DESIGN, SYNTHESIS AND ANTIMICROBIAL EVALUATION OF BENZODIOXANE-BENZAMIDES FtsZ INHIBITORS: MODIFICATION OF THE LINKER BETWEEN THE TWO SCAFFOLDS

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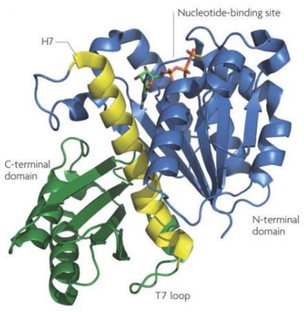
INTRODUCTION

FtsZ is a bacterial cell division protein, highly conserved among different species, which function is crucial for the viability of the cell. Therefore, FtsZ inhibitors are considered a viable tool as new antimicrobials to overcome antibiotic resistance [1].



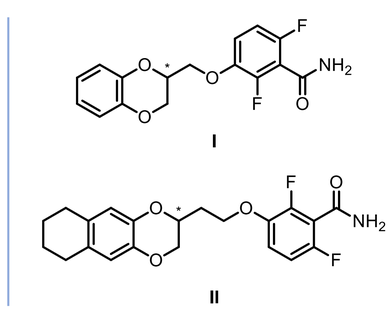


Figure 1: (left) Structure of FtsZ; (right) Structure of compounds **I** and **II**

Starting with 2,6-difluorobenzamide, over the years in our research laboratory the class of benzodioxane derivatives as FtsZ inhibitors has been developed, moving from guide compound **I** to **II** (Figure 1) [2,3], which is characterized by potent activity against different bacterial strains.

DESIGN

During the development of benzamides FtsZ inhibitors, Stokes and collaborators [4] started from the structure of PC190723, modifying it by changing the heterocycle and by including different pendants on the linker, achieving positive results (Figure 2).

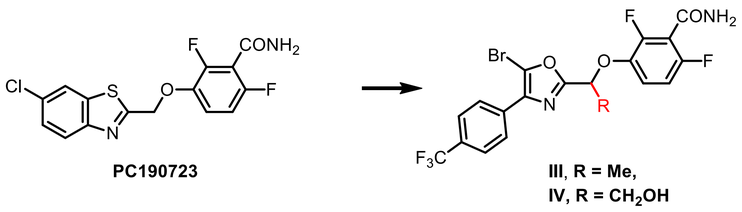


Figure 2: Structure of PC190723 and the modifications performed by Stokes and collaborators

Starting from these considerations, we designed, synthesized, purified and characterized compounds **1-3,** both *erythro* and *threo* (Figure 3).

