HARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of pharmaceutical chemistry and pharmaceutical analysis

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Fahim - Joe Rahi**
Thesis Tutor:	doc. PharmDr. Jan Zitko, Ph.D.
Consultant:	Mgr. Marek Kerda
Opponent:	PharmDr. Marta Kučerová, Ph.D.
Thesis title:	**Design, synthesis and evaluation of heterocyclic compounds with potential antimicrobial activity III**

Scope of work, number of 62 pages, 15 figures, 3 tables, 59 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Good
b)	The complexity of the methods used:	Excellent
c)	Preparation of the methodological part (clarity, comprehensibility):	Excellent
d)	The quality of the experimental data obtained:	Very good
e)	Processing of results (clarity):	Good
f)	Evaluation of results, including statistical analysis:	Very good
g)	Discussion of results:	Excellent
h)	Clarity, conciseness, and adequacy of conclusions:	Excellent
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Very good
l)	Formal level of the work (text structure, graphic design):	Very good

I recommend the thesis for recognition as a rigorous thesis ☐

Summary

The theoretical part of the thesis concerns with bacteria resistance, the reasons of its origin and its cellular mechanisms (they are mixed together in some parts of the Theory). Further, some novel drugs in the treatment of resistant bacterial infections are mentioned followed by the description of mycobacterial infections, their diagnosis and some anti-tuberculosis drugs.

The design of the prepared compounds is derived from the previous publication by student´s tutor (ref. 57) and two publications by Krátký M. et al. (refs. 56 and 58). Unfortunately, the inspiring structures from the latter ones are missing.

Within the synthetic part, the student prepared seven amides of 2-hydroxyphenylacetic acid and three acetyl esters thereof. He tested three methods for acetylation of phenolic hydroxyl group and three activation methods for the acid, that underwent the condensation with the corresponding aminopyrazines/-pyridines. All the final structures underwent in silico prediction of protonation and conformation in MOE software (described and commented only in Experimental) and pharmacokinetic analysis using SwissADME web service (results attached as a separate csv file, described and commented only in Discussion). The compounds have

been screened for antibacterial, antimycobacterial, and antifungal activity. Some antimycobacterial activity has been revealed.

Comments on the evaluation:

There is a shift in numbering the chapters in the text compared to the Table of contents. The abstracts should not be numbered, as it should be a stand-alone file and in case you mention a reference there, it should be cited at the end of the page. There is no chapter Results, the results are hidden partially in the chapter Experimental, partially in the chapter Discussion.

Title of subchapter "Mycobacterial diseases" (p. 21) is a little bit confusing, "Mycobacterial infections" would sound better.

The chapters "Aim of the study" and chapter "Design rationale" could have been joined, as their content is similar and the chemical structures (pp. 26 and 27) are identical.

It is not necessary to involve HCl in the abbreviations, as it is a chemical formula.

In caption of Fig. 9 (p. 22), it is not clear what number 10 means. In case you copy the pictures of other authors, there should be also the name of the first author in the caption, i.e., Irfan M. and Bisht D. and the reference number in upper index. The picture should always follow after the place, where it is mentioned in the text, not opposite (p. 27).

The information on mode for flash chromatography (p. 29) is missing (isocratic/gradient).

In Scheme 1 (p. 31) the acetic acid is indicated above the reaction arrow as the starting material, however, in the procedures of methods 1-3, acetic anhydride is mentioned.

The reaction yields in table I (p. 33) are wrongly calculated. It is not possible to verify the correctness of the yields for esters (compounds 8-10), as the weight gained is not listed.

It is unusual to interpret only some of the signals in IR spectra. It would be reasonable to interpret either all of them or none of them.

At NMR spectra interpretation, there is always 1H NMR and 13C NMR redundant. Writing of DMSO-D6 should be unified within the document. The reference signals for acetone-D6 should be also given.

The INNs should be written with small first letter (e.g., Cefepime p. 20, Rifampicin p. 21, delamanid p. 24), as well the names of chemicals (e.g., Diisopropylethylamine p. 32).

