Benzodioxane-benzamide FtsZ inhibitors: Synthesis of new derivatives and their biophysical and biochemical evaluation

Lorenzo Suigo[1], Marta Sobrinos-Sanguino[2], Silvia Zorrilla[2], Begoña Monterroso[2], Carlos Alfonso[2], Germán Rivas[2], Valentina Straniero[1], Ermanno Valoti[1]

lorenzo.suigo@unimi.it

[1]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Luigi Mangiagalli 25, 20133 Milano.
[2]Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior de Investigaciones Científicas (CSIC), Calle Ramiro de Maeztu 9, 28040 Madrid.

FtsZ: INTRODUCTION

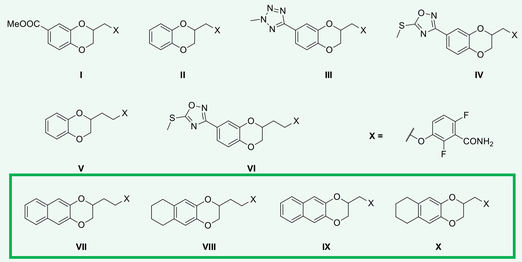
The bacterial cell division cycle is a fine-regulated crucial process that allows the bacterium to replicate. Among the various proteins which regulate this process, the main actor is recognized to be **FtsZ,** a GTPase protein functionally related to the human tubulin.

Normally, FtsZ is present in the cytoplasm forming discrete species, but when the process is triggered, it polymerizes at the centre of the cell, forming the **Z-ring**, which will recruit the other important proteins leading to the formation of the whole division complex, named **divisome.**

Polymerized FtsZ posses **GTPase activity,** and the consumption of the nucleotide is associated with the disassembly of the protofilaments, giving to the polymers a dynamic nature[1].

Considering the importance of FtsZ, it became an interesting target for antimicrobial therapy. Over the years, our research group developed a class of inhibitors: the **benzodioxane-benzamides**[2,3,4]**.** The antimicrobial activities of this class of compounds were tested over plenty of Gram-positive and Gram-negative bacteria, with good to great results, but an in-depth evaluation of the mechanism of action as well as a biophysical and biochemical characterization of the interaction with the protein were still lacking.

BENZODIOXANE-BENZAMIDES AS FtsZ INHIBITORS

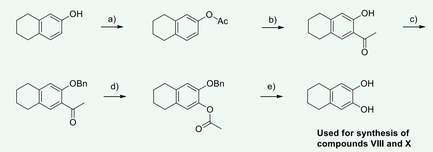


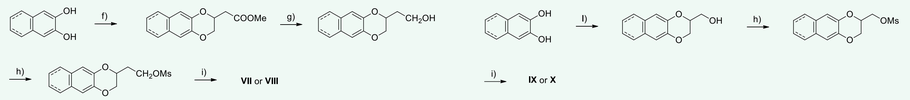
All these compounds show the development of this class over the years: Compounds **I-II**, which belong to the first generation of inhibitors, were characterized by none to simple 7-substitutions on the benzodioxane moiety and by one carbon atom as a linker to the benzamide. Compounds **III-IV** (second generation) bear a lipophilic and planar group in 7-position while compounds **V-VI** belong to the last generation and present two carbon atoms as linker.

The last developed compounds (**VII- X**) bear a naphthodioxane- or tetrahydronaphthodioxane moiety, with one or two carbon atoms as linkers. **All these compounds** were used and compared with each other to evaluate the interaction with the protein FtsZ and characterize the mechanism of action, observing also the differences between the generations.

CHEMISTRY

The synthesis of these compounds (**VII** to **X**) are all very similar. Nevertheless, while the 2,3-dihydroxynaphthalene is easly commercially available, the 2,3-dihyroxytetralin need to be synthesized. In the following schemes are depicted the synthesis of the precursor of the tetrahydronaphthodioxane derivatives and of the final compounds itselves.





