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A review of the Ph.D. thesis of MSc. Ghada Bouz entitled "Derivatives of Pyrazinecarboxylic Acid as Potential Antimycobacterial Active Drugs"

The Ph.D. thesis of MSc. Ghada Bouz was written under supervision of Professor Martin Doležal and Assoc. Prof. Jan Zitko (advisor). It deals with one of the most urgent healthcare topics worldwide – development of novel antimicrobial agents, here especially potential antimycobacterial drugs. Unfortunately, and despite partial recent advances, the research focused on antitubercular drugs has been disregarded largely for many years. Nowadays, eradication of tuberculosis (TB) belongs to WHO priorities deservedly. The treatment of TB complicated due to many reasons needs to be improved substantially.

The doctoral thesis, consistent with previous research of Doležal's group and reflecting it, contributes to this issue valuably. It is based on five scientific articles (P1-P5; one review presented in Critical Reviews in Microbiology, four experimental works published all in Molecules journal) and written as a commentary to them. G. Bouz is a first author four-times.

The Ph.D. thesis itself is composed of following sections: Introduction, Commentary on Published Results (containing extension of previous works and new series; in total 112 compounds), Ongoing Research and Future Plans, Conclusions and Recommendations, References (52), together on 27 pages. Then, the publications P1-P5 including their list are attached. Thus, almost all the results have already been published in journals with impact factor after careful peer review process facilitating my role of the thesis reviewer. Otherwise, I miss a list of conference contributions and also exactly defined goals of the Ph.D. project/thesis.

The short Introduction section reports the most basic facts about TB. It justifies the request for novel antitubercular agents, concerning obstacles for their development including a lack of proper animal model. Review P1 dealing with zebrafish model suitable not only for TB is a nice complement to the experimental part. Additionally, the introduction highlights pyrazinamide (PZA) as a unique drug for TB treatment.

The ambiguous and still not fully clarified mechanism(s) of PZA action complicates a rational design of novel derivatives, although docking studies were involved (probably not performed by the Ph.D. candidate). However, the determination of the mechanism of action only on the basis of molecular modelling would be inadequate. From synthetic point of view, the syntheses used are rather simple enabling a preparation of a wide range of the derivatives in a comparatively short time to determine structure-activity relationships (SAR). On the other hand, the straightforward synthesis could be advantageous because of its low cost; therefore, the approach chosen is quite adequate.

Section entitled "Commentary on Published Results" covers a very brief description of biological evaluation strategy and tests used, namely antimycobacterial activity (*M. tuberculosis*, four strains of nontuberculous mycobacteria), four Gram-positive and four Gram-negative bacteria, eight fungal strains (both yeasts and moulds), cytotoxicity (HepG2) and (here not mentioned) molecular modelling studies. At first, the expansion of previous works is

discussed, i.e., derivatives of 3-(phenylcarbamoyl)pyrazine-2-carboxylic acid (P2) and 3-aminopyrazine-2-carboxamides (P3), focusing on SAR that were identified. Then, new series are presented and discussed (ureidopyrazines P4 and pyrazine-based sulfonamides P5). This part interested me most, mainly biological activities of ureas. In contrast to the title of the thesis, a range of the derivatives from these two groups are not derivatives of pyrazinoic acid. In my opinion, this commentary section is overly short, and I prefer the discussion to be more complex.

The chapter entitled Ongoing Research and Future Plans reports preliminary results of synthesis and bioevaluation of quinoxaline-2-carboxylates, hybrids of PZA and 4-aminosalicylic acid (in general, the most promising compounds) and other pyrazine-based compounds. Having some results in hands, this part should be more detailed. However, I miss more specific future plans and structure(s) suggestions based on the findings cross-sectional of the thesis.

The last part (Conclusions and Recommendations) summarizes the results and the most active derivatives found; the recommendations are non-specific again.

From formal point of view, the thesis contains only a few typos and mistakes. It would be appropriate to present numbered formulas of the most active compounds in each chapter as you go along. List of abbreviations includes also physical units; on the other hand, it does not cover all the abbreviations used; *Mycobacterium tuberculosis* should be italicized.

My questions:

1) The thesis summarizes proposed mechanisms of action of pyrazinamide (Table 1). If PZA has truly a multiple mechanism of action, the incidence of acquired resistance should be significantly lower than for other anti-TB drugs. In reality, this is not the case. Please comment this fact.
2) Nontuberculous mycobacteria (*M. kansasii*, *M. smegmatis* etc.) are naturally resistant to PZA due to either reduced PZAase activity or highly efficient efflux pumps. Several of your derivatives (from P3-P5) exhibited activity also against nontuberculous strains, sometimes even at lower concentration that those for *Mtb*. Please try to explain this finding.
3) It is definite that the development of acquired resistance represents one of the most problematic aspects of TB treatment. Do you have any results about activity of the presented derivatives against PZA-resistant tuberculous strains?
4) Regarding aimed escalated lipophilicity of derivatives **1** in the P2, did you consider also cyclic imides formation?
5) Focusing on the article P3, why identical substitution pattern of phenyl ring was not used for homologous benzyl (**1-8**) and phenyl (**13-20**) derivatives?
6) The eliciting activity of two selected ureidopyrazines is interesting to me. Why just *Fagopyrum esculentum* and rutin production were chosen? Is it known a mechanism of action of used and structurally resembling 1-(2-chloropyridin-4-yl)-3-phenylurea?

Finally, the doctoral thesis of MSc. Ghada Bouz is an up-to-date and relevant work offering novel pyrazine-based derivatives with an interesting and promising biological activity. Unequivocally, it provides new findings of great interest and scientific soundness not only about mycobacteria-targeting compounds. The Ph.D. candidate has proved her erudition in the field of pharmaceutical chemistry.

In summary, I recommend this doctoral thesis of MSc. Ghada Bouz for further procedure and after a successful public defence, I advise awarding Ph.D. degree.

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