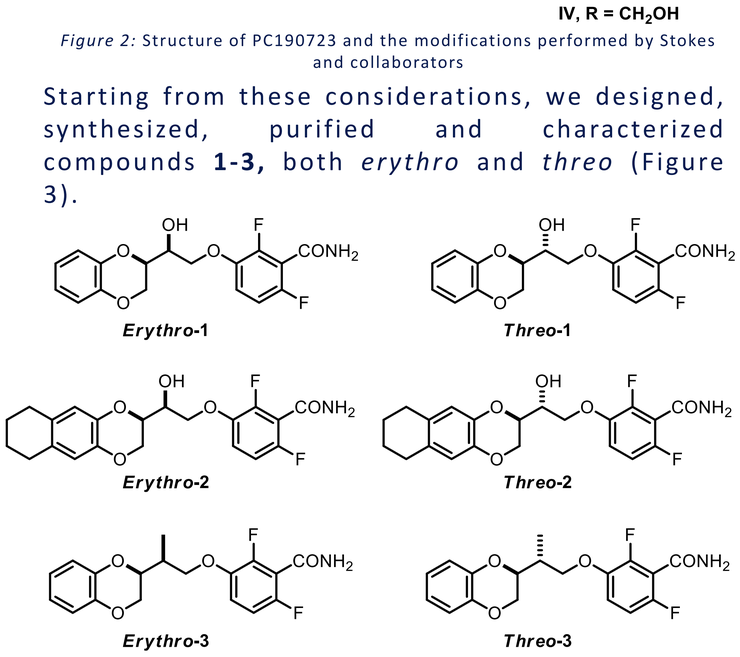
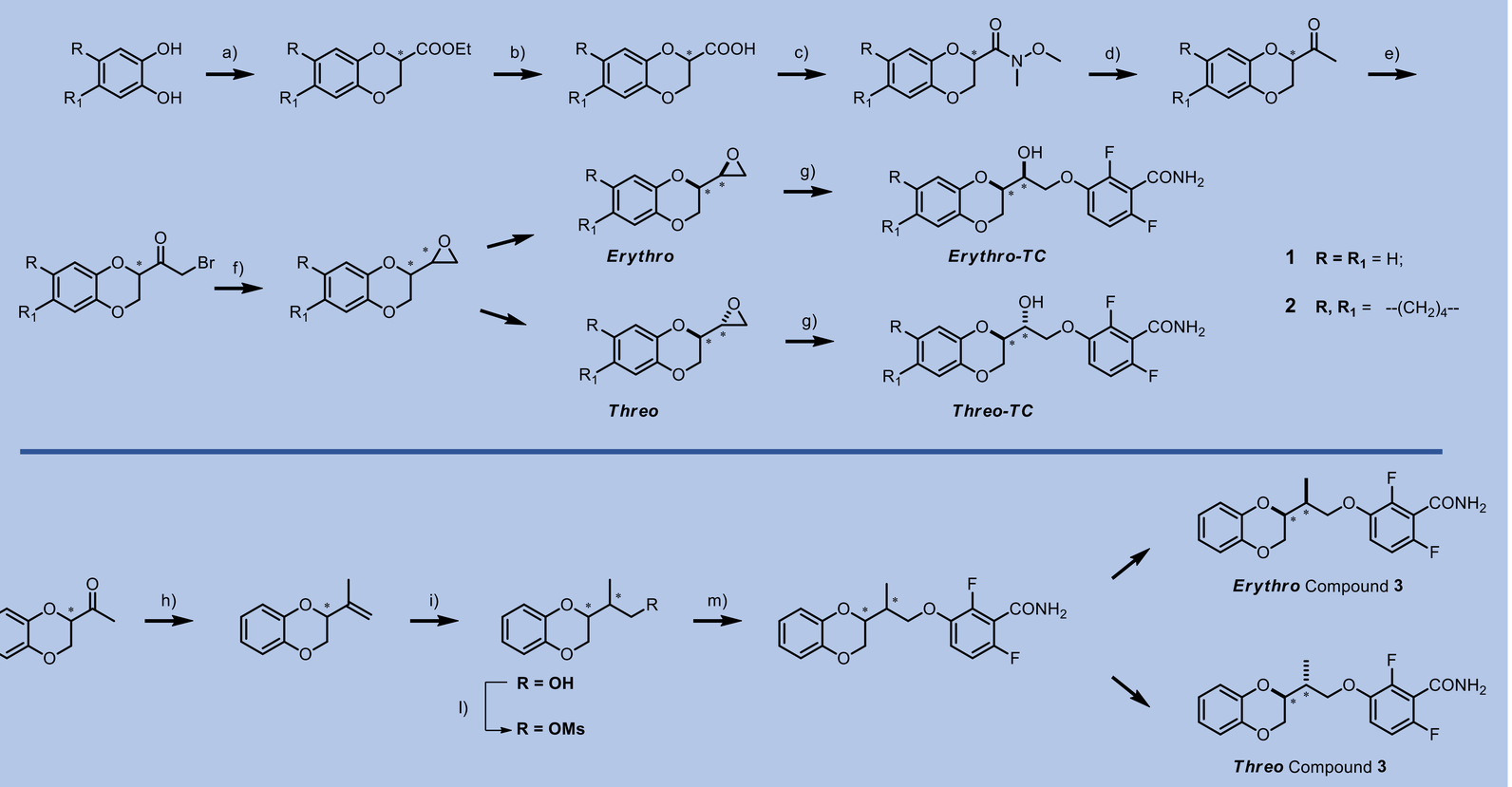


Figure 3: Structures of **1**, **2**, and **3,** objects of the present work

REFERENCES

[1] Hausser et al. *Nat. Rev. Microbiol*. **2016**, *14*(5), 305-319; [2] Chiodini, G. et al. *EJMC*, **2015**, *89*, 252-265; [3] Straniero, V. et al. *Antibiotics*, **2021**, *10*, 442; [4] Stokes, N.R.; et al. *Antimicrobial Agents Chemother.* **2013**, *57* (1), 317-325.

