REVIEW

by Assoc. Prof. Rumyana Ivanova Bakalska, PhD,

Plovdiv University "Paisii Hilendarski"

in the area of higher education 4. Natural sciences, mathematics and informatics

professional field 4.2. Chemical sciences

scientific specialty Organic Chemistry

For awarding the educational and scientific degree "Doctor"

in the area of higher education 4. Natural sciences, mathematics and informatics

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doctoral program Organic Chemistry

Author: Yordanka Dimitrova Sapundzhieva

Thesis theme: "Synthesis of Quinoline Derivatives with Potential Antibacterial Activity "

PhD Supervisor: Assoc. Prof. Plamen Angelov, PhD from Paisii Hilendarski University

1. General description of the submitted materials

By order № 22-265 of 4.02.2025 of the Rector of the University of Plovdiv "Paisii Hilendarski" (PU), I was appointed a member of the scientific jury to provide a procedure for the defense of a dissertation on "Synthesis of Quinoline Derivatives with Potential Antibacterial Activity" for obtaining the educational and scientific degree "Doctor" in the field of higher education 4. Natural sciences, mathematics and informatics; professional field: 4.2. Chemical sciences; doctoral program: Organic Chemistry. The author of the dissertation is Yordanka Sapundzhieva, a PhD student in full-time education at the Department of Organic Chemistry with supervisor Assoc. Prof. Dr. Plamen Angelov from Paisii Hilendarski University of Plovdiv.

The set of materials presented by Yordanka Sapundzhieva on electronic media is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of the University of Plovdiv. The doctoral student has attached 6 publications, which are indexed in the *Scopus* and *Web of Science* databases.

2. Relevance of the topic

The dissertation submitted for review on the topic "Synthesis of quinoline derivatives with potential antibacterial activity" is in a very interesting and rapidly developing area of the organic synthesis in recent years. The choice of the topic is based on the current problem of the

alarming increase in resistance to antibacterial agents established in medical practice. According to the World Health Organization (2014), the threat of the onset of the so-called “post-antibiotic era," in which the antimicrobial drugs known to date will have lost their effectiveness, is real. The current dissertation work is mainly focused on the development of new methods for the synthesis of quinoline derivatives, and more specifically of 2- and 4-quinolone-type derivatives with potential antibacterial action, structurally close to known medicinal substances and bacterial metabolites. Priority as target molecules are 4-quinolones, including known metabolites of the opportunistic pathogen *Pseudomonas aeruginosa*, as well as their new analogues with antibacterial activity. To a lesser extent, the preparation of 2-quinolone-type structures and some problems in the implementation of the Knorr synthesis with more complex functionalized substrates have been studied. As the main starting compounds and a common starting point for both types of target structures, *β*-ketoamides, accessible by a method previously developed in the Department of Organic Chemistry, were used.

The main part of the research for the dissertation was carried out under the scientific supervision of Assoc. Prof. Dr. Plamen Angelov at the Department of Organic Chemistry of the Plovdiv University Paisii Hilendarski. Only some specific measurements were performed in other laboratories, such as those for antimicrobial activity at the Faculty of Biology of the Plovdiv University and at the University of Food Technology in Plovdiv.

3. Knowing the problem

The literature review on the topic is excellently written and structured and covers 137 literary sources. The concise and logical exposition of the methods for the synthesis of 2- and 4-quinolones and their biological activity demonstrates a thorough knowledge of previous research on the subject. The doctoral student's knowledge of a huge number of named organic reactions, including the mechanism of their occurrence, as well as her impeccable handling of the specific terminology of organic synthesis, makes a very pleasant impression. This impression is complemented by the brilliantly executed graphic material, which makes it extremely easy for the reader to perceive the information. Citation of the latest publications in the researched scientific field (92% of the cited literary sources are after 2000) confirms my opinion that the doctoral student knows the specific scientific issues very well and handles the literature on the subject freely.

4. Research methodology

The dissertation work used modern and adequate methods for studying and proving the properties of the obtained organic compounds. The chemical structure and purity of the compounds was proven by proton and ^{13}C NMR, including two-dimensional techniques

(HSQC, HMBC, NOE correlations); HRMS- and URLC-MS analyses; IR-spectroscopy. The selected research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation.

5. Characteristics and evaluation of the dissertation work

The work with a volume of 144 printed pages is well structured and includes an introduction (2 pages), a literature review (50 pages), results and discussion (39 pages), an experimental part (48 pages), summarized results and conclusions (1 page). The dissertation work is illustrated with 100 schemes, 12 tables and 45 figures. The bibliography includes 155 cited literary sources, about 62% of which are from the last ten years. The introduction of the dissertation very skillfully leads the reader to the subject of the research and motivates the purpose of the research. The literature review begins with a general overview of the methods for constructing a quinoline ring, and then conditionally includes two parts: in the first, 4-quinolones with their biologically active representatives and methods of synthesis (classical and modern) are presented; and in the second part, the methods of synthesis and biological activity of 2-quinolones are considered. I liked the analytical follow-up and the depth of the comparative consideration of the different synthetic approaches. This logic in the structural organization is followed in the Results and Discussion section as well. The introduction to the dissertation very skillfully introduces the reader to the subject of the research and motivates the goal of the scientific pursuit. The literature review begins with a general overview of the methods for constructing a quinoline ring and then conditionally includes two parts: in the first, 4-quinolones with their biologically active representatives and methods of synthesis (classical and modern) are presented; and in the second part, the methods of synthesis and biological activity of 2-quinolones are considered. I liked the analytical follow-up and the depth of the comparative consideration of the different synthetic approaches. This logic in the structural organization is followed in the Results and Discussion section as well. To achieve the goal set in the introductory part, namely the synthesis of quinoline derivatives with potential antibacterial activity, two different approaches have been studied, from which the authors expect to lead to the preparation of quinoline derivatives of the 4-quinolone and 2-quinolone types, respectively. What these two approaches have in common is the use of *β*-ketoamides as precursors. Unlike the complex approach **A**, which is based on a multi-step methodology conceived by the team and includes several possible implementations, approach **B** is essentially an implementation of a one-step Knorr cyclization, which attracts interest with possible subsequent stages and competitive processes for some starting *β*-ketoamides with more

