

New synthetic cannabinoids (SCs) as AB-FUBINACA/Rimonabant hybrids have been reported by Stefanucci and coworkers; these new indazole based compounds contain the Lonidamine scaffold with structural analogies to both Rimonabant and Fubinaca derivatives (**Figure 1**), joined with hydrophobic amino acid residues (tert-Leu, Leu, Val). [4,5]

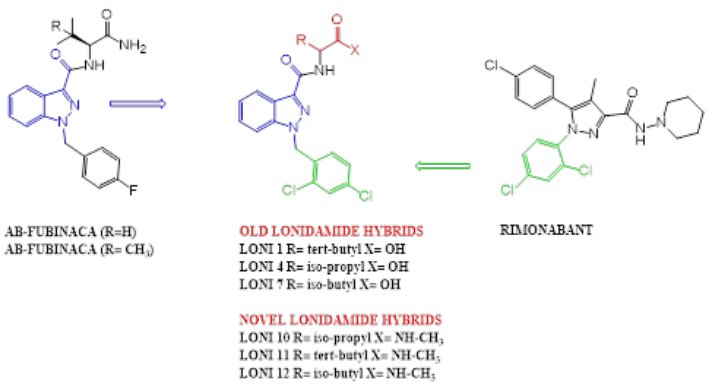

Figure 1. Structures of lonidamine hybrids and similarity with rimonabant and Fubinaca derivatives.

In this work we evaluated the ability of novel C-terminal methyl amide Lonidamine hybrids to bind and activate CB receptors; these hybrid compounds have been studied by *in vitro* binding assays to cannabinoid receptors and by functional receptor assays, using rat brain membranes. The most active among them as an agonist, (LONI 11), and an antagonist, were tested in *vivo* in mice, to evaluate their ability to stimulate or suppress feeding behavior after intraperitoneal (i.p.) administration.

We also investigated the antinociceptive property of LONI 11, at the central and peripheral levels using formalin test and a tail flick test after an administration by the subcutaneous (s.c.) and intracerebroventricular (i.c.v.) routes, *in vivo* in mice.

A significant orexant effect has been also observed for LONI 11 and an intense anorexant effect for (LONI2) and LONI4. In zymosan-induced edema and hyperalgesia, LONI 11 reduced the percent of paw volume increase and paw latency after s.c. administration, also suggesting a possible peripheral anti-inflammatory activity.

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New benzylpiperazine derivatives as selective σ_1 receptor ligands: synthesis, *in vitro* and *in vivo* pharmacological characterization

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The sigma-1 receptor (σ_1R) is a unique endoplasmic reticulum chaperone protein involving in the regulation of the trafficking of a variety of proteins. Due to its ability to regulate both voltage-gated ion channels and non-voltage-gated ion channels, the σ_1R is involved in intracellular calcium homeostasis and neurotransmission.[1] Therefore, the σ_1R has been proposed as a valuable therapeutic target for several diseases and conditions, including neuropathic pain, cancer, treatment of drug abuse, and neurodegenerative disease.[2]

In this work, a new series of selective σ_1R ligands, bearing the benzylpiperazine scaffold as a basic moiety, were developed and preliminarily evaluated for their *in vitro* and *in vivo* pharmacological properties (Fig.1). Among the newly synthesized compounds, 3-cyclohexyl-1-[4-(4-methoxybenzyl)piperazin-1-yl]propan-1-one (**8**), showed low nanomolar affinity towards the σ_1R, and high selectivity over the σ_2R subtype (σ_1 K_i = 1.6 nM, and σ_2 K_i = 1418 nM) in the radioligand binding assay. Besides, the *in vivo* antinociceptive effect exerted by **8**, in a mice model of induced inflammatory pain (formalin paw assay), suggested that it acts as an antagonist at the σ_1R.

Altogether, our results support the further development of benzylpiperazine-based derivatives as potential therapeutics and diagnostic agents for the pharmacological modulation of the σ_1R.

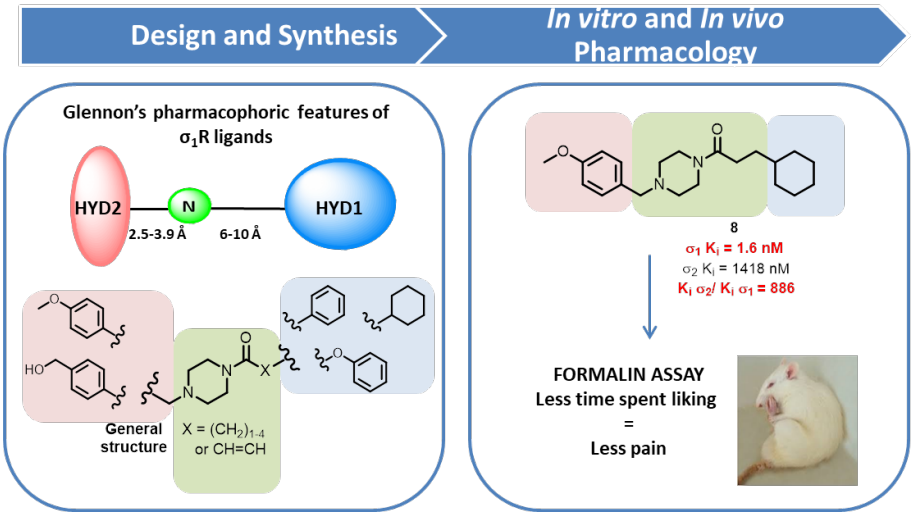

Figure 1. Design strategy and result highlights for the new benzylpiperazine-based σ_1R ligands.

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Modeling Epac1 Interactions with the allosteric inhibitor AM-001 by co-solvent molecular dynamics

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The exchange proteins activated by cAMP (EPAC) are implicated in a large variety of physiological processes and they are as considered promising targets for a wide range of therapeutic applications.[1] In particular EPAC1 is considered as a novel protein target for the treatment of various cardiac diseases.[2,3] In that context, we recently characterized a selective EPAC1 antagonist named AM-001.[2] This compound was featured by a non-competitive mechanism of action but the localization of its allosteric site to EPAC1 structure has yet to be investigated. Therefore, we performed cosolvent molecular dynamics with the aim to identify a suitable allosteric binding site. Then, the docking and molecular dynamics were used to determine the binding of the AM-001 to the regions highlighted by cosolvent molecular dynamics for EPAC1. These analyses led us to the identification of a suitable allosteric AM-001 binding pocket at EPAC1. As a model validation, we also evaluated the binding poses of the available AM-001 analogues, with a different biological activity. Finally, the complex EPAC1 with AM-001 bound at the putative allosteric site was further refined by molecular dynamics. The principal component analysis led us to identify the protein motion that resulted in an inactive like conformation upon the allosteric inhibitor binding (Figure 1).

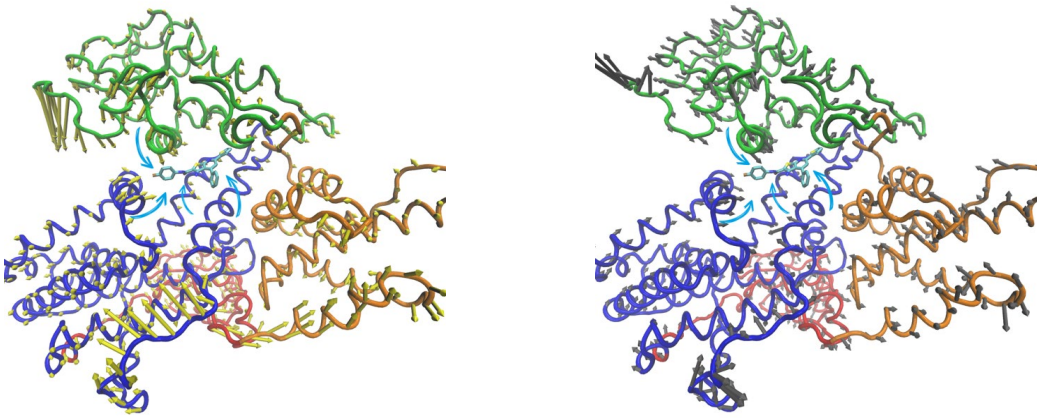

Figure 1. Porcupine plot of the top two eigenvectors. Right panel: eigenvector 1, left panel: eigenvector 2. Epac1 is reported as tube: CNBD and DEP green; REM orange; CDC25-HD blue and RA red. AM-001 is reported as cyan stick. The yellow and grey arrows attached to each a-carbon atom indicate the direction of the movement; the size of each arrow shows the magnitude of the corresponding movement.

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Shift in activity of ciprofloxacin and norfloxacin "no-light activated" derivatives from antibiotics to anticancer agents: preliminary results

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Quinolones represent an important family of antibacterial drugs, widely prescribed for the treatment of urinary and respiratory infections in humans with great activity and spectrum of action. Recent advances in the development of novel quinolone derivatives demonstrate that this chemical scaffold can be properly modified in order to obtain molecules with promising cytotoxic activity against different cancer cell lines and some of them have already been used in clinics or under clinical trials, making quinolone derivatives efficient candidates for cancer treatment. [1] Nitric oxide (NO) is a short-lived gas whose cytotoxic role depends on its timing, location and concentration. [2] In this work, we conjugated the fluorescent fluoroquinolones ciprofloxacin and norfloxacin scaffold with a proper NO photodonor (NOPD) to achieve a controlled release of NO using a specific light irradiation (Fig.1).

Shift in activity from antibiotics to anticancer agents of twelve new compounds was investigated performing MTT assays in the dark on HCT116, MCF-7, MCF-7/ADR and MDA-MB231 cell lines whereas the lack of any antimicrobial activity has been demonstrated on PAO1 strain.

Photochemical studies regarding NO release are in progress to strengthen the dual cytotoxic activity of these novel hybrids.

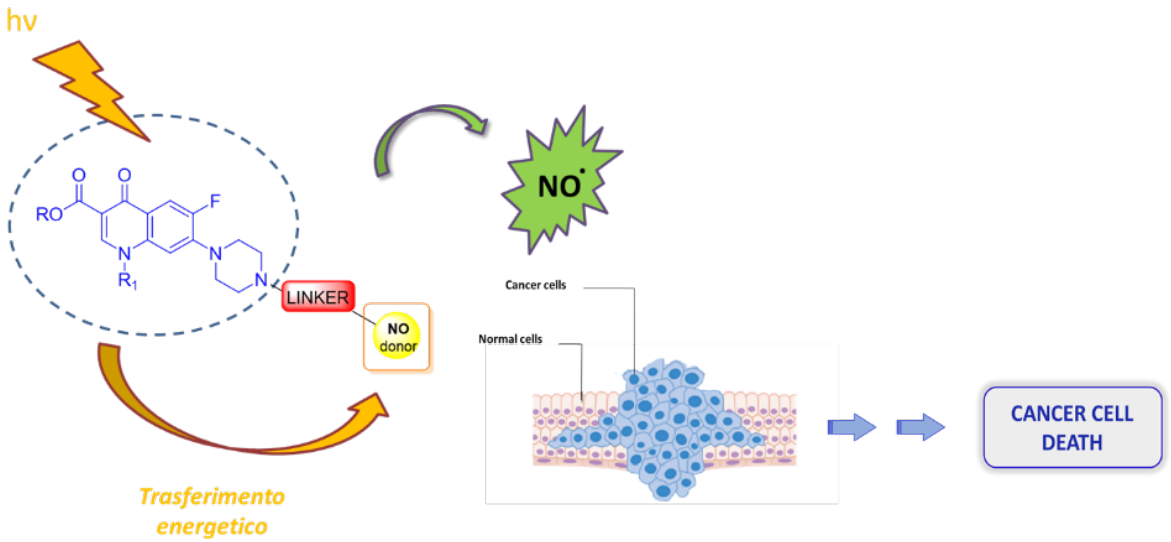

Figure 1. Rational design of "NO-light activated" fluoroquinolone derivatives.

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Screening of [1,2]oxazolo[5,4-*e*]isoindoles in lymphoma models

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Non-Hodgkin lymphoma (NHL) is one of the most common hematological malignancy in the world, with more than 30 distinct subtypes divided into aggressive and indolent. Diffuse large B-cell lymphoma (DLBCL) is the most widespread form of NHL, accounting for 30-40% of all cases. Anti-tubulin agents are widely used in the treatment of lymphoma both alone and in combination chemotherapy regimens such as ABVD and R-CHOP.[1] The [1,2]oxazolo[5,4-*e*]isoindole system gave excellent results in reducing *in vitro* cell growth of several cancer cell lines (GI_{50} 0.01-27 μM) and selected derivatives showed *in vivo* antitumor activity at well-tolerated doses in a diffuse malignant peritoneal mesothelioma (DMPM) xenograft model.[2,3] Moreover, they proved to affect microtubule assembly inducing cell cycle arrest at the G2/M phase and to inhibit the colchicine binding to tubulin up to 80%.

[1,2]Oxazolo[5,4-*e*]isoindoles were further screened in 4 lymphoma histotypes: germinal center B-cell and activated diffuse large B-cell lymphoma (GCB-DLBCL and ABC-DLBCL), marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL). After 72 h treatment, cell proliferation was measured with the MTT assay. Compounds were pre-screened at the dose of 1 μM in SU-DHL-10 (GCB-DLBCL), HBL1 (ABC-DLBCL), VL51 (MZL) and MINO (MCL) cell lines. Those with percentage of proliferating cells down to 60% were further screened with a wider range of concentrations (0.15-10 μM).

After the one-dose treatment, 8 out of 60 derivatives reduced the proliferation rate by 7-61%. At concentrations of 0.15-10 μM, they confirmed their anti-proliferative activity with IC_{50} values in the low micromolar - nanomolar range. The most potent compound reached nanomolar activity against MINO (IC_{50}= 0.07 μM), SU-DHL-10 (IC_{50}= 0.07 μM) and HBL-1 (IC_{50}= 0.08 μM), followed by VL51 with a slightly higher IC_{50} value (0.12 μM). Structure-activity relationship (SAR) suggest that 3,4 and/or 5 methoxy substituted benzyl groups at the pyrrole nitrogen are crucial in conferring potent activity (Figure 1).

