Opinion of the opponent

Mgr. Vinod Sukanth Kumar Pallabothula submitted a dissertation on the topic Synthesis, development and biological evaluation of new antimicrobial compounds, developed at the Department of Pharmaceutical Chemistry and Pharmaceutical Analysis the Faculty of Pharmacy of Charles University in Hradec Králové, under the supervision of Assoc. Prof. PharmDr. Jan Zitko, Ph.D. The thesis is a set of candidate´s journal articles provided with commentary.

The aim of the thesis has been formulated as the design, synthesis and *in vitro* biological evaluation of 3-amidopyrazine derivatives targeting Mycobacterial prolyl-tRNA synthetase (mtProRS) as weapons to combat the growing threat of antimicrobial resistance (AMR). Its objectives are then specified as the design of novel compounds with pyrazine scaffold with antimycobacterial activity targeting mtProRS, but without interaction with its human analogue, using computer-aided drug design, then synthesis of such compounds, their evaluation using various *in vitro* biological assays, and then formulation of the structure-activity relationships (SAR) within them followed by *in silico* investigation of the mechanism of action.

The theoretic part of the thesis consists of the chapters Introduction and Methodology. The chapter Introduction presents, after a short introduction to the issues of tuberculosis and drug resistance, a short overview of some important bacterial enzymes serving in this thesis as potential drug targets. The following chapter Methodology contains then a brief overview of some synthetic a biological evaluation methods used in this thesis.

The practical part of the thesis is replaced with the commentary to published journal articles of the candidate, and these 4 articles published in good scientific journals with Q2/Q1 JIF ranking themselves. The chapter Summary of this part of the thesis informs that the candidate has prepared 85 target compounds in total, of which 56 are 3-benzamidopyrazine-2-carboxamides and similar derivatives, marked by the author as series P1, and the additional 29 compounds, marked as P2 series, are 3-ureidopyrazine-2-carboxamides, disubstituted 3- benzamidopyrazine-2-carboxamides, and similar compounds. Some members o P1 series exhibited an excellent antimycobacterial activity exceeding the activity of the model compound – ligand “Candidate A” from the work of Adachi, while 3- ureidopyrazine-2-carboxamide derivatives from series P2 exhibited the lowest antimycobacterial activity. *In silico* conformational analysis and subsequent NOE NMR experiments then indicated that the latter compounds adopt conformation incompatible with the binding cavity. In conclusion, the aims and objectives of the thesis were clearly met.

Conclusion of the opinion:
The thesis meets all the requirements for this type of qualification work, therefore I recommend it for defense, and the candidate for a Ph.D. degree in Pharmaceutical Chemistry.

Comments:

1. Chapter 2., sub-chapter 2.1.1., Fig. 5: It is evident from only small differences between R_fs of starting material and crude product, that the chromatography system was not chosen properly.

Questions:

1. What was the criterion for the selection of methods included in the sub-chapter Synthetic Methods of the chapter Methodology? Also other synthetic methods were used in the synthesis of target compounds, not only reactions with acyl chlorides and reactions with ammonia in an alcohol.
2. What were the criteria for the choice of scientific journals for publishing of your results?
3. You mention prolyl-tRNA synthase, aspartate decarboxylase, and methionine aminopeptidase 1 as potential targets of antimicrobial drugs in the introductory part of your thesis. Which of these enzymes is, in your opinion, the most promising?

Brno, October 17, 2024 doc. PharmDr. Oldřich Farsa, Ph.D., the opponent