Abstract

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Doctoral thesis: Synthesis and development of new antimicrobial compounds using Computer Aided Drug Design

The resistance of microorganisms to commonly used antimicrobial agents is a natural phenomenon that complicates the effective treatment of infections. Improper use of effective medications has accelerated this process and highlighted the need for the development of new antimicrobial compounds, preferably with a novel mechanism of action, for which resistance has not yet developed. A key measure to slow down this process is also the responsible use of these compounds.

This dissertation utilizes a comprehensive approach to the development of new antimicrobial compounds aimed at overcoming the challenges associated with increasing bacterial resistance. The first part of the work focused on the synthesis of derivatives of benzamidopyrazin-2-carboxamide. A series of these compounds demonstrated solid antimycobacterial activity, with the highest activity observed in the derivative with a 4-Br substitution, achieving MIC value of 1.95 µg/ml against *Mycobacterium tuberculosis* H37Ra. Based on the design of the synthesized compounds, the target enzyme was proposed to be mycobacterial prolyl-tRNA synthetase. Since this enzyme has not been extensively studied as a therapeutic target, these compounds represent the first attempt at developing such inhibitors. Due to the unavailability of the crystallographic structure of the mycobacterial enzyme, homologous models were created and compared, which were then used in subsequent molecular docking and molecular dynamics studies.

Using the Ligand Growing method, a database of ligands with optimal interactions at the binding site of prolyl-tRNA synthetase was created. By simplifying these ligands, a second series of potential inhibitors of prolyl-tRNA synthetase was created. These compounds were tested on human prolyl-tRNA synthetase to verify their binding to the enzyme and the achieved interactions.

The second part of the dissertation was inspired by previous research on *N*-pyrazinylbenzamides, with hydroxy-substituted derivatives showing very promising antibacterial activity. Through systematic modifications, a series of compounds was developed with the goal of describing the structure-activity relationships. Although the originally very promising antibacterial activity values was not confirmed, some compounds exhibited mild antibacterial activity (MIC = 62.5 μM). These compounds showed also significant antimycobacterial activity, with the best value being 6.25 μg/ml against *Mycobacterium tuberculosis* H37Rv. In studies of the mechanism of action, significant membrane depolarization was observed in methicillin-resistant *Staphylococcus aureus* strains. Nevertheless, the compounds act bacteriostatically and do not exhibit cytotoxicity towards HepG2 cells. The identified effect of *N*-pyrazinyl-2-hydroxybenzamide on protein biosynthesis may play an important role. To rationalize further development, a project was proposed aimed at identifying a specific biological target for these compounds.

The third part of this work involved the preparation of derivatives of quinazolin-4(3*H*)-one, which exhibited antimycobacterial activity (MIC = 6.25 μg/ml against *Mycobacterium tuberculosis* H37Rv). Using molecular docking and molecular dynamics, a potential target, PonA1, a penicillin-binding protein in *Mycobacterium tuberculosis*, was proposed for these compounds.