In conclusion, [1,2]oxazolo[5,4-*e*]isoindoles are very promising compounds for the treatment of lymphoma, confirmed by the strong anti-proliferative effect. Elucidation of the mechanism of action indicates a strong inhibition of the colchicine binding to tubulin.

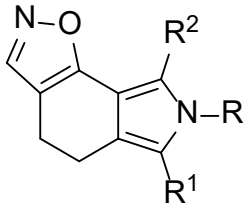

Figure 1. [1,2]oxazolo[5,4-e]isoindoles

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Neurotensin, a multi-faceted peptide

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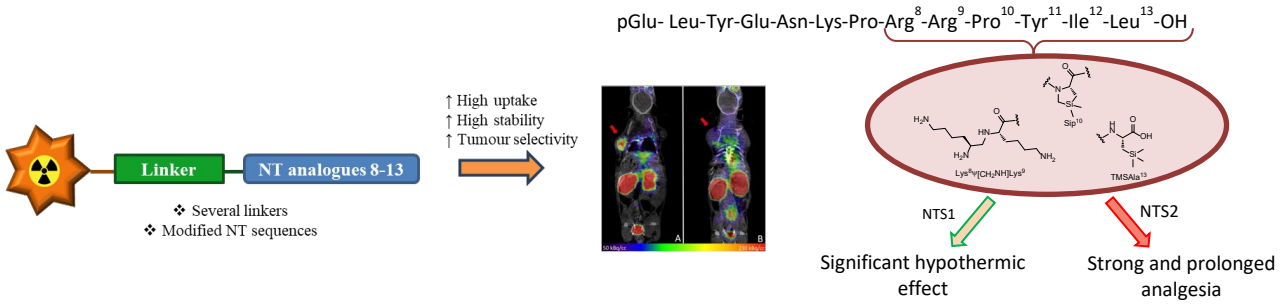
Neurotensin (NT) an endogenous tridecapeptide, firstly isolated from bovine hypothalamus,[1] is involved in various biological processes, such as blood pressure and body temperature modulation, analgesia, regulation of dopaminergic transmission and gastrointestinal function.[2] NT effects are mediated by the activation of two G protein-coupled receptors, NTS_1 and NTS_2, and NTS_3, a sortilin-like receptor.[3] Several studies have demonstrated that the C-terminal portion H-Arg^8-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH, namely NT(8-13), is the minimum peptide fragment required for producing NT effects. Given the overexpression of NTS_1 in various cancers, such as breast cancer, pancreatic adenocarcinoma, lung cancer and some prostate cancers, we developed three series of NT conjugates as potential agents for imaging and therapy of cancers. In more detail, NT(8-13) sequence was chemically modified and several linkers, along with a chelating agent, were inserted. Novel NT conjugates were radiolabelled with ^{68}Ga for PET imaging and ^{111}In as surrogate for therapy, and fully characterized on human cancer cells and small animals.[4]
Besides, a panel of proteolytically stable NTS_1 agonists was synthesized to target hypothermia. Backbone modifications and unnatural amino acids, such as pseudopeptide LysΨ[CH_2NH]Lys, trimethylsilylalanine (TMSAla) silaproline (Sip) and D-amino acids were inserted. Biological evaluation showed significant hypothermic effect (-3°C for 1h) and increased metabolic stability (up to > 24h).[5]
On the other hand, NTS_2-selective ligands led to analgesic effects without inducing vasodilation and hypothermia. For this reason, NTS_2-selectivity is promising for developing alternatives to opioid medications in pain treatment. A set of modified hexapeptides was developed following a rational approach. When evaluated in different pain models, JMV5296 (H-LysΨ[CH_2NH]Lys-Sip-Tyr-Ile-TMSAla-OH) showed a strong and prolonged antinociceptive effect without hypothermia.[6]



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Design and synthesis of small molecules to disrupt XIAP/Caspase interaction

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The ovarian cancer is the most lethal gynaecologic malignancy and one of the most causes of female death in the world.[1] From the factories that contribute to malignant transformation, there is a down-regulation of the apoptosis process, due to mutations of suppressor genes.[2]
In this contest, IAP (Inhibitor of Apoptosis Protein) proteins have an important role in the regulation of apoptosis, especially XIAP (X-linked Inhibitor of Apoptosis Protein). Among the different protein domains, XIAP-BIR2 domain mediates the interaction with Caspase-3 and Caspase-7, while XIAP-BIR3 domain binds Caspase-9, to block their activation. SMAC/Diablo protein, an endogenous peptidic inhibitor release by the mitochondria, can antagonize IAP proteins binding both BIR2 and BIR3 domains to carry out its function and to regularise the apoptotic cell process.[3] Several peptidomimetic compounds mimicking SMAC have been developed in recent years to inhibit XIAP or cIAP.[4] But the most difficult part is to achieve and to obtain selective compounds between members of the IAP proteins family, and between XIAP-BIR2 and XIAP-BIR3 domains to improve pharmacokinetics parameters.
Our aim is to design non-peptidic molecules that can mimic the interaction of the moiety (IBM), responsible of the interaction between XIAP and Caspases (Figure 1). Disrupting these interactions could promote the apoptosis of cancer cells and overcome the chemoresistance associate to the classical therapy in ovarian cancer.[5] As part of a fragment-based drug design (FBDD) approach, a previous library of compounds has been synthesized and screened against domains of XIAP. Actually, we are working on the scaffold optimization of the best hit to improve both its affinity and its selectivity. All our work is supported by molecular modelling and *in vitro* assays (Alphascreen, Fluorescence Polarization Assay).

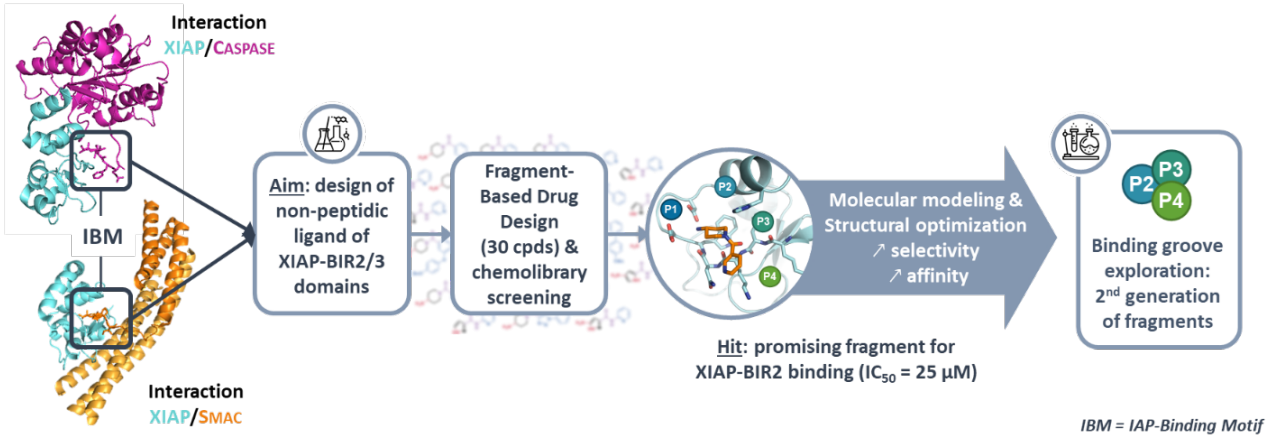

Figure 1. Design of new non-peptidic fragments to target XIAP/Caspases interaction.

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Molecular Docking Studies of Promising P-Glycoprotein Inhibitors obtained from *Plectranthus* spp

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The development of multidrug resistance (MDR) is a major cause of failure in cancer chemotherapy. Development of MDR is often associated with overexpression of P-glycoprotein (P-gp).[1] *Plectranthus* spp. (Lamiaceae) are important sources of bioactive compounds, namely, royleanone diterpenes.[2] The antitumoral compounds 6,7-dehydroroyleanone (DHR, 1) and 7α-acetoxy-6β-hydroxyroyleanone (AHR, 2), are lead compounds isolated from *Plectranthus* spp.[3, 4]

Several royleanone derivatives were prepared through hemi-synthesis from natural compounds **1** and **2**, to achieve a small library of products with improved P-glycoprotein inhibition potential. Molecular docking and molecular dynamics studies were performed on several derivatives in order to clarify the molecular mechanisms by which they may exert their inhibitory effect in P-gp activity.

Molecular docking and Molecular dynamics studies suggested that steric factors play a role when choosing which substituent can be placed at position C-12. Furthermore, results suggested that the presence of aromatic moieties increases the binding affinity of royleanone derivatives towards P-gp. Future generation of novel royleanone derivatives will comprise i) a selective modification of position C-12 with chemical moieties smaller than unsubstituted benzoyl rings and ii) the modification of the substitution pattern of the benzoyloxy moiety at position C-6.

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In silico ADME and inverse docking of A,B-modified steroidal D-homo lactones with cytotoxic properties

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According to World Health Organisation, cancer is the second leading cause of death globally. Finding new potent anticancer compounds is one of fundamental goals of modern medicinal chemistry. This has led to large number of structurally diverse compounds that are used in treatment of this vast group of diseases. Some of those compounds have steroidal structure. For example, testolactone was one of the first compounds used in breast cancer treatment. This steroid contains D-homo lactone ring and its was motive for synthesis of large number of A,B-modified 17-oxa-17a-homoandrost-16-ones.[1-3] These compounds have shown significant cytotoxic activity on a number of cancer cell lines, eg breast, lung, prostate etc. In order to gain further insight in possibility of medicinal use for this compounds, we have selected 30 most active and evaluated their *in silico* ADME profile. This was done comparing phisicochemical propreties calculated by SwissADME web tool with five different sets of criteria (Lipinski, Veber, Egan, Ghose and Muegge), as well as using the BOILED-Egg model to analyse possibility of the gastrointestinal absorption and brain penetration. Finally, ligand-based 3D similarity search was used to propose molecular targets potentially responsible for their cytotoxic activity.

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N-Acylethanolamine Acid Amidase (NAAA): Mechanism of Palmitoylethanolamide Hydrolysis Revealed by Mechanistic Simulations

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N-acylethanolamines (NAEs) are a large family of endogenous lipid transmitters characterised by an acyl chain, which differs among the NAEs for length and degree of unsaturation, linked to ethanolamine through an amidic bond. NAEs exert several biological effects, and among them *N*-palmitoylethanolamine (PEA) takes a major role in analgesia and in anti-inflammatory and neuroprotective processes mediated by the activation of PPARα.[1] The activity of PEA is terminated by its degradation to palmitic acid and ethanolamine catalysed by *N*-acylethanolamine acid amidase (NAAA), a member of the N-terminal nucleophile (Ntn) hydrolases superfamily, mainly expressed in B lymphocytes and macrophages,[2,3] where it concentrates in lysosomes and shows an optimal activity at acidic pH.[4] NAAA is produced as proenzyme autocatalytically activated through the proteolysis that releases the N terminal cysteine C126, which is the catalytic residue.[3] As revealed by recent X-ray experiments,[5] the catalytic cysteine is placed at the entrance of a narrowed and hydrophobic channel that accommodates the benzothiazole-piperazine scaffold of the non-covalent inhibitor ARN19702,[6] and the lipophilic chain of the covalent inhibitor ARN726.[7] On the solvent-exposed access of the channel, the N-terminal amino group is caged between the side chain of D145, R300, and N287, which forms the oxyanion site with the backbone of E195. In this work, the mechanism of NAAA-mediated catalysis was investigated at the atomic level using a model based on the X-ray structure of the human enzyme by applying molecular dynamics simulations and quantum mechanical/molecular mechanics simulations coupled with enhanced sampling. The attribution of the correct protonation state of carboxylate groups surrounding the catalytic site was critical for the identification of a stable model. Starting from a stable complex of the Michaelis complex, the free-energy surfaces of the acylation and deacylation reactions were estimated through Umbrella sampling simulations. These calculations outlined that the acylation is the rate limiting step, with the catalytic residue C126 acting both as an acid, protonating the ethanolamine leaving group, and as a nucleophile attacking the PEA carbonyl group. Consistently with experimental data, showing that NAAA hydrolyzes *N*-methylpalmitoylamide with high catalytic efficiency, the ethanol moiety of did not appear to contribute to the acylation reaction.

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Preparation and HuR interaction studies of BOPC1 enantiomers combining DEEP-STD NMR and molecular modeling analysis

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The Human antigen R protein (HuR) is an RNA binding protein which plays a pivotal role in the stabilization of various RNAs (Figure 1).[1] This protein is considered an intriguing target for discovering novel anticancer drugs.[2,3] From our previous data, N-(2-(benzylamino)ethyl)-2-(3,5-dimethoxyphenyl)-1-isobutyl-6-oxo-piperidine-3-carboxamide (**BOPC1**) compound was found able to bind HuR.[4]
In this work, we carried out the enantio-resolution, the assignment of the absolute configuration and the recognition study of enantiomerically pure *trans*-**BOPC1** with HuR.[5] The DEEP (differential epitope mapping)-STD NMR were applied to study the interaction between **BOPC1** and HuR. Starting from the crystal structure of the two N-terminal RRM domains of HuR complexed with RNA, deposited in the Protein Data Bank (PDB) with the 4ED5 PDB code,[6] we performed our modeling analysis in order to investigate the binding mode and the theoretical binding affinity of the two trans-**BOPC1** enantiomers towards HuR protein.[5]

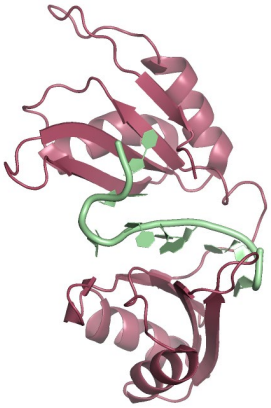

Figure 1. 3D representation of HuR-mRNA complex; the protein and the m-RNA are shown as raspberry and palegreen cartoon, respectively.