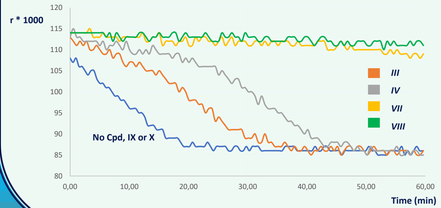
Reagents and solvents: a) Acetyl chloride, DCM, RT, 2 h; b) $AlCl_3$, 1,2-dichlorobenzene, 100°C, 5 h; c) Benzyl bromide, TBAB, 2,5 N aq. NaOH, DCM, RT, 6 h; d) *m*-CPBA, DCM, RT, 18 h; e) 1) 2,5 N aq. NaOH, MeOH, RT, 18 h; 2) H_2, Pd/C, MeOH, RT, 18 h; f) Methyl 3,4-dibromobutyrate, K_2CO_3, acetone, reflux, 18 h; g) $LiAlH_4$, THF, 0°C, 1 h; h) Mesyl chloride, TEA, DCM, RT, 3 h; i) 2,6-difluoro-3-hydroxybenzamide, K_2CO_3, DMF, 80°C, 4 h; l) Epibromohydrin, K_2CO_3, acetone, reflux, 4 h;

BIOPHYSICAL AND BIOCHEMICAL CHARACTERIZATION

Considering the high degree of characterization[5], we started the study of the effects of these compounds on ***E.coli* FtsZ**. To do so, we proceeded by evaluating the *E.coli* FtsZ **polymerization/depolymerization properties** by fluorescence anisotropy in the presence and absence of the compounds. We also observed if the **GTPase activity** of the protein and/or the **polymers dimensions** were altered by the putative inhibitors.

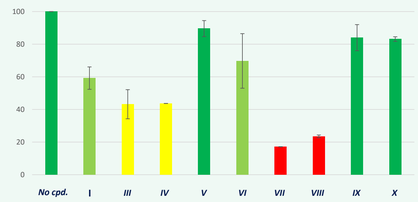
1) Polymerization properties: kinetics

Through **fluorescence anisotropy,** we evaluated the kinetics of FtsZ depolymerization (after 2 mM GTP addition) in absence and in presence of our compounds: The blue trace represents FtsZ in absence of any inhibitor. As we can see, the compounds exert different degrees of stabilization, with a total inhibition of the depolymerization within the time interval measured in presence of 20 μM **VII** or **VIII.**



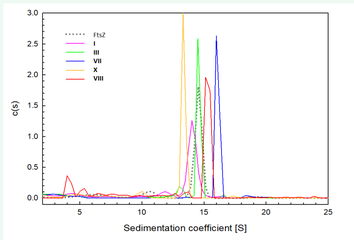
2) GTPase activity

After observing the stabilization effect of these compounds, we decided to assess if this was related to an inhibition of the GTPase activity. In this graphic is represented the **residual GTPase activity of FtsZ** (expressed as %) in presence of the different inhibitors. As we can see, the majority of the compounds mildly interfere with the GTPase activity, while compounds **VII-VIII** strongly reduce it (~ 80%).



3) Polymers dimensions

Lastly, we wanted to evaluate if these inhibitors were also able to affect the size of FtsZ polymers. Nevertheless, from the **analytical ultracentrifugation** assays, it seems that the dimensions of the protofilaments are not significantly affected by these compounds.



CONCLUSIONS

- The antimicrobial activity of the majority of these compounds seems to be related to a **moderate to strong stabilization** of the FtsZ protofilaments, which would result in the formation of a non-functional divisome.
- This stabilizing effect is correlated with a **lowering of the GTPase activity** of FtsZ, crucial for the depolymerization.
- Compounds **IX** and **X**, even if characterized by a strong antimicrobial activity towards both Gram-positive and Gram-negative bacteria related to the inhibition of FtsZ, **do not present the same profile of inhibition** of the rest of the compounds, suggesting a different mechanism of action.
- None of the compounds, regardless the generation at which they belong, are able to modify the protofilaments size.

These preliminary data will be followed by the evaluation of the capability of these compounds to interfere as well with **FtsZ bundling** and/or with **FtsZ hetero-associations** with other crucial proteins for the bacterial cell division cycle (I.e. SlmA).

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