REVIEW

by Prof. Dr. Milen Georgiev Bogdanov

Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski"

of a dissertation for the award of the educational and scientific degree "Doctor" in:

Scientific field: 4. Natural Sciences, Mathematics and Informatics

Professional field: 4.2. Chemical Sciences

PhD program: Organic Chemistry

Author: Yordanka Dimitrova Sapundzhieva

Title: Synthesis of quinoline derivatives with potential antibacterial activity

Scientific supervisor: Assoc. Prof. Dr. Plamen Angelov, Faculty of Chemistry at the University of Paisiy Hilendarski

1. General description of the materials presented

Per order No. P22-265 dated February 4, 2025, from the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been appointed to the scientific jury responsible for evaluating the dissertation titled "Synthesis of Quinoline Derivatives with Potential Antibacterial Activity." This dissertation aims to achieve the educational and scientific degree of "Doctor" in the field of higher education 4, focusing on Natural Sciences, Mathematics, and Informatics, within the professional field of 4.2. Chemical Sciences under the doctoral program in Organic Chemistry. The dissertation's author is Yordanka Dimitrova Sapundzhieva, who is a part-time doctoral student in the Department of Organic Chemistry, supervised by Assoc. Prof. Dr. Plamen Angelov from the Faculty of Chemistry at PU "Paisii Hilendarski".

The materials provided by the PhD student on paper comply with Art. 36 (1) of the Regulations for the Development of the Academic Staff of the University of Plovdiv, and consist of the following documents:

– a request to the Rector of the University of Plovdiv for the opening of the procedure for the defense of a dissertation;
– a CV in European format;
– a report from the departmental council, related to reporting the readiness to open the procedure and to a preliminary discussion of the dissertation;
– dissertation;
– an abstract (Short Thesis);
– a list of scientific publications on the topic of the dissertation;
– copies of scientific publications;
– a list of noted citations;
– a declaration of originality and authenticity of the attached documents;

PhD student Sapundzhieva has included six scientific publications, along with certificates and attestations for participating in scientific conferences, qualification courses, and international exchange programs (Erasmus+).

2. Brief biographical data

Yordanka Sapundzhieva, born on December 8, 1999, graduated from the University of Plovdiv's Faculty of Chemistry (FCH) in 1999, majoring in "Chemistry, Food Chemistry, Pedagogy". She also attended part-time studies in the Master's program for Medical Chemistry from 2019 to 2020. In February 2021, she enrolled as a PhD student in the "Organic Chemistry" program within the same department. From 2004 to 2023, she worked at the Ministry of Defense of the Republic of Bulgaria, where she held various roles, such as Commander of the Security Department, Commander of the Nuclear, Chemical, and Biological Intelligence Department, and Head of the Calculation and Information Group. In February 2024, she started her current position as a researcher at the Faculty of Chemistry at the University of Plovdiv.

3. Relevance of the topic and appropriateness of the set goals and objectives

The problem of increasing antimicrobial resistance, acknowledged by the WHO as a major health concern since 2014, continues to be crucial today. This highlights the need for thorough research into both natural and synthetic biologically active compounds to develop new and more effective antimicrobial agents. A key part of this research is to innovate synthesis methods and create structural analogues, which will improve our understanding of how structure relates to biological activity, ultimately resulting in the development of superior pharmaceuticals.

Quinoline derivatives, especially 2- and 4-quinolones, remain a focus of ongoing research due to their established diverse biological activities and structural variety. These quinolones form a fundamental structural component of various natural compounds, such as alkaloids and microbial metabolites, as well as synthetic entities. This dissertation aims to develop novel synthesis methods for quinoline derivatives, particularly focusing on 2- and 4-quinolones with potential antibacterial properties. Utilizing β-ketoamides as starting materials is an effective approach for synthesis, and the project addresses the pressing issue of antimicrobial resistance. The research contributes new insights by broadening the range of existing synthesis techniques for biologically active quinolones and establishing innovative methods and compounds with significant value. It also holds promise as a foundation for future studies aimed at creating new and potent antibacterial agents.

4. Knowing the problem

PhD student Sapundzhieva possesses a thorough grasp of her dissertation subject. Her literature review examines methods for creating quinoline rings and explores the synthesis and biological properties of 4- and 2-quinolones, drawing upon 137 sources, a significant number of which were published in the past ten years. The methods discussed align closely with her dissertation focus and effectively underscore her contributions from independent research. The manner in which she presents the references is clear and proficient. Sapundzhieva skillfully evaluates the strengths and weaknesses of different approaches, categorizing them as either classical or modern, while also differentiating between those that use transition metal complexes as catalysts and those that do not. This illustrates her comprehensive expertise in the field, highlighting her capability to search for, organize, and critique scientific literature..