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A follow-up study on new synthetic thyroid hormone analogues: identification of new treatments for Transthyretin Amyloidosis

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Wild-Type Transthyretin Amyloid (WTTA) is a systemic amyloidotic disease, with high impact on elder's life conditions and a growing incidence; to date, no pharmacological solution has been found for this pathology.[1] WTTA is characterized by amyloid formations following the misfolding and aggregation of Transthyretin (TTR), a 55 kDa homotetramer functioning as a transporter of thyroxine (T4) and holoretinol-binding protein (RBP). Amyloid formation by TTR requires rate-limiting tetramer dissociation into monomers, which subsequently yields the aggregation-prone amyloidogenic intermediate. Among the several therapeutically approaches proposed, stabilizing the TTR structure and so preventing its dissociation into monomers seems a valuable strategy to be pursued in the drug discovery process of WTTA treatments. Recently, we designed and synthetized a chemical library of approximately thirty synthetic compounds able to exert a selective agonist action towards the Thyroid Hormone Receptor Beta.[2] All the synthetized compounds have been screened against a full panel of ADME-tox in vitro assay, including cytotoxicity, CYP450-related metabolic stability, cardiotoxicity, and off-target liability. Most of this chemical library showed a safe profile on all assays performed. Among this set of compounds we identified two new lead candidates, namely TG68 and IS25, able to interfere with the misfolding and aggregation of TTR by pharmacologically stabilizing its tetramer. Moreover, administration of TG68 and IS25 demonstrated a highly safe profile in vivo, without the side effects commonly associated with thyroid hormones administration.[3] Finally, preliminary data acquired by computational studies and ^{1}H-^{15}N NMR technique showed a high capacity of these two molecules to stabilize the TTR tetramer, suggesting their potential role as new pharmacological treatments for WTTA.

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Enzymatic and cell-based characterization of the inhibitory mechanism of UPR1444 on EGFR by TR-FRET-based assays and liquid chromatography-mass spectrometry analysis

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Osimertinib is a third generation EGFR inhibitor which targets activated EGFR mutants such as L858R/T790M and del19/T790M, while it spares the wild type form. This drug exerts its biological activity by covalently modifying Cys797. However, the clinical use of osimertinib in non-small cell lung cancer (NSCLC) induces mutations conferring drug resistance, including C797S. [1] Design of EGFR inhibitors able to bind Lys745, a residue fundamental for the kinase activity, emerged as a promising strategy [2] to overcome acquired resistances. UPR1444 (Figure 1) is a novel compound which possesses the 2,4-dianilino-pyrimidine scaffold found in third generation EGFR inhibitors and incorporates a benzensulfonyl fluoride warhead designed to selectively react with Lys745 of activated EGFR. In the present work, we characterized the inhibition mechanism of UPR1444 on recombinant L858R/T790M/C797S (TM) and wild type (WT) EGFR by combining time resolved fluorescence energy transfer (TR-FRET) and high-performance liquid chromatography coupled with high resolution mass spectrometry (HPLC-HRMS) techniques. In TR-FRET-based activity assays, UPR1444 inhibited EGFR TM in a time-dependent manner with a progressive decrease of its half-maximal inhibitory concentration (IC_{50}) that reached 1.6 nM after 3 h of pre-incubation, a value at least 150-fold lower than osimertinib IC_{50} (Figure 2). In the TR-FRET-based rapid dilution assay UPR1444 irreversibly inhibited EGFR TM activity after 30 min from dilution while it allowed 70% recovery of EGFR WT activity, thus providing EGFR TM selectivity. To corroborate the covalent modification of Lys745, EGFR TM was incubated for 3 h with and without UPR1444 and then subjected to tryptic digestion overnight before HPLC-HRMS analysis. The peptide carrying Lys745 was identified by MS/MS analysis in both control and UPR1444-treated samples. In the latter, the targeted peptide showed a 42% decreased intensity but no covalent adduct was detected, suggesting the need to optimize experimental conditions. After characterizing UPR1444 inhibition mechanism on the isolated enzyme, the compound was tested in PC9 cells expressing del19/T790M/C797S. UPR1444 showed a moderate activity by inhibiting autophosphorylation at 72% after 1 h incubation at 2 µM, a result comparable to osimertinib. Conversely to what observed for osimertinib, intracellular dosages by HPLC-MS/MS indicates UPR1444 poorly penetrated the cellular membrane: in that sense an optimization of the physicochemical properties of UPR1444 may lead to inhibitors able to block EGFR C797S activity also in cells.

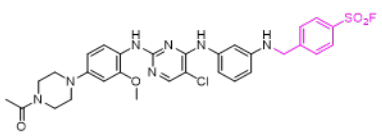

Figure 1. UPR1444.

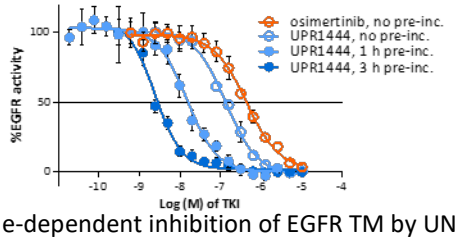

Figure 2. Time-dependent inhibition of EGFR TM by UNIPR1444.

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(-)-(*S*)-usnic acid from *Cladonia foliacea*: microwave assisted extraction and cytotoxic profile

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Due to their centennial uses, lichens have attracted the attention of the scientific community, and many secondary metabolites with interesting biological activities have been identified.[1] The most studied one is usnic acid, a dibenzofuran derivate characterized by a stereogenic center (Figure 1).
Both enantiomers occur in nature, depending on the producing organism and show different biological properties and toxicity. In the present work we focus on (-)-(*S*)-usnic acid, an underestimated metabolite produced by many lichens.[2] Being the less abundant enantiomer in nature and owing very high commercial costs, so far it is poorly investigated. In the present work, we studied the lichen *Cladonia foliacea*, as a potential potent source of this metabolite. Once it has been verified that only the (-)-(*S*)-usnic acid is present in the matrix, we optimize a Microwave-Assisted Extraction (MAE) to exhaustively extract the metabolite of interest. For selecting the best extraction conditions, we applied a Design of Experiment approach. The resulting procedure was then applied to extract and isolate (-)-(*S*)-usnic acid. So far, MAE approach has never been applied to the extraction of usnic acid from natural sources.
Lastly, (-)-(*S*)-usnic acid was then subjected to preliminary vitality tests on four different cancer cell lines. Of particular interest is the activity against glioblastoma U87-MG, and that this activity is strictly related to the chiral center. Indeed, the eudismic ratio ($IC_{50(R)\text{-usnic acid}}/IC_{50(S)\text{-usnic acid}}$) is about 5000.[3]

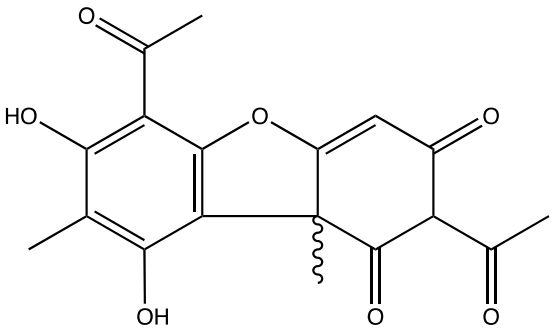

Figure 1: Chemical structure of racemic usnic acid

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Rational design and synthesis of heme oxygenase 2 (HO-2) inhibitors by targeting the secondary hydrophobic pocket of the HO-2 western region

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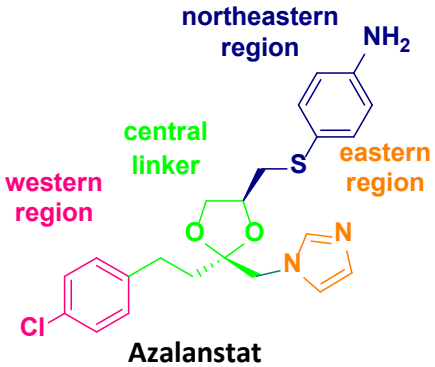
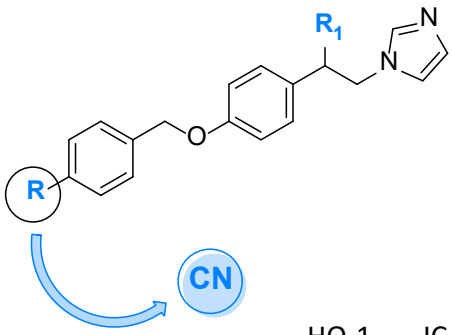
The cytoprotective heme oxygenase (HO) family of enzymes is accountable for heme breakdown. Two isozymes have been currently detected: the stress-induced isoform HO-1 and the basally expressed isoform HO-2. [1] Although the effects of HO-1 induction and inhibition have been well documented, HO-2 specific role and its druggability have not been clarified because of the lack of selective ligands. [2] HO inhibitors, whose chemical structure derives from the lead compound azalanstat (Fig. 1) principally differ for the nature of the central linker and the substituent in the western region of the molecule. This portion interacts with a highly flexible lipophilic pocket in both enzymes. Moreover, crystallographic studies highlighted the presence of a smaller secondary hydrophobic pocket in both isozymes, whose interaction with proper substituents seems beneficial for the inhibitory activity. [3] Our research team recently reported that the HO-1 binding site is better able to host molecules with an average Van der Waals volume of 274.34 Å^3. At the same time, the catalytic pocket of the HO-2 is able to interact better with larger molecules, with an average Van der Waals volume of 284.10 Å^3. [4] With this in mind, we rationally designed three novel HO-2 inhibitors by selecting two of the most potent and selective HO-1 inhibitors (compounds **1**,**2**, Fig. 1) and increasing their volume by adding a cyano group in the *para* position of the benzyloxy moiety (Fig. 1). Synthetic strategies, inhibitory potencies, and docking studies of these novel HO-2 ligands will be described.



Azalanstat

IC_{50} HO-1 = 5.3 μM
IC_{50} HO-2 = 24.4 μM



	R	R_1	IC_{50} HO-1	IC_{50} HO-2
1	H	OH	0.5 μM	11.7 μM
2	H	H	0.9 μM	ND

Figure 1. Azalanstat and HO-1 selective inhibitors **1** and **2** used for this work.

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Identification of new inhibitors of Protein Arginine Methyltransferases by a Multi-substrate-adduct approach

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The methylation of arginine residues is a common post-translational modification, performed by a family of methyltransferases known as PRMTs (Protein Arginine Methyltransferases). In humans, methylation is carried out by nine members of the PRMTs that are ubiquitously expressed. Arginine methylation is known to play a key role in gene regulation due to the ability of the PRMTs to deposit activating or repressive "histone marks".[1] This modification correlates PRMTs to several biological processes, including transcription, DNA repair, protein stability, cell signaling, pre-mRNA splicing and receptor trafficking. Therefore, aberrant activity of PRMTs is involved in many pathological conditions like inflammation, neurodegeneration and cancer. According to these evidences, PRMTs have been identified as promising therapeutic targets. [2,3]

Starting from **EML108**, an inhibitor of PRMTs previously identified by us,[4] we deconstructed our compound and then performed a medicinal chemistry optimization campaign.

Here we report a multi-substrate-adduct approach to develop new powerful and selective compounds (Figure 1).

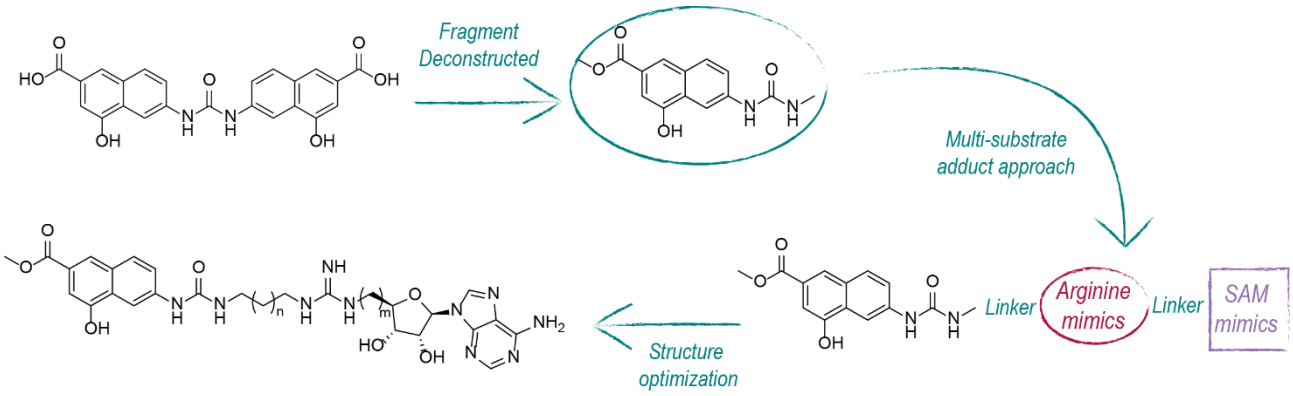

Figure 1. Multi-substrate-adduct approach

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Looking for new agents against multiple myeloma: exploration of the chemical space of the PAN-sigma receptor modulator RC-106

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Cancer is a dangerous threat for human health due to its multifactorial origin. The cancer molecular basis has not been completely explained yet, but nowadays it is well known that proteasome plays a crucial role in cancer. So far, three proteasome inhibitors (above all, Bortezomib should be mentioned) are used for treating cancer and particularly Multiple Myeloma (MM), a hematological disorder caused by an uncontrolled proliferation of monoclonal plasma cells in bone marrow.[1] Nevertheless, the use of proteasome inhibitors is accomplished with several side effects in central nervous system and it is also responsible for drug resistance. For all these reasons, new effective therapies are still considered an urgent medical need. Since several years our group was focus on the identification and development of new active molecules against MM. Starting from the engagement of Sigma Receptors (SRs) in proteasome dysfunction and in molecular cascades of cell proliferation, we investigated the anticancer potential of **RC-106,** a pan-sigma receptor ligand recently discovered by our research team.[2,3] Briefly the **RC- 106** capability to inhibit proteasomeand to impair the cell viability of RPMI8226 cell line has been evaluated. The promising results obtained prompted us to investigate the chemical space around **RC-106.**

The synthesis and *in vitro* evaluation of the piperazine analogue **RC-206** proved that the substitution od piperidine with piperazine moiety does not affect the activity. Therefore, a compounds library of 40 piperazine derivatives have been designed and their *in silico* druglikeness properties evaluated. Afterwards, starting from the specific key intermediate, the library was synthesized applying a combinatorial approach. All compounds have been tested on RPMI 8226 cell line and the most effective compounds have been subjected to proteasome inhibition assay. Above all, three new molecules showed a good *in vitro* cytotoxic properties and have been selected for further investigation.[4] Particularly, our efforts are now directed to better understanding their mechanism of action.

