CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Pharmaceutical Chemistry and Analysis

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Hanieh Kamangar**
Thesis Tutor:	Assoc. Prof. Dr. Jan Zitko, Ph.D.
Consultant:	Ghada Bouz, Ph.D.
Opponent:	Assoc. Prof. Dr. Martin Krátký, Ph.D.
Thesis title:	**Synthesis and evaluation of novel quinazolones as potential antimicrobial compounds**

Scope of work, number of 65 pages, 9 figures, 14 tables, 81 citations

Evaluation of the work:

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

Comments on the evaluation:

The work focuses on the synthesis and evaluation of novel quinazolinone derivatives, a highly relevant topic due to the increasing bacterial resistance, particularly MRSA. Quinazolines are well-established scaffold for development of potential bioactive agents. Research on new antimicrobial agents is crucial, up-to-date, and has a potential for clinical application. That is why the topic of the thesis is important and deserves to be studied.

The thesis was completed under the supervision of Assoc. Prof. Jan Zitko, Ph.D., at the Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, with Ghada Bouz, Ph.D., as the consultant. The work partially draws on previous research in the research group. The structure follows a traditional format: Aim of the work (that is clearly defined), Introduction (theoretical background, focuses on Staphylococcus aureus, its resistance and treatment, particularly methicillin-resistant strain; it also covers quinazolinone-containing drugs, their biological activities, and the rationale for designing new antimicrobial agents), Experimental part (general methods in both chemistry and biology, synthesis, and characterization), Results and discussion including in silico evaluation and comparison of findings with structurally related compounds from previous diploma thesis, concise

conclusions, abstracts, and references. This structure ensures a logical flow and adheres to the academic standards for a research thesis in pharmaceutical chemistry.

The thesis contains formal errors, such as issues with punctuation, missing or extra characters, incorrect use of hyphens versus dashes, inconsistent use of italics, subscript notation, and typographical errors like "biologicsl", "lineyolid", "choloro" etc.
Further notes and comments:

• In Chapter 3.2, Methicillin-resistant Staphylococcus aureus, there is limited information provided about this pathogen; much of the chapter covers other topics. Given the focus of the thesis, a more detailed discussion would be beneficial.
• In the overview of drugs effective against MRSA, key agents such as rifampicin, cotrimoxazole, and fosfomycin are missing.
• Table 1 lacks stereochemical information for some drugs.
• The section on quinazolines relies heavily on older references and a review from 2019 - are there any significant updates since then? Could Figure 4 be revised to reflect newer findings?
• Page 19 - an incorrect formula for etaqualone. Additionally, I am curious about how GABAA receptor modulation by cloroqualone can suppress cough?
• For several figures, it is mentioned that they were taken without modifications from the respective sources - was this always done with the authors' permission or in accordance with licensing conditions?
• Table 4: There is no reference to it in the text - what compound does it refer to?
• I would suggest omitting the last paragraph of Chapter 3.
• Page 26 - Is 3.6 g really 36 mmol of the starting acid? Please describe in detail the recrystallization process using 500 mL of hexane and 5 mL of ethyl acetate. Additionally, not all benzylamines were halogenated.
• In the preparation of the lactone intermediate, you mention heating the reaction mixture to 130 °C and refluxing it - what is the boiling point of acetic anhydride? What was the yield of this reaction?
• What was the reason for generating SMILES for the synthesized compounds?
• Regarding spectral characterization - where can the N-H stretch vibration be found in your compounds?
• Each compound was prepared in quantities of 200 mg or more - was there truly not a one crystal left for measuring IR spectra in some cases?
• In the 13C spectra of compounds GDM20 and GDM22, a quartet is present - please explain its origin.
• In the methodology section, it is stated that the compounds were tested against Serratia marcescens, but this is not reflected in the results - please explain.
• Where was the activity against Mtb. H37Ra tested? This information is missing in the thesis.
• According to the newer terminology, Mycobacterium aurum and Mycobacterium smegmatis are now referred to as Mycolicibacterium.
• Table 14 contains Czech words.
• The abstract states: "Future studies will explore whether the active compound's target is the mycobacteria penicillin binding protein 2a." However, do mycobacteria actually possess this form of PBP?
• There are several deficiencies in the literature section, with some references being incomplete, for example 37 and 47.

Another issue that needs to be addressed and explained is the similarity rate in the plagiarism check, which came out to be 41% and 42%. It is entirely understandable that similarity occurs in the description of biological tests methodologies, which were not conducted by the student herself and are properly cited. Similarly, the similarity is expected in the list of abbreviations, declarations, citations, dedication, and other formal issues. The problem, however, lies in the similarity with the previous diploma thesis by A. Askari, not only from a formal perspective (e.g., identical chapter titles) but also in terms of content. This is

not an isolated case - there are several relatively long sections in the thesis that, apart from these two works, I was not able to found elsewhere on the internet. It is also unusual for two individuals to describe the methodology of chemical compound preparation in exactly the same words, especially with the same typographical error. On the other hand, the thesis is original in many respects, both factually and textually.

Questions and comments to student:

1. You refer to a docking study of quinazolines with PBP2a that was performed by your colleagues. Could you please present its results during your defense? Additionally, I am curious why you did not synthesize derivatives with the substituents proposed in Figure 8?
2. Please explain why you chose the specific benzylamine substituents presented in the thesis as well as their positions.
3. Please try to explain why, in comparison with the previous diploma thesis of A. Ascari, which focused on analogous derivatives without chlorine, consistent results of biological activity were not achieved. Sometimes your compounds performed better, and other times the opposite was true. Additionally, what effect(s), apart from the mentioned increased lipophilicity, does the introduction of chlorine atom into the molecule have?

In summary, I recommend the thesis for defense, but the above-mentioned comments should be carefully addressed and the similarity issues must be convincingly explained. Despite the fact that no compounds effective against MRSA were successfully prepared, the thesis provides valuable insights and represents a significant contribution to the field of developing potential antimicrobial agents, with the opportunity for repurposing them as antimycobacterial compounds.

Evaluation of the thesis: choose evaluation		**For the defense:** **Recommend**
In Hradec Králové	6. září 2024	signature of the opponent