complex functionalized residues. I was very impressed by the in-depth look at solving problems of dynamic and static stereochemistry that arose during the research.

The contributions of Yordanka Sapundzhieva's dissertation are undeniable, and the most significant ones can be summarized as follows: 1) three different approaches for the preparation of quinoline derivatives were investigated, each of which started from *β*-ketoamides as starting compounds; 2) a new method was developed for the preparation of 2-alkyl-4-quinolones, 2-alkyl-4-quinolone-3-carboxamides and their *N*-hydroxy derivatives by reductive cyclocondensations of *ortho*-nitrobenzoylated enamine intermediates, and this synthetic method allowed for the first time to isolate and characterize as individual compounds the two tautomeric forms of the bacterial toxin 2-nonyl-4-quinolone-*N*-oxide (NQNO); 3) a method for the preparation of 1,2-dialkyl-4-quinolone-3-carboxamides by intramolecular aromatic nucleophilic substitution in *ortho*-fluorobenzoylated enamine intermediates has been developed; 4) using the newly developed methods, six natural compounds from the group of 4-quinolones, which are known metabolites of *P. aeruginosa* or plant alkaloids, have been synthesized, and thirty-seven new and unstudied structural analogues of them have been synthesized, with varying substituents in positions 1, 2 and 3 in the 4-quinolone ring system; 5) the behavior of *γ*-aminophenyl-functionalized *β*-ketoamides under Knorr-cyclization conditions in polyphosphoric acid was studied, in which, in addition to the expected 2-quinolones, two more types of products of competing cyclization processes were isolated, and it was proven that the competing indole-type product undergoes additional dearomatative spirocyclization; 6) the obtained new 4-quinolone derivatives were tested for antibacterial activity, in which several substances with strong activity against *S. aureus* and one substance with broad-spectrum activity against gram-positive and gram-negative bacteria were found.

6. Evaluation of the publications and personal contribution of the doctoral student

The results included in the dissertation have been published in 6 scientific articles in specialized journals *Molecules* (Q1), *Beilstein Journal of Organic Chemistry* (Q2), and *Molbank* (Q4) (3 manuscripts), with the sixth article being at the stage of preprint preparation, but it has a DOI in *ChemRxiv* (no impact factor). All the articles are in English; as in the articles above, the doctoral student is the first and second author, respectively.

I have no significant comments on the dissertation, the abstract and other materials submitted by Yordanka Sapundzhieva. On the contrary, I would like to express my satisfaction with the completeness, orderliness and precision of the materials submitted to me for review, which not only fully satisfy the requirements of the regulatory documents, but also ease the work of the members of the scientific jury. I would only like to mention the technical inaccuracy

on page 23 (the comment concerns scheme 16, not scheme 14) and some spelling errors, but their number is insignificant. The inevitable inaccuracies noted do not change the overall excellent impression of the research conducted and their presentation in the dissertation. According to these data, the scientometric requirements for acquiring the educational and scientific degree "Doctor" are fully covered and significantly exceeded.

7. Abstract

The abstract, in a volume of 32 pages, is prepared in accordance with the requirements and presents the content of the dissertation in a short and informative form. It reflects the goals and objectives, the main results and their discussion, the conclusions of the achievements, as well as the list of publications. A good impression is made by the thanks expressed to funds, without whose funding the present work would be impossible.

8. Recommendations for future use of the dissertation contributions and results

From the point of view that doctoral student Yordanka Sapundzhieva has clearly mastered and successfully applied in her work the most modern methods for the synthesis and study of heterocyclic organic compounds, and also because of the great potential of these compounds, I would recommend that research in this area be continued with an expanded composition of the scientific group.

CONCLUSION

The dissertation of Yordanka Sapundzhieva represents a thorough and systematic study in a current field of organic synthesis and **contains original scientific studies** on the synthesis of 2- and 4-quinolones from *β*-ketoamides as starting compounds. **The scientific and applied results** in the dissertation work are related to the conducted studies on the antibacterial activity of the obtained compounds. The work performed is significant in volume and diverse in nature, which gives reason to assume that the doctoral student Yordanka Dimitrova Sapundzhieva **possesses** in-depth theoretical knowledge and professional skills in the scientific specialty of organic chemistry, **demonstrating** qualities and skills for independent conduct of scientific research.

In conclusion, the dissertation **meets all the requirements** of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the relevant Regulations of the University "Paisiy Hilendarski."

Due to the above, I confidently give my **positive assessment** of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results, and contributions, and

I propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to Yordanka Dimitrova Sapundzhieva in the field of higher education: 4. Natural Sciences, Mathematics and Informatics; professional field 4.2. Chemical Sciences; doctoral program Organic Chemistry.

25.02.2025

Reviewer: .....

/Assoc. Prof. Rumyana Bakalska, PhD/