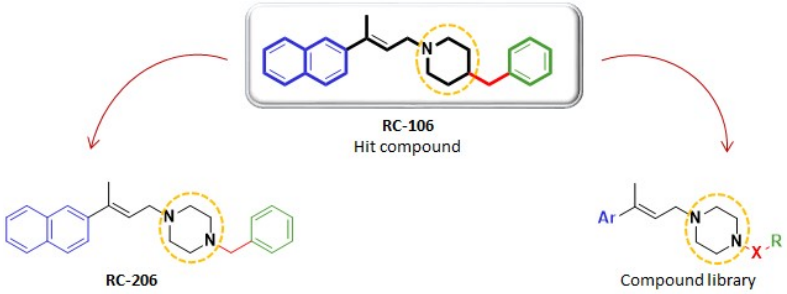

Figure 1. RC-106 derivatization

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Investigating the inhibition mechanism and catalytic cycle of MbtI, the salicylate synthase from *Mycobacterium tuberculosis*

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According to the latest annual report published by the World Health Organisation, tuberculosis is the first cause of death from a single infectious agent worldwide. Moreover, the growing emergence of resistant strains of *Mycobacterium tuberculosis* (Mtb) poses a serious threat to the public's health. Therefore, the discovery of new antitubercular agents has still a critical importance.[1]

In this context, the mycobacterium-specific salicylate synthase MbtI has been recently validated as a promising pharmacological target. This Mg^{2+}-dependent enzyme is involved in the siderophore-mediated iron uptake, a key pathway for the survival and pathogenicity of Mtb in the host.[2]

A structure-based virtual screening allowed us to identify a competitive furan-based inhibitor of MbtI, which was taken as a starting point for a thorough structure-activity investigation. Our studies led to the development of potent enzymatic inhibitors, also exhibiting a promising antimycobacterial action.[3,4]

In an attempt to deeply understand the inhibition mechanism of this class of compounds, we performed biochemical investigations: these studies suggested the possibility of a Mg^{2+}-independent binding, despite the interaction with the catalytic metal had been a cornerstone of MbtI inhibition for years. Further computational analyses and experimental data seemed to support our hypothesis, but it was only with crystallisation studies that we obtained a definitive characterisation of the binding mode. Our structural investigations also provided new insights into the conformational shifts of the active site, in relation to the catalytic state of the enzyme.[5]

Overall, these results pave the way for the rational modification of our scaffold, which will hopefully lead to the obtainment of improved antitubercular candidates.

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Dihydroquinolone pyrazoline-based compound as a new RAD51-BRCA2 protein-protein disruptor to trigger synthetic lethality in pancreatic cancer

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Synthetic lethality is an innovative framework for the discovery of new anticancer drugs. Cancer cells are strictly dependent on efficient DNA repair pathways due to their pervasive genomic instability. Targeting different DNA repair pathways provides the opportunity to apply the synthetic lethality as a novel anticancer therapeutic strategy.[1]

In this context, we proposed to trigger a fully small-molecule-induced synthetic lethality, combining a RAD51-BRCA2 protein-protein interaction (PPI) disruptor with Olaparib, a known PARP inhibitor, to target pancreatic cancer, one of the major unmet oncological needs. RAD51-BRCA2 PPI is essential to repair DNA double strand breaks (DSBs) through the homologous recombination (HR). According to our working hypothesis, a RAD51-BRCA2 PPI inhibitor would chemically mimic the enhanced sensitivity to Olaparib observed in BRCA2-defective tumors, leading to a synthetic lethal effect (Figure 1A).[1] The RAD51-BRCA2 complex is mediated by two critical "hotspots" on RAD51 surface, zone I and II, which lodge eight highly conserved BRCA2 motifs.[2] These two pockets make the RAD51-BRCA2 interaction suitable for a structure-based design of PPI small molecule disruptors.

Herein, we focused on zone II, which has proved to be crucial in RAD51's mechanism of action.[2] To date no inhibitors targeting zone II have been reported. Through a virtual screening campaign, we identified a dihydroquinolone pyrazoline-based molecule **1** as promising hit compound, which showed an inhibitory activity of RAD51-BRCA2 PPI in the competitive ELISA assay (EC_{50}= 16 ± 4 µM).[1] To discover more effective compounds and depict general structure-activity relationship (SAR) studies, we explored the chemical space around **1** by optimizing a general synthetic strategy and building a library that contains a variety of aromatic substitutions (green region) in combination with modifications of the acyl chain moiety (red region) (Figure 1B).[1] SAR efforts yielded **2** with the desired biological profile. As expected, **2** proved to disrupt the RAD51-BRCA2 PPI, inhibiting HR in pancreatic cancer cell line BxPC-3 and reproducing the paradigm of synthetic lethality in combination with Olaparib (Figure 1B).[1]

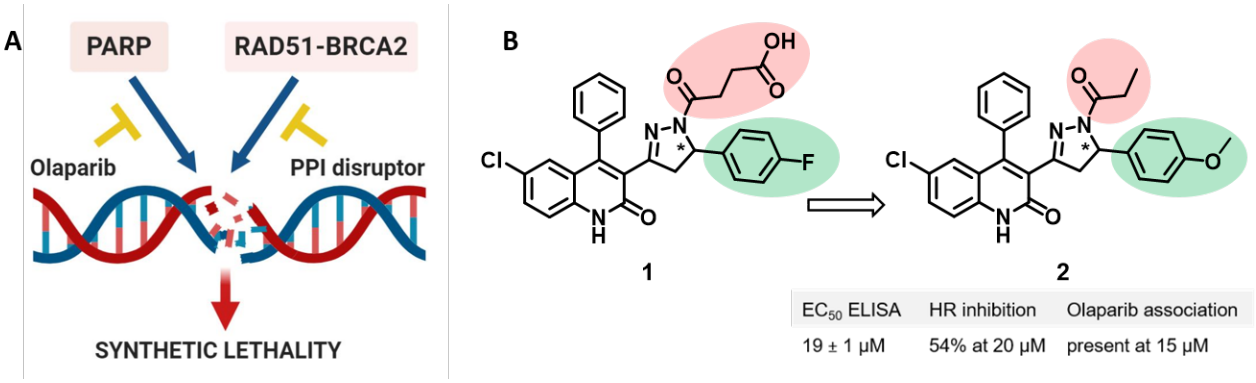

Figure 1. A) Fully small molecule-induced synthetic lethality concept; **B)** Identification of compound **2**.

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Isolation and pharmacological evaluation of Δ^9-tetrahydrocannabiphorol, the new heptyl homologue of tetrahydrocannabinol: new insights on cannabis research

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Cannabis sativa L. produces characteristic terpenophenolic compounds called phytocannabinoids, among which the most studied is Δ^9-tetrahydrocannabinol (Δ^9-THC). The wide structural variety of such compounds derives from the combination of a terpene and a resorcinyl moiety, the latter bearing an alkyl side chain.[1] It has been reported that the length of this carbon chain influences the biological activity of tetrahyddrocannabinol.[2] In particular, synthetic analogues of Δ^9-THC with a longer side chain have shown cannabimimetic properties far higher than Δ^9-THC itself.[3] To date, no phytocannabinoid with a linear side chain longer than five carbon units has been reported. In this work, a new phytocannabinoid with the same structure of Δ^9-THC but with a seven-term alkyl side chain was identified in a medicinal cannabis variety. Isolation, full characterization and stereochemical configuration assignment of the natural compound were carried out. This new phytocannabinoid has been called (-)-*trans*-Δ^9-tetrahydrocannaphorol (Δ^9-THCP) (Fig. 1a). Similarly, the heptyl homologue of the well known phytocannabinoid cannabidiol (CBD), named cannabidiphorol (CBDP), was unambiguously identified. The binding activity of Δ^9-THCP against human CB_1 receptor *in vitro* resulted over 30-fold higher than that of Δ^9-THC (K_i=1.2 nM *vs* K_i=40 nM respectively). *In vivo* tests showed that Δ^9-THCP reduced locomotion (Fig. 1b-c), decreased rectal temperature (Fig. 1d) and induced catalepsy (Fig. 1e) and analgesia (Fig. 1f) suggesting a THC-like cannabimimetic activity.

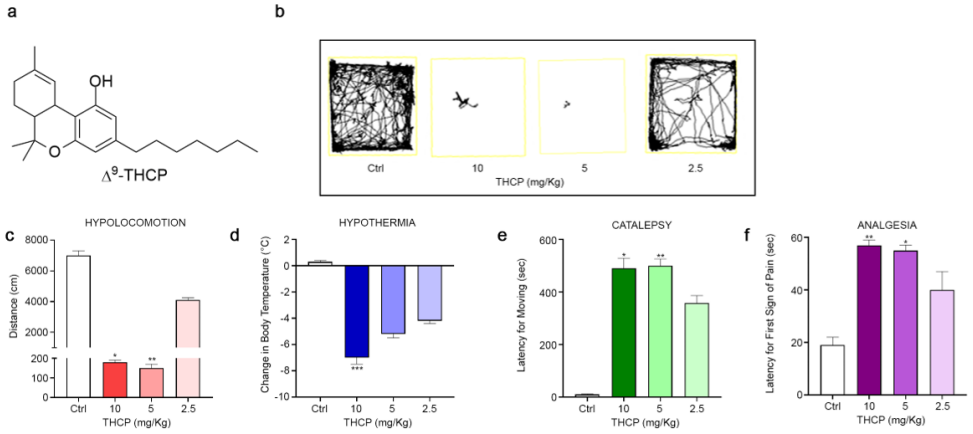

Figure 1. a) Structure of Δ^9-THCP. b-f) Dose-dependent effects of Δ^9-THCP administration (2.5, 5, or 10 mg/kg, i.p.) on the tetrad phenotypes in mice in comparison to vehicle.

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Targeting the viral envelope: synthesis and biological evaluation of novel broad-spectrum antivirals

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Some of the most infectious emerging and re-emerging viruses are endowed with a lipid membrane called envelope. Despite the viral envelope derives from the lipid membrane of the host cell, several differences can be highlighted between them, such as the lack of biogenic and reparative pathways that makes these viruses vulnerable to envelope's injury.

In our previous papers, [1,2] we reported some rhodanine and aminothiazolone derivatives endowed with submicromolar activities against HIV-1 infected cells. The compounds were found to be only moderately active on HIV-1 integrase and HIV-1 gp120, but their submicromolar activity in vitro on HIV-1 replication and time of addition experiment suggested a diverse mechanism of action.

New thiobarbituric derivatives [3] displayed a broad-spectrum antiviral activity against different enveloped viruses (i.e. HSV-1, HCMV, RSV, ZIKV, INFLUENZA A, VSV) and resulted to be completely inactive against non-enveloped ones (i.e. Ad5, HPV and HRoV), suggesting that their mechanism of action could involve the viral envelope, affecting the dynamics of viral fusion and altering the fluidity and integrity of the lipid bilayer.

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Hit optimization for the development of novel RNF5 inhibitors as therapeutic strategy in cystic fibrosis

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In cystic fibrosis (CF), deletion of phenylalanine 508 (F508del) in the CF transmembrane conductance regulator (CFTR) anion channel is associated to misfolding and premature degradation of the mutant protein. RNF5 is an ubiquitin-ligase promoting F508del-CFTR degradation. Recently, our group reported that genetically suppressing *in vivo* RNF5 increases CFTR activity in intestinal epithelial cells, suggesting that RNF5 inhibition could trigger F508del-CFTR rescue.[1] Therefore, through computational methods, we discovered **inh-2**, a drug-like small molecule that efficiently inhibits RNF5 ligase activity (Fig. 1 - A). Evaluation of **inh-2** efficacy on CFTR rescue showed that **inh-2** decreased ubiquitylation of mutant CFTR and increased chloride current in human primary bronchial epithelia.[2]
Based on the promising biological results obtained with **inh-2**, we focused on the design and synthesis of a library of **inh-2** analogues. The series of new derivatives show structural variants introduced around the central 1,2,4-thiadiazol-5-ylidene core (Fig. 1 - B), in order to explore the structure activity relationship of this class of compounds. A preliminary biological evaluation of the activity of the new analogues was performed by using the microfluorimetric YFP-based assay on CFBE41o$^-$ cells.
Some of the new analogues displayed a greater efficacy than **inh-2**, demonstrating that the 1,2,4-thiadiazolylidene scaffold is a versatile architecture for the identification of RNF5 inhibitors, able to rescue F508del-CFTR trafficking defect in human bronchial epithelia. These finding validate RNF5 as a drug target for CF and provide evidences to support its druggability.

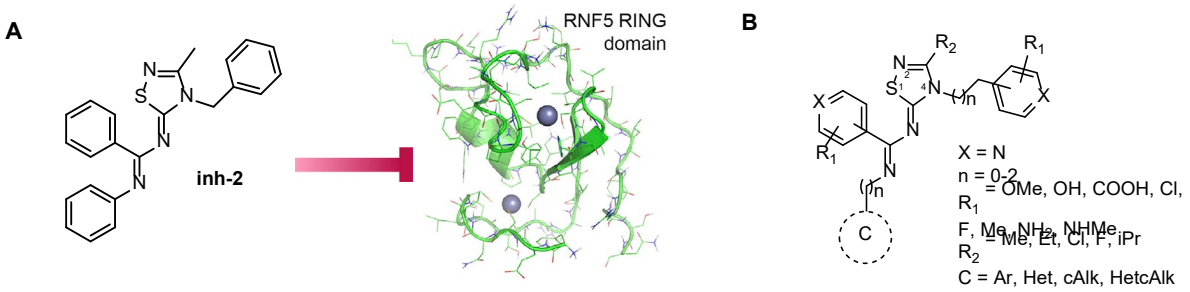

Figure 1. A) inh-2 stucture and activity; B) library of planned **inh-2** analogues.