CHEMISTRY



Reagents and solvents: a) Ethyl 2,3-dibromopropionate, K_2CO_3, Acetone, 80°C; b) 2,5 N, NaOH, Methanol, RT; c) I)$SOCl_2$, II) *N,O*-Dimethylhydroxylamine, DCM, TEA, RT; d) MeMgBr, THF, RT; e) Br_2, DCM, Reflux; f) I) $NaBH_4$, II) NaH, THF, 0°C to RT; g) 2,6-difluoro-3-hydroxybenzamide, K_2CO_3, DMF, 70°C; h) $MePh_3P^+Br^-$, *t*-BuOK, Toluene, 80°C; i) BH_3-THF, H_2O_2, NaOH, H_2O, RT; l) MsCl, TEA, DCM, RT; m) 2,6-difluoro-3-hydroxybenzamide, K_2CO_3, DMF, 80°C;

BIOLOGICAL EVALUATION

ANTIMICROBIAL ACTIVITY

Compound	MIC vs MSSA (μg/mL)	MIC vs MRSA (μg/mL)	MIC vs *E.coli* N43 (μg/mL)
I	**5**	**5**	**>30**
II	**0.1**	**0.1**	**>30**
***Erythro*-1**	***Inactive***		
***Threo*-1**			
***Erythro*-2**	**1<MIC<10**	**1<MIC<10**	**1.88**
***Threo*-2**	**10<MIC<100**	**10<MIC<100**	**7.5**
***Erythro*-3**	**32**	**32**	***n.d***
***Threo*-3**	**128**	**128**	***n.d***

Table 1: Antimicrobial activities of **1-3**

As we can see, compounds **2** show the strongest antimicrobial activity vs *E.coli* N43. Thus, **2** was selected for further *in vitro* studies with *E.coli* FtsZ.

IN VITRO POTENCY EVALUATION

Fluorescence anisotropy with *E.Coli*-FtsZ-Alexa488 is a technique that allows to follow dimension and/or rigidity changes of the protein over time, when in presence or in absence of the putative inhibitor. FtsZ treadmilling is altered in presence of both *Threo* and *Erythro* **2** (Figure 4).

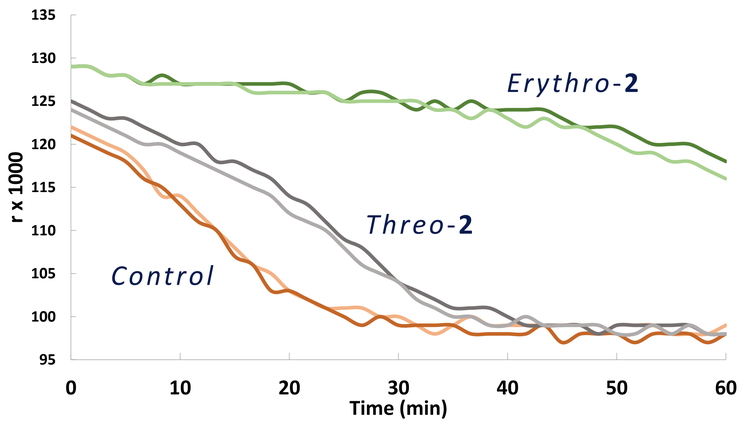


Figure 4: Fluorescence anisotropy trays (in duplicates) with Alexa488-FtsZ. Orange trays: FtsZ alone; Gray trays: FtsZ + *Threo*-2; Green trays: FtsZ + *Erythro*-3

CONCLUSIONS

A comparison of the activities of compounds **2** and **3** (*erythro* and *threo*) suggests a positive contribution of the OH to the interaction with *E.coli* FtsZ. In addition, the insertion of this free OH will allow us:

- to develop fluorescence probes for studying the interaction with the protein;
- to improve the bioavailability of compounds by formation of appropriate prodrugs.