Abstract of the doctoral thesis entitled

"Contributions to the design, *in silico* study and obtaining of fluoroquinolone hybrids with biological potential"

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Introduction

Bacterial infections have been and continue to be a major challenge for humanity despite significant advances in vaccines and antibiotics. They continue to affect the population globally, including through healthcare-associated infections. In addition, the increase in bacterial resistance and the decrease in the effectiveness of antibiotics are a serious threat worldwide. In this context, research on developing new effective antibiotics becomes essential for managing bacterial infections.

Fluoroquinolones (FQNs) are a major class of antibacterial agents effective against Gram-negative and Gram-positive bacteria. They have extensive therapeutic applications, including viral and fungal infections, cancer, and neurodegenerative diseases. However, the excessive and sometimes inappropriate use of fluoroquinolones in human and veterinary medicine has led to the rise of bacterial resistance. To counter this problem, research has focused on developing new effective derivatives with optimized pharmacokinetic and pharmacodynamic properties, thus strengthening the therapeutic value of this class of compounds.

FQNs are ideal candidates for developing hybrid antibacterial compounds due to several advantages: bactericidal effect, high potency, slow development of bacterial resistance, and dual activity against two bacterial target enzymes (DNA gyrase and topoisomerase IV). In addition to these functional benefits, FQNs present significant structural advantages, being synthetic compounds that are easy to structurally optimize, with an excellent ability to complex metal ions and combine with other active molecules. These characteristics have facilitated their inclusion in numerous hybrids with antibacterial effects, both with other antibiotics (e.g., tetracyclines and aminoglycosides) and with non-antibiotic molecules (e.g., benzimidazole, triazoles). Thus, FQNs have an essential role in the research for developing new antibacterial agents, reinforcing the hybridization strategy as a promising approach against global bacterial resistance.

Tetracyclines (TC) are broad-spectrum antibiotics effective against Gram-positive and Gram-negative bacteria and pathogens such as parasites, protozoa, rickettsiae, mycoplasmas and chlamydiae. They act by inhibiting protein synthesis by interacting with the 16S ribosomal RNA of the 30S ribosomal subunit. In addition to antibacterial activity, TC exhibit additional biological effects, such as antiapoptotic, anti-inflammatory, antiviral and neuroprotective activities, the mechanism of action of which is still under investigation.

Used for treating various infections, including respiratory, urinary and genitourinary infections, and in preventing malaria, the widespread use of TC has led to bacterial resistance through mechanisms such as ribosomal protection proteins and efflux pumps. However, TC continue to be essential in the management of bacterial infections.

Hybrid antibiotics, obtained by covalently linking two pharmacophores with distinct bacterial targets, offer a promising strategy for combating bacterial resistance. These molecules have the potential to exhibit efficacy against resistant bacteria, a broad spectrum of activity, and prolonged effectiveness even after the emergence of resistance. Also, the likelihood of favoring the further development of bacterial antibiotic resistance is reduced. The hybridization strategy confers additional benefits over the separate administration of the components, strengthening the therapeutic potential of this type of antibiotic.

Study no. 1. Evaluation of the potential inhibitory effects on bacterial DNA gyrase (*Escherichia coli*) of some hypothetical tetracycline-fluoroquinolone hybrids

The main objective of this study was to evaluate the ability of some hypothetical TC-FQN hybrids to inhibit bacterial DNA gyrase (*Escherichia coli*). The synthesis of these hybrids, based on the method of Sriram D. et al. (2007), follows principles of green chemistry by using microwave radiation and relatively non-toxic solvents such as ethanol. Until now, no structures have been obtained by cryo-electron microscopy or X-ray crystallography of complexes between this type of hybrid and DNA gyrase. Thus, the study focused on the computational design of TC-FQN hybrids and evaluating the interactions with the target enzyme DNA gyrase by molecular docking. In addition, the study also aimed to compare the hybrids with albicidin, a known DNA gyrase inhibitor, in terms of molecular similarity and physicochemical properties.

A series of 40 hypothetical TC-FQN hybrids were created, whose structures had balofloxacin, besifloxacin, ciprofloxacin, delafloxacin, finafloxacin, moxifloxacin, nemonoxacin, norfloxacin, sitafloxacin or zabofloxacin as the FQN component and doxycycline, minocycline, tetracycline or tigecycline as the TC component. A methylene linker connected the two antibiotic components through the amide group at C2 of TC and the secondary or primary amine of the C7-position substituent/primary amine of the N1-position substituent (delafloxacin) of FQN.

Among the co-crystallized structures available in the Protein Data Bank database at the time of the study, a target enzyme whose ligand was bound in the same site as that of FQN was selected: bacterial DNA gyrase (*Escherichia coli*) with albicidin as ligand. The determination of the similarity between TC-FQN-type hybrids and the co-crystallized ligand, the calculation of the physicochemical parameters of the designed structures of the hybrids and the co-crystallized ligand to compare them, and molecular docking were carried out using the FORECASTER platform.

The Do-Ba (doxycycline-balofloxacin), Mi-Fi (minocycline-finafloxacin), and Te-Ba (tetracycline-balofloxacin) hybrids showed the closest physicochemical similarity to albicidin and similar docking scores. However, some hybrids outperformed albicidin with better scores; the Te-De (tetracycline-delafloxacin) hybrid was especially noted. The studied hybrids likely inhibit DNA synthesis by binding to enzyme-DNA complexes, similar to the FQN component. Future studies could examine the role of the 30S ribosomal subunit as a target within a possible dual mechanism of action. These hybrids could provide a broad spectrum of activity and reduce the development of bacterial resistance, although further *in vitro* and *in vivo* research is needed to confirm these hypotheses.

Study no. 2. Obtaining tetracycline-fluoroquinolone hybrids to discover compounds with potential antibacterial effect – preliminary study

This study aimed to optimize a synthesis method to obtain TC-FQN hybrids using a methylene linker derived from formaldehyde. Starting from the research of Sriram D. et al. (2007), who synthesized Mannich bases of TC with FQN, the current study used the Mannich condensation reaction to obtain hybrids between FQN (ciprofloxacin, moxifloxacin, and norfloxacin) and TC (doxycycline and tetracycline). Synthesis methods included both classical reflux and microwave-assisted reflux processes, and variation of reaction parameters, including the use of a catalyst, was explored for method optimization.

The preliminary study of the synthesis of the norfloxacin-doxycycline hybrid was successful; DSC and MS analyses supported the formation of a new compound distinct from the "parent" compounds. Both reflux synthesis processes (classical and microwave-assisted) produced similar results. It is unclear whether the $CuCl_2 \cdot 2H_2O$ catalyst used favored the Mannich reaction to obtain TC-FQN hybrids. However, one of the samples in the catalyst series showed the m/z ratio closest to the theoretical mass of the hybrid.

The synthesis method is simple and involves a single step using non-toxic solvents. The encouraging results represent valuable information for optimising these methods for obtaining the targeted hybrids.

These TC-FQN-type hybrids based on two antibiotic molecules can potentially contribute to developing new compounds that are effective in combating bacterial resistance due to the possible dual mechanism of action conferred by the two "parent" components.