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Design, synthesis and structural studies of novel piperazine-based sulfonamides as potent human carbonic anhydrases (hCAs) inhibitors

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Human carbonic anhydrases (hCAs, E.C. 4.2.1.1) are metalloenzymes catalyzing the reversible hydration of carbon dioxide to bicarbonate and proton. The hCA family comprises fifteen genetically distinct α-CA isozymes which differ for kinetic properties, oligomeric structure, structural fold as well as tissue and cellular distribution. A part for physiological processes, hCAs are involved in numerous pathological events such as epilepsy, obesity, and tumorigenicity[1]. Therefore, several hCAs represent well-recognized targets to develop hCA inhibitors (hCAIs) as innovative theranostic agents. Among them, acetazolamide (AAZ) and topiramate (TPM) are well-known hCAIs, that are considered useful for the treatment of neurological disorders, whereas the 4-ureido-benzenesulfonamide derivative SLC-0111 recently entered cancer drug clinical trials for the treatment of hypoxic tumors. However, the classical hCAIs are not selective towards druggable hCAs; do that they might induce unwanted side effects. On this basis, the identification of new hCAIs with a lower affinity toward the ubiquitous off-target isoforms has become the goal of research in the field of hCAIs.

Herein we reported the synthesis and biochemical evaluation of a series of 4-(4-aroylpiperazine-1-carbonyl)benzenesulfonamides, that were designed by decorating the benzoyl tail with hydrophobic/hydrophilic groups able to establish additional interactions within the middle/top area of hCA catalytic site[2]. We focused our interest on the development of newer analogs bearing hetero aroyl systems in place of aroyl-substitued one. All synthesized compounds were screened against selected druggables isoforms (e.g. hCA VII, IX, XII and XIV) and the K_i values were compared with the inhibitory effects against ubiquitous hCA I and II. Notably, this hCAI series was effective at low nanomolar range and several compounds demonstrated an unexpected favorable selectivity ratio for inhibition of the target isoforms over the ubiquitous hCA I and II. X-ray crystallography and docking studies confirmed that this class of benzenesulfonamides binds hCAs through the canonical anchoring of the benzenesulfonamide moiety to the zinc ion (Figure 1), while further interactions between the tail and the protein residues located in the middle/top area of the active site cavity stabilize the binding. Overall, this study furnished new suggestions about the structural requirements controlling affinity and or selectivity towards druggable hCAs.

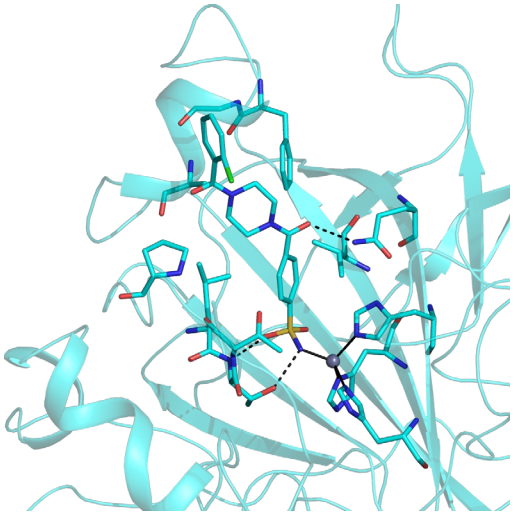

Figure 1. Active site view of hCA VII isoform in complex with benzenesulfonamides inhibitor.

Acknowledgements

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Small-molecule inhibitors targeting AKT1 active and allosteric sites

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AKT (also known as protein kinase B - PKB) is a serine/threonine protein kinase, belonging to the AGC kinase family and involved in PI3K-AKT pathway. Activated by the phosphorylation of PI3K, AKT plays a pivotal role in essential signaling cellular mechanisms including cell growth, cell cycle progression, invasion, angiogenesis, cell apoptosis inhibition, regulation of glucose metabolism, genome stability, transcription, protein synthesis and neovascularization.[1] Given its prominent role, a dysregulationof AKT activity is associated with several disorders like cancerogenesis, neurological diseases, insulin signaling and diabetes, cardiovascular processes, pulmonary fibrosis, inflammation and autoimmune diseases.[2] This plethora of effects makes AKT an attractive target for drug discovery.

Although allosteric and ATP-competitive small molecule inhibitors targeting AKT are currently in clinical trials, to date no drugs against this protein have yet been approved by the FDA.[3]

It should also be noted that the protein AKT comprises three isoforms (AKT1, AKT2, and AKT3), AKT1 being the most expressed and best characterized isoform in many tumors, reflecting its tissue versatility and context-specific effect.[4]

Against this backdrop, our research project is focused on the identification of new chemotypes of selective and potent AKT1 inhibitors. To this end, an in-depth analysis of the available crystal structures of AKT1 in complex with its inhibitors was conducted, obtaining useful insights both on the impact of inhibitor binding to target protein conformation and the key ligand chemical features.

Then, different protein conformations were selected as target for a funnel-like virtual screening campaign of our proprietary UNIPG chemical library. Virtual hits were submitted to biological testing to measure the inhibitory potency against the explored target AKT1.

As results, we identified three promising novel AKT1 inhibitors showing IC_{50} values ranging from 1.6 µM to 2.12 µM and associated ligand efficiency (LE) ≥ 3. Further experiments carried out on the validated hits in order to reveal their mode of inhibition, i.e. competitive, allosteric o mixed, confirmed the validaty and predictivity of the *in silico* inhibitor discovery approach.

The promising results have paved the way for Hit-to-Lead-optimization strategies, and the chemical synthesis of rationally designed derivatives is now ongoing.

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Straightforward synthesis of highly functionalized benzo[c]chromenes by Diels-Alder cycloaddition/aromatization sequence

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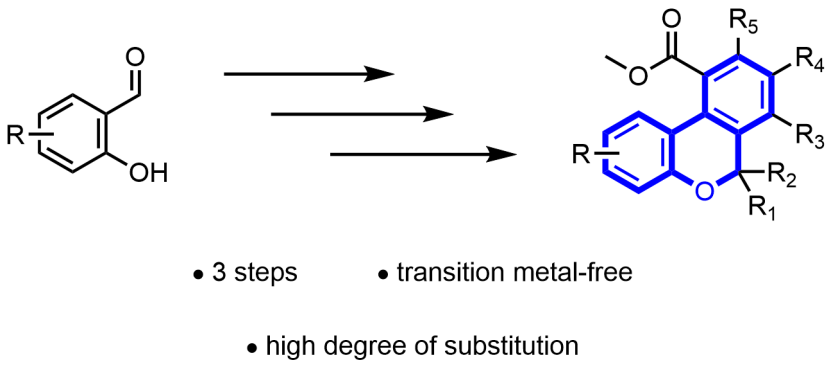
The 6*H*-benzo[c]chromene core is a common motif observed in numerous natural products and synthetic biologically active molecules.[1,2] A few notable examples can be found in the cannabinoid family, of which Tetrahydrocannabinol (THC) and cannabinol (CBN) are well known representatives. Due to its considerable pharmacological importance, the development of an efficient protocol for its synthesis is an ambitous target for both medicinal and organic chemists.

The classical synthesis is based on transition metal chemistry, where two aryl fragments are coupled using a metallic catalyst and then cyclized. Although this approach allows for the rapid synthesis of variably substituted compounds for screening purposes, the low functional group tolerance and the required purification from the catalyst hinder the possibility for production on a larger scale.[3]

Here we report our results towards the development of a modular and transition metal-free synthetic protocol for highly substituted benzo[c]chromene. We envisioned a Diels-Alder cycloaddition disconnection for a *de novo* construction of the 3,4-fused benzene ring, follower by oxidation/aromatization of the intermediate cycloadduct to obtain the desired substituted 6*H*-benzo[c]chromene compounds.

The chromene core is quickly assembled in a 3-step sequence from commercially available salicylaldehdes, α, β-unsaturated carbonyl compounds and alkynes substituted with an electron withdrawing group. Due to this modular nature of this approach, the degree of substitution of the final product can be easily modulated by choosing the appropriate starting material.



Scheme 1. General scheme for the synthesis of benzo[c]chromenes.

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Further exploration of different nitrogen heterocycle scaffolds as potent HNE inhibitors

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Chronic inflammatory diseases represent a wide number of common and highly disabling pathologies that are characterized by a perpetuating and persistent long-term inflammation. These pathologies, such as rheumatoid arthritis, chronic obstructive pulmonary disease, psoriatic arthritis, and inflammatory bowel disease, are strongly debilitating and have a deep impact on people's quality of life.[1] Furthermore, there are not effective therapies to eradicate chronic inflammation, and new methods are required to treat and control these types of diseases. Human neutrophil elastase (HNE) represents a relatively new target in chronic inflammation treatment. It plays an important physiological role in many processes, such as inflammation, and it is involved in a variety of pathologies affecting the respiratory system.[2] In medicinal chemistry, nitrogen heterocycles scaffolds play an important role in drug design, being part of more than 85% of biologically-active chemical molecules. Thus, we designed, synthesized, and performed pharmacological evaluation of new HNE inhibitors as further elaboration of indazole and 7-azaindole nuclei, which were previously studied in our research laboratory.[3-6] We obtained potent HNE inhibitors, whose structure is presented in Figure 1, exhibiting IC_{50} values in the low nanomolar range (10-89 nM) and good chemical stability ($t_{1/2}$ > 6h). *In silico* ADMET studies suggested that most of the new compounds are optimally absorbed, distributed, metabolized, and excreted. Finally, molecular modeling studies highlighted the differences in interaction of these inhibitors with the enzyme depending on the number and position of the nitrogen atoms.

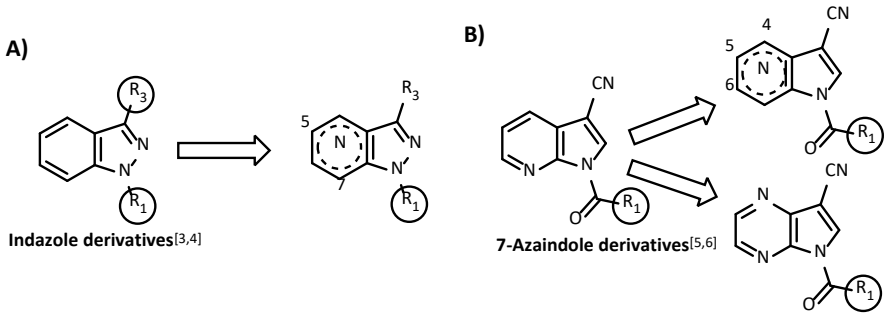

Figure 1: Indazole and 7-Azaindazole derivatives elaboration

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Capillary volumetric microsampling for the evaluation of methadone use and misuse

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In recent years, the synthetic opioid methadone ((RS)-6-(dimethylamino)-4,4-diphenylheptan-3-one, MTD, Figure 1) is being increasingly used for recreational purposes with several health and safety problems. At the same time, MTD is an important drug prescribed in different therapy such as methadone maintenance treatment (MMT) programs. For these reasons, it is essential monitoring the use and misuse of this drug. Traditionally, assessment of MTD levels is performed by blood plasma analysis and samples are collected by venepuncture. The patients are often reluctant to this invasive practice which also requires proper facilities and specialised personnel.

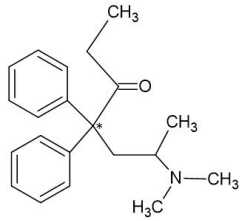

Figure 1. Chemical structure of MTD

For these reasons, the development of novel and accurate sampling strategies able to increase subject compliance is of great relevance in MTD level monitoring process. To this aim, the use of biological fluid microsamples collected and dried on specific supports represents a valid alternative with several advantages, regarding sample storage and shipment (controlled temperature precautions are not required and samples can be stored in a reduced space), increasing sample stability and patient compliance with the possibility for home-sampling. Microsampling strategies have found several applications in several fields in these last years, as in the analysis of illicit drugs and therapeutic drug monitoring (TDM).[1] In this study, microsample collection was designed and developed by exploiting a novel handheld technology, hemaPEN, able to simultaneously generate four replicates of fixed-volume dried blood samples (DBS) from a single drop of capillary blood obtained by finger pricking.[2] All sample collection and pretreatment steps were thoroughly optimised for the extraction and evaluation of MTD in spiked blood samples. The developed strategy was validated in terms of sample extraction, selectivity, precision and sample stability, also evaluating possible haematocrit effects on sampling and analysis performances. Microsamples were analysed by an original method based on HPLC coupled to coulometric electrochemical detector (ED) and successfully validated in terms of extraction yield (always higher than 90%), sensitivity (LOQ = 5.0 ng/mL) and precision (RSD% < 10.0). Different electrochemical conditions were carefully studied and set up in order to define and optimise the best parameters for MTD detection. In particular, the detection was carried out in oxidation mode, using a potential of +0.700 V and a pH of 6.4 for the buffer solution. Once extensively applied to the analysis of real samples from patients or abuser subjects, obtaining a significant amount of data allows the correlations between MTD levels assessed in dried microsamples with those obtained by employing reference methods on fluid matrices. At the same time, ED detection, compared to other advanced analysis systems such as mass spectrometry (MS), showed comparable accuracy and sensitivity, also providing satisfactory reliability and robustness. This can allow to propose the strategy developed herein as a valuable alternative for MTD monitoring, to be applied in a range of fields, such as the prevention of illicit use or in the TDM in patients undergoing MMT in order to adjust and personalise therapy regimens.