For the systematic name of esters (pp. 42-44), the proper brackets should be used: round brackets, square brackets, and curly brackets (braces).

In the second paragraph of description of antibacterial screening (p. 47), it is ambiguous, what is "they" (bacteria vs compounds).

There is inconsistency in script font (p. 27), script colour (p. 46) and style of the chemical structures is not consistent (e.g., p. 19, 25).

Citing literature: The details on the ref. 2, 51, and 53 should be given (article, webpage?); in fig. 1, the ref. 5 as Nature is cited, however Nature is ref. 3; name of the journal is missing in citation of ref. 4; ref. 27 in caption of Fig. 3 (p. 16) is mentioned before the ref. 25); the complete title of ref. 57 is missing.

Mistyping: abbreviation for NTM, Stenotrophomonas (p. 20), Figure 8 (last sentence on p. 21), missing links to Fig. 12 for bedaquiline and delamanid (p. 24), Lipniski (p. 53), small letters in the beginning of sentences (p. 46, 55), capital in the middle of a sentence (e.g., p. 11, 48), pyridine in a ratio of 4:1 (p. 51, not clear what ratio), missing full stop (e.g., p. 27, 56).

In my opinion, the persons who were involved in identification of the prepared compounds should be acknowledged at least in the defense presentation.

I suggest Errata file including: 1.) correction to the abbreviation MS-ASAP, 2.) information on mode for flash chromatography, 3.) correction to Scheme 1, 4.) correction to the incubation for

antibacterial screening, 5.) adding results of antifungal screening, 6.) corrections to the Table I, and 7.) adding weight of the products 8-10.

Similarity check in Theses generated an overall similarity of 26% with 27 documents (maximum individual agreement of 8%) and Turnitin evaluated an overall similarity of 37% with 168 documents (maximum individual agreement of 2%).

Questions and comments to student:

1. In association with the secondary bacterial resistance described in the Theory, also bacterial biofilms should be mentioned. Could you shortly comment on it?

2. In subchapter 6.4 "Novel drugs in the treatment of resistant bacterial infections" (p. 18), there is mentioned, that eight new drugs have been approved by FDA. Could you please list them? Is zoliflodacin really still under clinical trials? The statement, that bedaquiline is under clinical trials is in contradiction with the mentioned therapy in the following paragraph (p. 24)? The same situation happened to delamanid in the following paragraph.

3. Could you comment on the chirality of ethambutol in Fig. 11 (p. 23)?

4. On the pp. 26 and 54, the potential target receptor for your compounds is mentioned. What type it could be?

5. In subchapter Materials and instruments, there is a statement: "all chemicals fit the parameter set by the manufacturer." How did you prove it?

6. The way of sample application and method of ionization MS spectra measurement are missing. Name correctly the meaning of the abbreviation MS-ASAP. You could have included the measured mass of the final compounds.

7. What does Stuart SMP30 "spot meter" mean (p. 29)?

8. What does it mean "All structures were put through Molecular modelling software MOE…" did you draw the structures in MOE?

9. What was the incubation time for MIC values in Table II (p. 49)?

10. Can you explain the discrepancy in names Mycolicibacterium aurum (p. 46) and Mycobacterium aurum (p. 50)?

11. In the synthetic procedures, sodium sulphate is mentioned. The form of the salt should be also mentioned in the procedures: decahydrate, heptahydrate or anhydrous. Was drying of the organic fraction performed also in method 6 after extraction (p. 33)?

12. How did you check the purity of the prepared compounds?

13. In method 1 for preparation of 2-acetoxyphenyl acetic acid (p. 30), the molar excess of acetic anhydride is not mentioned, only the added volume. Was it equivalent to the excess in the following tested methods?

14. Can you more comment on the inhibition of cytochrome enzymes by the compounds, which was found in SwissADME analysis, what it could influence?

Disregarding all the above-mentioned objectives, the thesis has brought new knowledge on the biological activity of the final compounds, that served for comparison with the activity of the previously published compounds. I appreciate the discussion and the calculation study.

Evaluation of the thesis: Very good		**For the defense: Recommend**
In Hradec Králové	29. května 2024	signature of the opponent