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Review of the doctorate thesis entitled

„ Study of Pyridine Derivatives as Potential Antimycobacterial Active Drugs" written by MSc Daria Nawrot under supervision of Prof Martin Doležal

The tuberculosis is an infectious disease of bacterial origin, that continues to be a leading cause of death globally. Despite losing some prominence to modern-world ailments such as diabetes and cardiovascular disorders, tuberculosis remains a formidable and deadly bacterial infection. It is disheartening that, in the contemporary era, over a million people surrender to Mycobacterium-caused infections each year, more than a century after the advent of the first antibacterial drugs. The persistently high mortality rates can be attributed to the low efficacy of existing drugs, the emergence of resistance, and the scarcity of innovative treatments. However a paramount contributing factor is the inadequate accessibility to healthcare services in developing countries, where tuberculosis is most prevalent. This, in turn, diminishes the motivation within the industry to invest in the development of new drugs, posing a hindrance to research initiatives. Consequently, independent research conducted in universities becomes crucial and highly valuable. The esteemed research group led by Professor Dolezal at the Pharmaceutical Faculty of Charles University is renowned for its outstanding contributions to this field, evident in numerous publications, projects, and invaluable insights into the chemistry and pharmacology of potential antimycobacterial agents. The thesis, submitted for my evaluation also serves as an example of excellent scientific work and a full display of humanistic sensitivity towards pressing issues in developing countries.

Technically the dissertation is a commentary on articles of which three experimental works has been published in good scientific journals and two supplementary publications that explore the adjacent field. In most of these papers Ms Nawrot was first author. It deserves to note here also

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that she has presented her results in ten conference materials. The volume of the thesis is kept minimum counting 31 pages including 95 references and abstract. Introduction count 7 pages only, but covers broad range of aspects including problem of tuberculosis particularly in the light of resistance and drug design. The latter consist also bioisosterism and halogen bond as those issues of fragment based design that that were exploited in this work. Excluding the small volume and, consequently, the applied abbreviations or simplifications, this section is written in correct language, is logically structured, and adequately explains the addressed issues.

Biological testing relevant to this research have been conducted in cooperation and was discussed separately in the introduction section. Author presented the scope of the testing and reasons for selection of particular bacterial strains and cell lines. From the methodological point of view those test have been correctly designed and performed. Typical *Mycobaterium* strains have been used as panel of slow- and fast-growing bacteria with different virulence supplemented also by drug resistant strains. Another bacterial strains as well as human cancer cell lines were used to evaluate broader scope of activity and cytotoxicity respectively. Beside, some preliminary in vivo evaluation of toxicity and effectivity for selected compounds is a good proof of concept for the research. The experimental part delves into the design, synthesis, and biological evaluation of newly developed compounds. It has been divided into consecutively published results: N-pyridinylbenzamides, 2-aminooxazoles and pyridinyl carboxamides. The research involved the screening of these compounds against various mycobacterial, fungal, and bacterial strains. Notably, the mycobacterial strains included M. avium, M. aurum, M. kansasii, M. smegmatis, and the strains of primary interest—Mtb H37Ra and Mtb H37Rv. Additionally, the compounds were tested for their cytotoxicity against the HepG2 cancer cell line and, where applicable, using the Galleria mellonella in vivo model. Further evaluations included in vitro antiproliferative activity against specific cancer cell lines, such as A498, PC-3, and U-87MG. Advanced in vivo testing was conducted in a murine tuberculosis model.

Generally speaking the core of this research is bioisosteric shift from pyrazine to pyridine moiety and its further modification in fragment based design approach. The noteworthy outcome of this work was the observation that these subtle isosteric changes yielded considerable influence on biological activity and physicochemical properties.

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In the first publication discussed in this dissertation, the isosteric exchange from pyrazine to pyridine resulted in improved activity in 8 out of 10 comparable pairs of compounds, with the most significant change in MIC reaching two orders of magnitude. Moreover this trend persisted in the second publication, further affirming the beneficial impact of the pyrazine-to-pyridine exchange on biological activity. The hypothesis regarding the physicochemical benefit of isosteric exchange from thiazole to oxazole was also validated. Apparently the introduction of sulfur into the heterocyclic ring resulted in lower activity and worse pharmacokinetic properties.

Third work was focused on modification of the salicylic acid fragment and exchange of the pyrazine core to pyridine and quinoline. These modifications resulted in conclusions such as the lack of hydroxyl worsening the compound's biological efficacy, supporting existing literature findings. The pyrazine-to-pyridine exchange improved biological activity and marginally influenced the mechanism of action. Additionally, unpublished results involving 2-phenyl-N-(pyridin-2-yl)acetamides further emphasized the potential viability of isosteric exchange as a strategic chemical modification for influencing biological activity against targeted pathogens. The research also delved into the significance of halogenation, particularly chlorine substitution on the pyridine ring, with varied outcomes across publications. While the effect was robust in P1 and unpublished 2-phenyl-N-(pyridin-2-yl)acetamides, suggesting positive influences on biological activity, P2 and P3 provided inconclusive evidence on the benefits of such structural modification. These results and whole dissertation are further strengthen by chapter entitled ongoing research and future plans, that clearly indicate various direction of development of pyridine based anti-mycobacterial agents. This chapter underscores the depth of scientific research expertise gained by Daria Nawrot and her adept collaborative skills, demonstrated across various groups and diverse topics

The editorial work carried out on this thesis, in my opinion, deserves commendation. I was able to identify only a few misspelled words. In the page 14 two references are missing – author probably forgot to replace [ref] with the number. Figure 14 is barely visible and quite difficult to follow. The text related with this picture e.g. "Fig 14a summarized the current status of eight monoclonal antibodies targeting SA" do not help in this manner

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In conclusion, Daria Nawrot's doctoral thesis makes a substantial contribution to the field of medicinal chemistry, particularly in the development of pyridine derivatives as potential antimycobacterial agents. The multidimensional evaluation of the compounds against various pathogens and cancer cell lines underscores the potential clinical relevance of the research. The thesis stands as a commendable effort in addressing the pressing challenges associated with tuberculosis and antibacterial resistance. In my opinion the scientific achievements of MSc Daria Nawrot fully confirm her development and attitude as young scientists. Therefore it is my great pleasure to request the Scientific Council of Pharmaceutical Faculty of Charles University to award her with the PhD degree.

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