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A dual-target approach in anti-tuberculosis therapy: new MptpB/MbtI inhibitors

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Tuberculosis (TB) is an ancient disease that still remains the world's top killer among infectious illnesses, despite the considerable global effort to reduce its incidence.[1] TB is mainly caused by *Mycobacterium tuberculosis* (Mtb) and spreads through respiratory droplets, produced by infected individuals.[2] As shown in the last WHO Global TB Report, 7 million people were diagnosed and treated for TB in 2018, and 1.5 million people died.[1] New and more effective anti-TB drugs acting on novel targets are needed to shorten treatment time, and to contrast drug resistance and progressive loss of antibiotic efficacy.

An innovative strategy to achieve this goal may consist in the development of selective multi-target drugs. In this context, MptpB, a mycobacterial protein tyrosine phosphatase (PTP), and MbtI, a salicylate synthase, were found to be promising targets for the development of innovative antitubercular agents. MptpB dephosphorylates a variety of substrates, playing a key role in the survival of the *bacilli* during the infection. Moreover, it can attenuate the immune response of the host, by interfering with signal transduction pathways; it inhibits macrophage apoptosis, by activating Akt and blocking caspase 3 activity, and depletes PI3P, arresting the maturation of the vacuoles.[3] MbtI is a Mg-dependent salicylate synthase, which catalyses the first step of the synthesis of siderophores, iron-chelating molecules that bind iron and internalize it into the mycobacterial cell. Since iron is essential for the survival of the *bacilli* during the infection, the inhibition of siderophore synthesis is an important target for novel antitubercular agents.[4]

On these bases, the aim of this study was the design, synthesis, characterization, and biological evaluation of innovative MptpB/MbtI inhibitors. With this purpose, we selected a representative group of 5-phenylfuran-2-carboxylic acid derivatives from our in-house library of MbtI inhibitors and assayed them against MptpB. Based on the results of these tests, we synthesised some analogues to improve their activity. The results of these preliminary studies, which led to the selection of some promising dual-targeting candidate inhibitors, will be presented here.

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Nutraceutical Extract from autochthonous Tuscan olive leaves incorporated in poly(Hydroxybutyrate-co-Hydroxyvalerate) (PHBHV) for medical application

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Olive leaves extract (OLE), derived from discarted leaves of olive tree, represents an important biowaste source of phenolic compounds whose the main representative is oleuropein. Oleuropein is a polyphenol endowed with nutraceutical properties, such as antiproliferative, cardioprotective, antidiabetic and gastroprotective actions, due to its antioxidant and anti-inflammatory activities.[1] Besides oleuropein, in olive leaves there are also natural compounds such as flavonoids and simple phenols which are also endowed with antioxidant activity. The aim of this project is to incorporate an OLE, obtained from *Olivastra seggianese* groves, in a biobased polymer scaffolds, i.e., poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) (Figure 1A), and demonstrate the potential use of this agrifood waste in biomedical applications. PHBHV is a natural polyester produced by a great variety of microorganisms through fermentation and endowed with biocompatibility and biodegradability properties which production and degradation have no negative ecological impact.[2]

Initially the qualitative and quantitative analysis of phenols present in OLE were performed by High Performance Liquid Chromatography (HPLC) confirming that the main phenolic compounds present were oleuropein and luteolin-7-O-glucoside. Then the OLE-loaded PHBHV (Figure 1B) was prepared by electrospinning technique, and characterized by FT-IR and Scanning Electron Microscopy (SEM) analysis. Moreover the release profile of phenols from OLE-loded in PHBHV was evaluated.

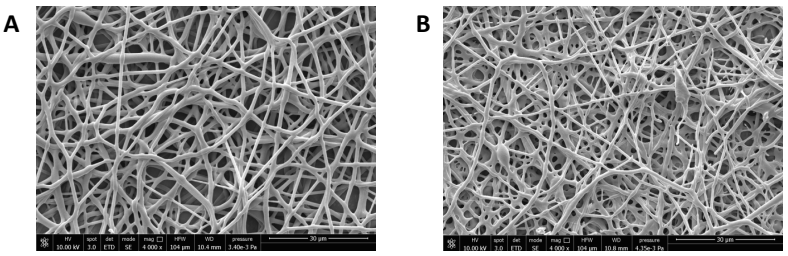

Figure 1. SEM images of PHBHV fiber (**A**) and PHBHV + OLE fiber (**B**). The scale bar in inserts is 30 µm.

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Oligo(ethylene imine)-grafted Linear and Star Homopolymers for DNA delivery: Odd-Even Correlated Transfection Efficiency

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The development of safe and efficient gene carriers is one of the prerequisites for successful gene therapy.[1] Synthetic amine-containing polycations, such as branched and linear poly(ethylene imine) (PEI) of different molecular weights, have been widely studied toward DNA and siRNA delivery *in vitro*. In particular, DNA complexes with high molecular weight PEIs (molecular weight ~ 25 000 g mol^{-1} (PEI25k)) show high transfection efficiencies. However, this high efficiency is accompanied by high cytotoxicity due to the strong interactions with the cytoplasmic and mitochondrial membranes. In contrast, DNA complexes with low molecular weight PEIs (oligo(ethylene imine)s, OEI] present a lower cytotoxicity but also an insufficient transfection efficiency, with the latter limiting their usefulness for gene transfer. Studies in the past years indicated that a combination of a high transfection efficiency with a low cytotoxicity could be achieved if high molecular weight uncharged polymers grafted with OEIs were employed. These studies showed that transfection and cytotoxicity depended on the number of ethylene imine repeating units, and the presence of other functional groups on the OEI grafts.[2]

The present study aims to further investigate the effect of the number of ethylene imine repeating units on transfection efficiency and cytotoxicity. In particular, the odd-even effect of the number of ethylene imine units in the side-groups of totally abiotic synthetic polymers on their efficiency in DNA transfection is studied. One star homopolymer and one linear homopolymer series based on glycidyl methacrylate (GMA) were used as these synthetic polymers. Each polymer separately grafted with one of five linear oligo(ethylene imine)s (OEI), comprising from one to five ethylene imine repeating units: ethylene diamine (EDA), diethylene triamine (DETrA), triethylene tetramine (TrETA), tetraethylene pentamine (TEPA) and pentaethylene hexamine (PEHA), thereby resulting in ten different polymers. All polymers were evaluated in terms of their efficiency to transfer to C2C12 mouse myoblast cells plasmid DNA encoding firefly luciferase. The transfection efficiency indeed displayed an odd-even pattern, with all OEI-grafted polymers with an odd number of ethylene imine repeating units exhibiting higher transfection efficiency compared with those possessing an even number of ethylene imine repeating units. This trend was the reverse from the one originally observed by Kataoka and coworkers[2] on a series of polyaspartamides *N*-substituted with the same OEI. This difference can be attributed to one of the OEI nitrogen atoms participating in the formation of a non-ionizable amide group in Kataoka's polymers compared with the amine nitrogen atoms in the polymers in the present study that are all ionizable. The odd-even effect was more pronounced for the star polymers, while in case of the linear polymers, the odd-even effect was only observed for the lowest polymer loading. The cytotoxicity of all OEI-grafted polymers also followed an odd-even pattern.[3]

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Identification of benzimidazole-based compounds as selective ligands for the first bromodomain of BET proteins: rational design, synthesis and in vitro evaluation.

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Bromodomains (BRDs) are epigenetic readers able to selectively recognize the acetyl-lysine group on histone and non-histone proteins. The Bromo and Extra-Terminal Domain (BET) family is perhaps the most important group of BRD-containing proteins, whose members (BRD2, BRD3, BRD4, and BRDT) contain two highly homologous bromodomains: BD1 and BD2.[1] Their role in different biological processes makes them attractive therapeutic targets.[2] To date, several ligands have been identified as potent BET ligands and some compounds have been entered in clinical trials.[3] However, most of these compounds are pan BET ligands, with no selectivity between each BET member and/or their singular BD1/BD2 domains.[4]
Herein, we report the design, the synthesis and the in vitro evaluation of a small library of benzimidazole-based compounds (Figure 1). The benzimidazole nucleus was formally obtained applying a frozen analogue approach to **MS436**, a potent and selective BD1 ligand of BETs. [5]

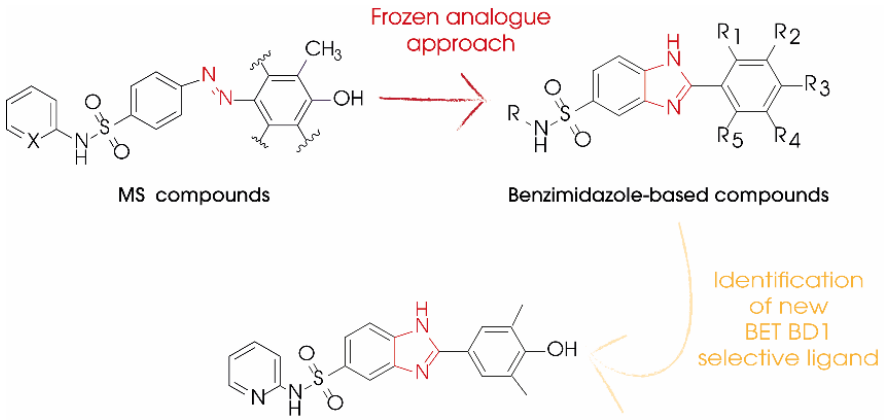

Figure 1. Aim of work: Identification of benzimidazole-based compounds as BD1 selective ligands.

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Biocatalytic synthesis of two pharmacologically active compounds: (*S*)-pramipexole and its enantiomer, dexpramipexole

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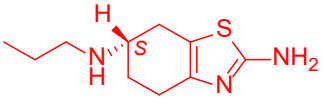
Many pharmaceutically active compounds contain a chiral core inside their structure. Therefore, compounds formed highly selectively are valuable products. The chemo-, regio-, and stereo-selectivity required could be achieved using biocatalysts (enzymes or microorganisms), which can work on a wide range of substrates, in mild reaction conditions and not only in aqueous solutions, but also in organic solvents. Moreover, biocatalysis meets the green chemistry principles.

Our research work focuses on the biocatalytic synthesis of key building blocks affording to pharmaceutically active compounds, currently used in therapy [1, 2].

Following this approach, we were able to synthetize the enantiopure key intermediates of (*S*)-pramipexole, a synthetic dopaminergic agonist utilized as anti-Parkinson drug, and (*R*)-pramipexole, which has been studied as therapeutic agent against Amyotrophic Lateral Sclerosis (ALS) and now it has found new interest for the potential treatment of Eosinophilic Asthma and Hypereosinophilic Syndrome.



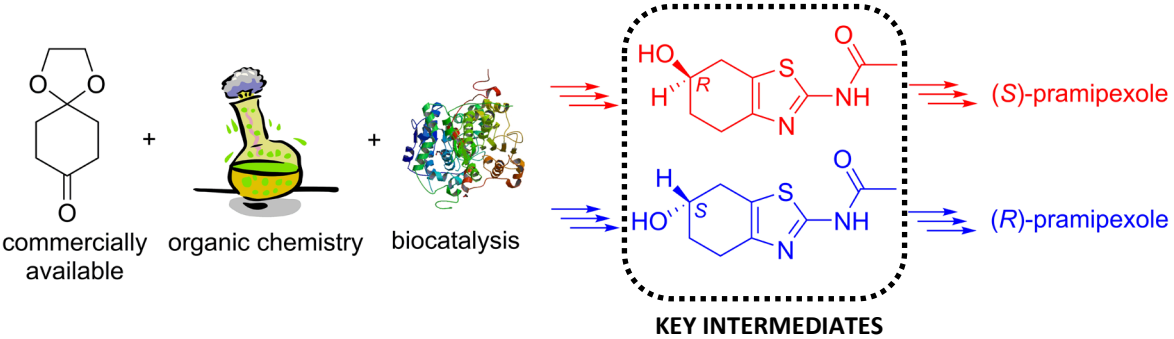
(*S*)-pramipexole

(*R*)-pramipexole

Two different biocatalytic approach allowed us to stereoselectively synthesize these compounds:

1) After the investigation of the activity and selectivity of different microorganisms (especially yeasts), we obtained the enantiomerically pure synthons for the preparation of (*S*)- and (*R*)-pramipexole by means of *Saccharomyces cerevisiae*, the common baker's yeast, a cheap and easy to handle microorganism.

2) The two enantiomerically pure synthons were achieved by means of a double kinetic resolution catalized by a commercially available purified enzyme, Lipase A from *Candida antarctica*, under irreversible transesterification conditions.



The definition of the stereochemistry of the two enantiomers was also carried out by means of single crystal X-ray analysis.

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Molecular Recognition Properties of IDO1 Crystal Structures: Computational and Biophysical Studies

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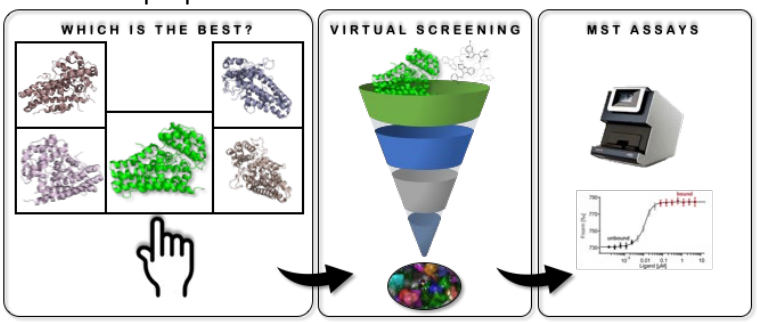
Indoleamine 2,3-dioxygenase 1 (IDO1) works as oxidoreductase along the Kynurenine Pathway (KP), catalyzing the first and rate limiting step responsible for the convertion of L-Tryptophan (L-Trp) into N-Formylkynurenine (NFK). IDO1 is a heme-containing enzyme and is involved in the oxidative cleveage of the indole C2-C3 double bond of L-Trp through the incorporation of O_2.[1] A dysregulation of IDO1 levels is observed in patients affected by neoplasia, with high levels of IDO1 expression, or auto-immune/neuroinflammatory diseases, where IDO1 levels are defective.[2] Considering the failures behind the drugs in clinical trials as IDO1 inhibitors, f.e. Epacadostat, the challenge in the area of IDO1 drug discovery is still open and an improvement is needed.[3]At this point in order to find and optimize different chemical scaffolds, Strucuture-Based Drug Design (SBDD) becomes a pivotal strategy. SBDD has been widely applied on IDO1 drug discovery, and since 2006 many crystals have been published. The large available crystallographic data of IDO1 allowed the chance to analyze their features. In this poster, computational studies are used to analyze which is the structural information that can still be exploited for successful SBDD, and biophysical studies are carried out in order to prove the *in-silico* results. Only 54% of the released crystals has been used in computational studies, and among them only 25% has been employed in the SBDD and Virtual Screening (VS) methods. Computational studies assessed the presence of secondary structural motifs, caused by the presence of different ligands, which affect the binding site and the small domain features.[4] These structural differences led us to deeply analyze how these differences can influence docking results. Employing the docking results as input in a Principal Component Analysis (PCA), a grouping of the proteins based on the crystallized ligands emerged. The PCA results have been analyzed critically highlighting which are the structures already used in SBDD and VS, showing that not all the conformational space of IDO1 has been exploited. According to these results, a VS has been performed employing three different IDO1 proteins from different groups. Next, the top 100 and 1000 ranking poses have been compared. Finally, the results have been clusterized and the cluster centroids have been tested through a MicroScale Termophoresis (MST) protocol, and the obtained data have been analyzed both *in-vitro* and by evaluating their physicochemical properties.



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A challenge in the treatment of neuropathic pain: synthesis of new photostable dihydropyridines

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Voltage-gated calcium channels play a fundamental role in essential body functions and the molecules that block these channels are of potential use in the treatment of various diseases. L-type calcium channel blockers, are widely used in the treatment of cardiovascular diseases, particularly hypertension and angina pectoris [1]. T-type calcium channels also attract great interest because of their potential in treating a variety of disorders, including pain and epilepsy [2]. 1,4-Dihydropyridines (DHPs) are always been used as L-type calcium channel blockers in the hypertension specially. Ongoing efforts to modify the DHP scaffold to modulate calcium channel blocking activity have led to a new class of compounds with a condensed ring system (hexahydroquinoline) [3]. Among them, the compound benzyl 4-(2-hydroxy-5-nitrophenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate has been verified to block the L-type calcium channel (Cav1.2), the main target of DHPs, but also the T-type calcium channel isoform Cav3.1. It was concluded that this compound could serve as a new scaffold for the treatment of hypertension by reducing aldosterone secretion through inhibition of the T-type calcium channel along with the well-known antihypertensive effect by inhibition of the L-type calcium channel. Modification of the ester moiety determined the blocking affinity for both L- and T-type channels. Exemplarily, 3-pyridylmethyl moiety as the alkyl group in the ester function provided the development of DHPs with high selectivity for T-type calcium channels over L-type [3-4]. These findings suggested that focusing on the modifications of the ester side chain is a rational approach to identify more efficient calcium channel blockers in the treatment of neurological diseases. Exposure of these molecules to natural or artificial light leads to a significant production of singlet oxygen, superoxide, or both of them, which in most cases are responsible of photosensitive/phototoxic effects [5]. All the synthesized compounds were subdued to photodegradation tests, in accordance with the ICH international rules [6]. Concentration of parent compounds and by-products was calculated by multivariate curve resolution - alternating least squares (MCR-ALS) applied to the spectral data. The kinetic degradation parameters of all compounds were calculated and all the DHPs photoproducts estimated by MCR-ALS [5]. Because of their well-known instability to light, several studies have been proposed for producing formulations able to provide a valid photoprotection for this class of drugs. The incorporation in supramolecular systems has been proposed as a means to increase the stability of drugs. In particular, cyclodextrins have shown the most promising results due to their ability to improve aqueous solubility, chemical stability and bioavailability for several drug molecules by incorporating them in their core. The use of edible polymeric matrices as new photoprotective systems is under investigation.

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(2-Imidazolin-4-yl)phosphonates as Imidazoline I_2 Receptor Ligands for Neurodegenerative Diseases

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Imidazoline I_2 receptors (I_2-IRs) are distributed in the CNS and altered in brain disorders such as Huntington, Parkinson's and Alzheimer's diseases and depression. So far, few I_2-IRs ligands have been used to decipher the pharmacological implications of these receptors. We propose to add new I_2-IR ligands to the scarce arsenal and study the pharmacological potential of I_2-IRs ligands to treat unmet neurodegenerative diseases (ND). In this context, we have described an efficient and user-friendly synthetic process involving the combination of isocyanide-based multicomponent reaction and microwave heating that generates unprecedented (2-imidazoline-4-yl)phosphonates. Radioligand binding studies in human brain samples showed that the new family displayed a higher affinity for I_2-IRs than standard idazoxan, and high I_2/□$_2$ selectivity. [1] Theoretical studies were carried out for designing compounds with enhanced activity and selectivity upon I_2-IRs based on created 3D-QSAR model. *In vivo* studies in the female senescence accelerated mouse-prone 8 mice (SAMP8) with two representative compounds, **MCR5** and **MCR9**, showed beneficial effects in behavior and cognition. Changes in molecular pathways implicated in oxidative stress, inflammation, synaptic plasticity, and apoptotic cell death were also studied. Treatments with these I_2-IR ligands diminished the amyloid precursor protein processing pathway and increased Aβ degrading enzymes in the hippocampus of SAMP8 mice. These results collectively demonstrate the neuroprotective role of these new I_2-IRs ligands in a mouse model of brain aging through specific pathways and suggest their potential as therapeutic agents in brain disorders and age-related ND. [2] Herein, we present further studies in the effect of **MCR5** on behavioral and psychological symptoms of dementia (BPSD) and cognitive decline in SAMP8. **MCR5** oral administration improved cognitive performance and BPSD-like phenotype, demonstrating anti-depressant and anti-anxiety-like effects in older SAMP8 mice. In line with the behavioral improvement, changes in molecular pathways underlying depression and anxiety phenotype were observed after MCR5 treatment. This is the first study in which both cognitive and non-cognitive effects have been demonstrated in AD mice for an I_2-IR ligand. [3]

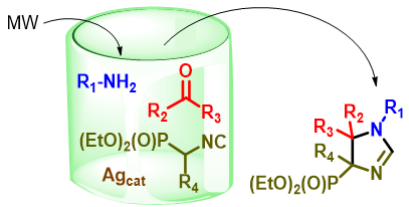

Figure 1. Synthesis of (2-imidazoline-4-yl)phosphonates

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Benzofuranylimidazoles: synthesis, affinity for imidazoline I_2 receptors and improvement in the cognition of 5xFAD mouse model

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Imidazoline I_2 receptors (I_2-IR) are widely distributed in the CNS and appear increased in the patients that suffer from Alzheimer's disease (AD). Since structural data for I_2-IR remains unknown, the discovery of selective I_2-IR ligands is necessary for their pharmacological characterization. [1] Recently, we focused our attention in the development of structurally new families of compounds endowed with an outstanding affinity and selectivity upon I_2-IR. [2,3] Here, we describe the synthesis and full characterization of 10 members of a family embodying a 2-(2-benzofuranyl)-2-imidazole nucleus, we assessed their pharmacological profile and selectivity through competition binding studies in human tissues against the selective I_2-IR radioligand [^{3}H]-2-BFI, and we performed 3D-QSAR studies of the family and *in silico* ADME properties. Then, a representative compound, LSL60101, was selected to carry out preliminary DMPK studies, including chemical stability, PAMPA-BBB permeability assay, solubility, cytotoxicity, microsomal stability, and cytochromes inhibition. Finally, we assessed the neuroprotective effect of LSL60101 by evaluating specific oxidative stress markers under oxidative damage and transcription factors related with OS machinery in 5xFAD, an early-onset mouse transgenic model of AD. We found a significant cognitive improvement in the treated animals and the biomarkers related to neurodegeneration (Figure 1). To sum up, we propose a new I_2-IR ligand that supports that I_2-IR constitute a relevant pharmacological target for the therapeutic strategy against AD.

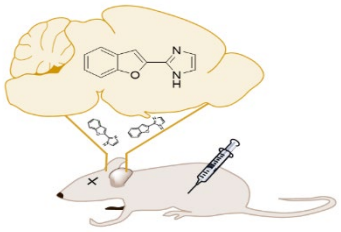

Figure 1. Neuroprotection studies of LSL60101

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A single-molecule based polypharmacological approach against Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia worldwide, only in Europe, it affects 10 million people and its incidence is expected to double by 2030.[1] Nevertheless, the research of an effective treatment still represents a critical challenge. Indeed, because of the multifactorial nature of AD, the classic drug discovery paradigm *one-target-one-drug-one-disease* results ineffective, suggesting a polypharmacological approach as the most promising to treat complex diseases.[2] Currently, one of the most promising polypharmacological approaches in AD is an ongoing phase 3 clinical trial focused on the synergistic modulation of neuroinflammation by co-administration of two well-known anti-inflammatory drugs, i.e., ibuprofen (IBU) and cromolyn (CR). In the trial, the two drugs are administrated by two different pharmaceutical forms, IBU as oral tablet and CR as inhaled powder formulation.[3] Inspired by the clinical results, this project aims to harness the IBU-CR drug combination to develop a polypharmacological approach based on codrugs,[4] aimed to obtain an anti-neuroinflammatory effect synergistic with the advantages of a single molecule. With this in mind, we rationally designed and synthesized a small library of IBU-CR co-drugs, by introducing cleavable bonds, which can be released *in vivo,* after hydrolysis.[4] The new molecules have been designed by fusing or linking strategies, reproducing a 1:1 (**1**-**4**) or a 2:1 IBU:CR (**5**-**8**) ratio (Figure 1). Moreover, the designed co-drugs might improve the pharmacokinetic profile of the two starting drugs. Indeed, the negative charges of the acid groups of both IBU and CR have been masked and the overall co-drugs' lipophilicity has been improved favoring blood–brain barrier (BBB) permeation and oral administration avoiding the CR inhalation powder, which seems not suitable due to the loss of coordination typical of AD patients. In detail, the design of the 1:1 co-drugs exploited a fusing strategy by the direct esterification of the carboxylic group of IBU with the hydroxyl function of CR. In the case of the 2:1 co-drugs, a linking strategy between the two carboxylic groups of CR and the amine or hydroxyl function of the ethylene glycol, ethanolamine, and ethylenediamine linkers, with different metabolic stability. Co-drugs' (**1**-**8**) metabolic stability (up to 6 hours) has been evaluated in human plasma. The results demonstrated that 2:1 co-drugs having amide bonds are at least 75% stable, suggesting their availability in crossing the BBB without metabolic modifications, and the possibility to release the parent drugs directly in the same target neuronal cells and at the same time. Concomitantly, **1**-**8** cytotoxicity assay has been performed on primary cell line of cerebellar granule neurons (CGNs) showing comparable or lower toxicity to the parent drug combinations. Future studies will include the evaluation of the immunomodulatory effect of the synthesized molecules compared to the IBU-CR combination. In addition, based on the antiaggregating properties of CR, the study of co-drug anti-amyloid profile will be pursued.

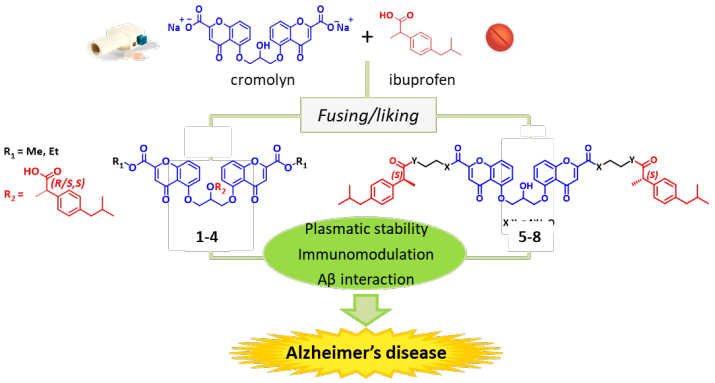

Figure 1. IBU-CR co-drug design strategies and biological assays.

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Fluorine Impact on the Drug-like Profile of MTDLs against Alzheimer's Disease

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Among neurodegenerative diseases, a major role is played by Alzheimer's Disease (AD), the most common cause of senile dementia. Given the multifactorial nature of this pathology and the lack of curative treatments, the multitarget approach represents a promising strategy [1] aiming at disease-modifying effects through the simultaneous modulation of several mechanisms with a unique chemical entity. Our work has long been devoted to rationally designed coumarin-based Multi-Target-Directed Ligands (MTDLs), leading to highly active dual selective inhibitors of two key enzymes in AD, monoamine oxidase B (MAO B) and acetylcholinesterase (AChE).[2, 3]

Fluorine and fluorinated motifs are widely used in medicinal chemistry to improve a molecule's efficiency and drug-like features.[4] In this contest we were interested on studying the impact of fluorine atom(s) on both the *in vitro* activity and *in vitro* early-ADME profile of our most potent and selective dual hits, by placing *gem*-difluoromethyl groups as bioisosters for primary alcohols and by introducing a *m*-fluoro substituent on the phenyl ring (Figure 1) to mitigate its metabolic liability.

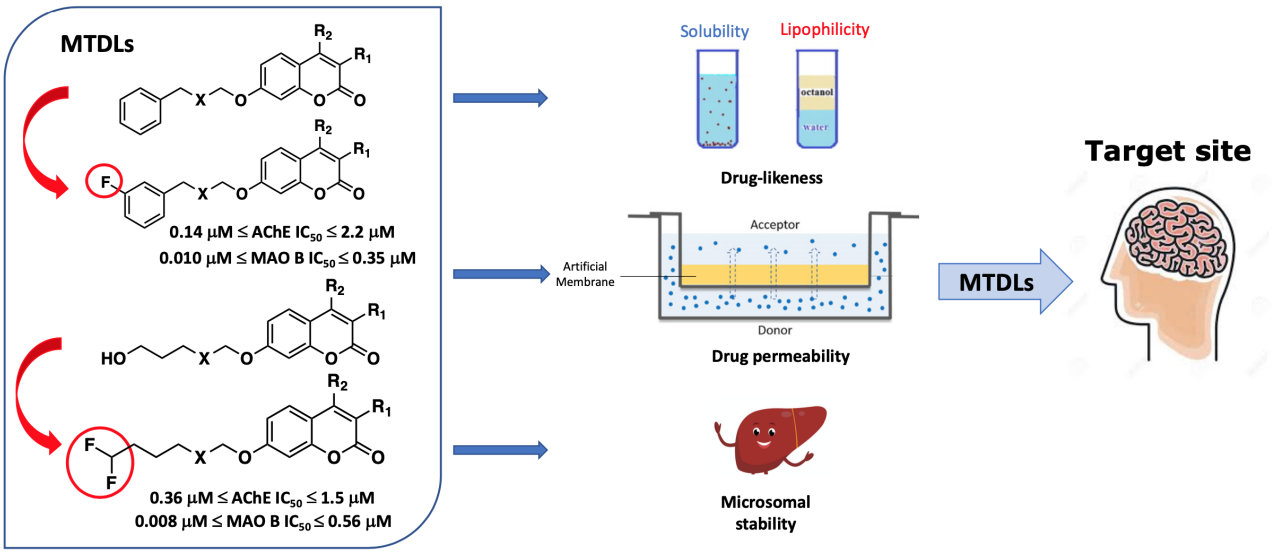

Figure 1. Fluorinated MTDLs and non fluorinated analogues included in the *in vitro* drug-like evaluation.

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A new therapeutic approach for alzheimer's disease: novel P2X7 receptor antagonists

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Aetiology of Alzheimer's disease (AD) is not fully understood. It has been revealed that the inflammation in the early stages is its major component. Therefore, the identification of anti-neuroinflammatory drugs could lead to novel treatments for AD. [1]

The P2X7 receptors (P2X7R) are implicated in the regulation of inflammatory response playing a crucial role in the neurodegeneration process. These receptors belong to the family of ionotropic receptor being present on neurons, microglial cells and macrophages. The activation of the P2X7R by ATP in high concentration leads to increased cytokine IL-1β, making this receptor an attractive therapeutic target to reduce neuroinflammation. [2] Despite a variety of potent antagonists P2X7R have become available, and several of them have entered clinical trials, so far, none has been tested against Alzheimer's disease. [3]Bearing in mind the aforementioned problem, the knowledge that adamantane moiety is a common structural feature in several P2X7 antagonists and the expertise of our group in polycyclic hydrocarbons, we decided to design, synthesize and pharmacologically evaluate a series of analogues of the known Abbott's adamantane P2X7 antagonist **1**. [4] Replacement of the adamantane subunit of **1** led to our new hit **2**, endowed with higher potency (IC_{50} = 0.3 nM) (Figure 1). Herein, we will present the synthesis and *in vitro* proof of concept of our new P2X7 antagonists as well as the screening cascade in order to identify the best candidate for an *in vivo* study in an animal model of AD.

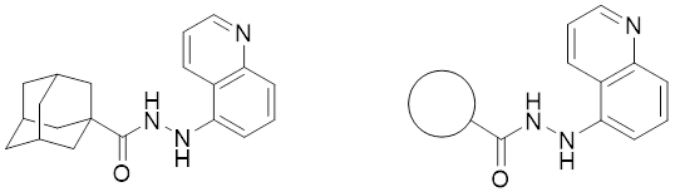

Figure 1. Structure and activity of the known Abbott's P2X7 antagonist and, of our new potent hit **2** provided with low nanomolar activity in P2X7R

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Novel 3-Aroyl-1,4-diarylpyrroles against Solid Tumors and Hematological Malignancies

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Microtubules (MTs) are an attractive target for the development of efficient anticancer agents. Evidence has accumulated correlating inhibition of tubulin polymerization and leukemic cell proliferation. [1] The activity of colchicine site agents in chronic myeloid leukaemia (CML) has not been adequately explored yet. [2] Recently, starting from previously reported aroylindoles and aroilpyrroles, we developed novel 3-aroyl-1,4-diarylpyrrole (ARDAP) derivatives to explore structure-activity relationships at the phenyls at the position 1 and 4 of the pyrrole. [3] Several compounds strongly inhibited tubulin assembly through binding to the colchicine site, the bests derivatives present the amino group in both phenyls (Table 1). Compound **1** was generally more effective as an inhibitor of the glioblastoma, colorectal and urinary bladder cancer cells (Table 2). whereas **2** consistently was more active as an inhibitor of the CML cell lines (Table 3). In animal models, the first promising compound exhibited significant inhibition of the growth of T24 bladder carcinoma and ES-2 ovarian clear cell carcinoma tumours (Figure 1). These derivatives represent robust lead compounds for the design of new anticancer agents active in different types of solid and haematological tumours.

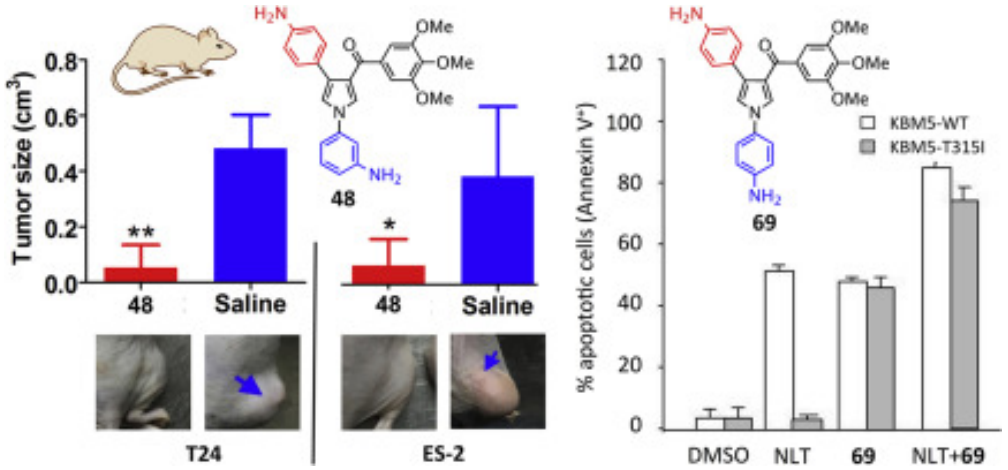

Figure 1. Biological activity of compounds **1** and **2**.

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Natural Cytotoxic Diterpenoids, as Potential Source of Drug Leads for cancer Therapy

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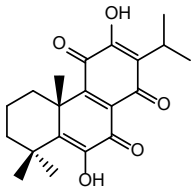
Natural products from medicinal plants represent a major resource of novel therapeutic substances for combating serious diseases including cancer. The *Plectranthus* genus (Family: Lamiaceae) represents a large and widespread group of species with a diversity of traditional uses for the treatment of several ailments. Cytotoxicity screenings have identified *Plectranthus* plants as potential sources of antitumor lead compounds [1,2]. *P. mutabilis* Codd. chemical constituents have been cited on its HPLC analysis that revealed the presence of Nepetoidins A and Nepetoidins B [3]. We therefore aimed to study the composition and biological activity of this plant to reinforce the low phytochemical information. In this study, the air-dried *P. mutabilis* whole plant was extracted in acetone using the ultra-sound assisted extraction method. Furthermore, a bio-guided fractionation was performed followed by an *Artemia salina* general toxicity assay [4]. The extract was subjected to different column chromatographies using silica or polyamide with increasing polarity to afford the metabolite Coleon U quinone (**1**), 8α,9α-Epoxycoleon U quinone (**2**), coleon U (**3**) and 7-hydro,14-deoxycoleon U (**4**). The fully structure characterization was done by 1D- and 2D-NMR, LC/MS and comparison with literature data. The *P. mutabilis* extract chromatographic profile and corresponding quantification of the four isolated compounds was performed. All the compounds were identified in the extract with Coleon U and Coleon U quinone being the major compounds in the *P mutabilis* extract.



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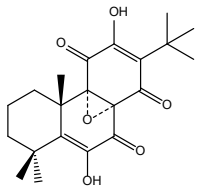

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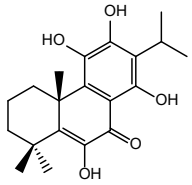

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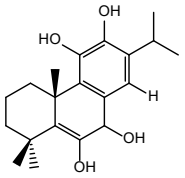

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Bio-Inspired G-Quadruplex Binders Powered by Multicomponent Reactions

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The G4s are a heterogeneous class of structural topologies composed of hydrogen-bonding arrangements of four guanine bases, namely, the G-tetrads. G4-forming motifs have also been identified in oncogene promoter regions, bringing to light the therapeutic potential for targeted gene regulation at the transcriptional level.

In the search for new drug-like selective G-quadruplex binders, a bio-inspired design focused on the use of nucleobases as synthons in a multicomponent reaction (MCR) proved to be viable and successful. Hence, a new class of multi-functionalized imidazo[2,1-*i*]purine derivatives, easily synthesized with a convergent approach, allowed the identification of the first dual *BCL2*/*c-MYC* gene promoter G-quadruplex ligand (Figure 1). Biophysical studies along with biological investigations have been carried out to assess the potency and to characterize the binding mode of the newly identified lead compound. The absence of toxicity towards normal cells, together with the small molecular weight (≅ 500), the water solubility, the ease of functionalization, and the selectivity profile are promising and desirable features to develop G-quadruplex binders as safe and effective anticancer agents.[1]

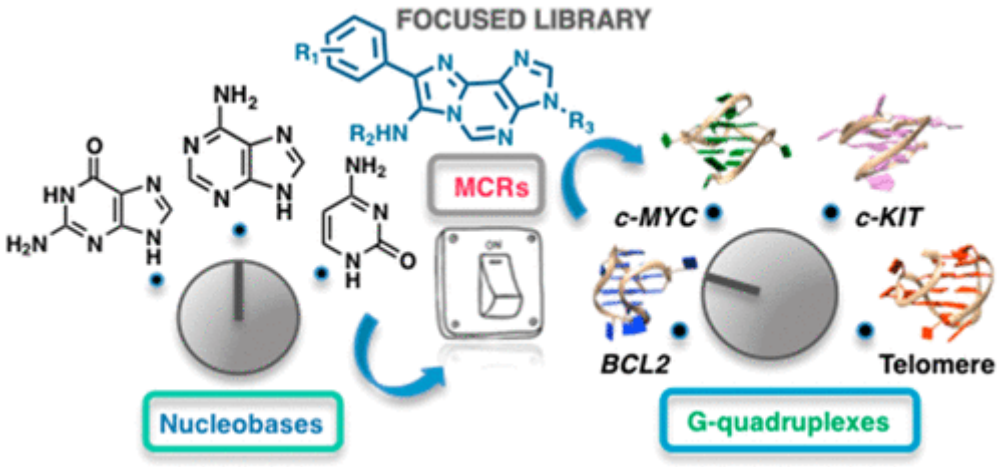

Figure 1. Multi-functionalized imidazo[2,1-*i*]purine derivatives as first dual BCL2/c-MYC G4 Binders.

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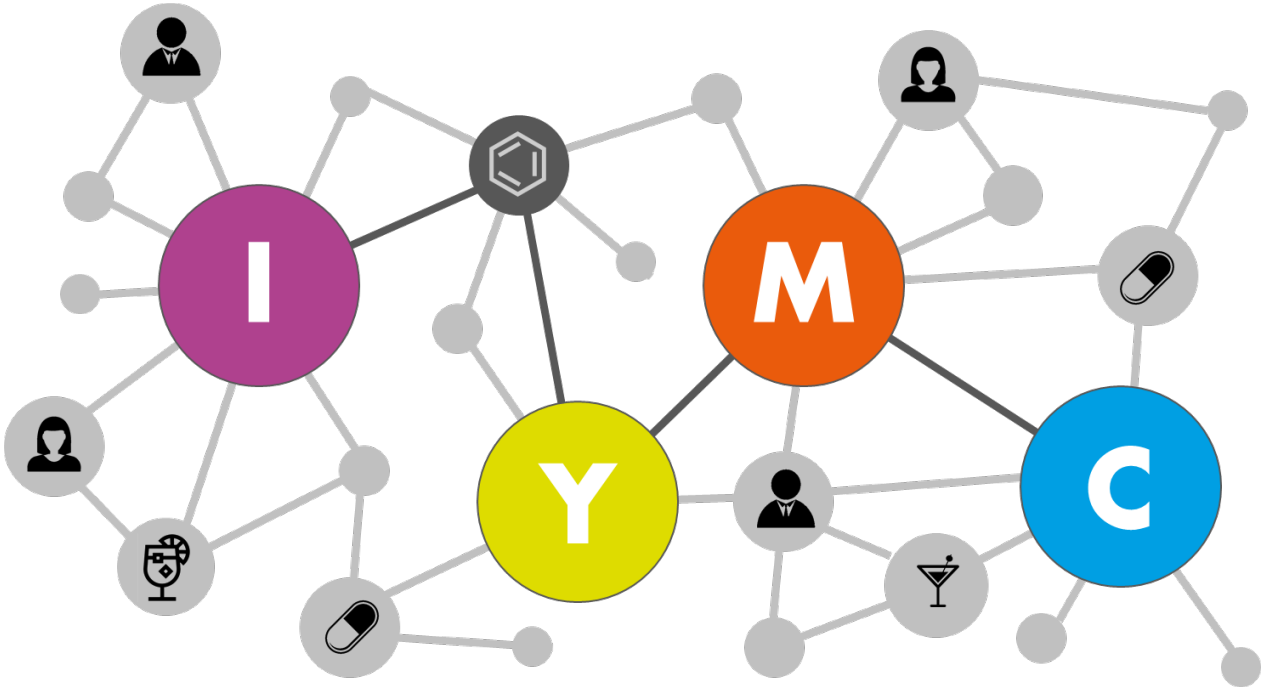

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