5. Research methodology

The primary contributions of this dissertation are rooted in organic synthesis. For synthesizing intermediates and the final functionalized compounds, well-established methods were utilized alongside the development of new and innovative techniques. The resulting products underwent purification and isolation through column chromatography, and when feasible, additional recrystallization. The synthesized compounds were thoroughly characterized using physical methods, including 1H- and 13C-NMR, as well as HRMS. I believe that the selected research methodology aligns perfectly with the defined goals and objectives, ensuring the reliability of the results obtained.

6. Characteristics and evaluation of the dissertation work

The dissertation is written on 144 pages, of which introduction (2 pages), literature review (50 pages), results and discussion (39 pages), experimental part (39 pages), summarized results (1 page), list of publications (1 page). The results are illustrated with 12 tables, 45 figures and 100 schemes. 155 literary sources are cited, 111 (71%) of which were published after 2010.

In the implementation of clearly defined tasks, doctoral student Sapundzhieva initially synthesized beta-ketoamides, selected as precursors of the corresponding 2- and 4-quinolone derivatives. Regarding acetoketoamides, an acetoacetylating reagent was chosen for work, decomposing to diketene upon heating, then treated with the corresponding amine to obtain ketoamides. For long-chain ketoamides, a strategy developed in the department was used, using Boc-monoprotected ethylenediamine to obtain beta-enaminoamides, which are susceptible to alpha-acylation in the presence of DMAP as a catalyst. Upon removal of the protective group, aza-Michael addition and fragmentation sequentially occur, leading to diversely and appropriately substituted beta-ketoamides. The reactions proceed with excellent yields. The strategy used allows the inclusion in the structure of the intermediates of fragments that determine biological activity for natural products.

The next part deals with the synthesis of 4-quinolone derivatives. The phenyl fragment is incorporated by acylation of the ketoamides synthesized in the first part. Nitro- and fluoro- ortho-substituents in the benzene nucleus are examined in more detail, which also suggests a specific enamination of the ketoamides. The properties of the acylated products are studied using spectral methods, including the differences in the chemical shifts of characteristic signals and the rotameric-tautomeric mixtures in solution, which are given special attention. The subsequent cyclization involves two substeps, depending on the ortho-substituent in the phenyl ring. In the case of nitro-substituted products, an initial reduction and subsequent condensation are required. The reduction is carried out by two alternative approaches. Analysis of the results shows the presence of unexpected products – N-oxides, accompanying the first step. The conditions are optimized so that this by-product is avoided, and its targeted preparation is carefully studied subsequently. In the case of 4-fluorobenzoylated products, the condensation occurs in a single step, but this requires the initial formation of the nitrogen nucleophile. Although it is assumed that the amide group in the compound can also be deprotonated, it turns out that only the deprotonation of the enamine N-H group leads to condensation, which has been confirmed by NMR spectroscopy. In addition to C-3 substituted 4-quinolones, they have been successfully synthesized by heating in acidic medium, reduction and subsequent cyclization of ortho-nitrobenzoylated intermediates and decarbamoylated products resembling biologically active structures. 2D NMR experiments allow us to conclude that there is a hydrogen bond stabilizing the Z-isomer of the enaminoketones. The partially reduced N-oxides observed as an unexpected product are the subject of a separate study. Optimal conditions for their preferred preparation have been found and 14 derivatives have been synthesized in good yields. An interesting phenomenon was observed for one of the synthesized compounds, namely, that the purification method led to the isolation of two separate tautomeric forms with clearly different physical properties, such as solubility, melting points, NMR and IR spectra. The synthesis of 2-benzyl-4-quinolone derivatives was also studied, in which an unexpected product was isolated, for the preparation of which an electrocyclic mechanism was assumed. The reduction of the newly obtained compound leads to four-cyclic condensed products with a naphthalene ring.

The third part concerns 2-quinolone derivatives, the synthesis of which expands the scope of the Knorr approach. It has been established that, depending on the protecting group, different ratios of compounds with 5-membered or 6-membered rings are preferentially obtained. The formation of a spirocyclic product is interesting to note.

The last part of the dissertation work is on the antibacterial activity of the synthesized substances. Tests were conducted against Gram-positive and Gram-negative bacteria, and the fungal strain C. albicans. It was found that the extension of the alkyl chain increases the activity and that N-

hydroxy-4-quinolones are more active than the initially assumed quinolones. Subsequent analysis against a wider range of microorganisms confirmed these conclusions and highlighted the n-nonyl substituent as the most promising for further studies.

7. Contributions and significance of the development for science and practice

In line with the established goals and tasks, many intermediates and new compounds have been synthesized and characterized - an endeavor that demands patience, dedication, precision, and a significant investment of time and effort. As a result of this research, PhD student Sapundzhieva outlined six contributions, summarized as follows:

1. **Expanding the scope of the methodology for the synthesis of β-ketoamides** – three new approaches for the synthesis of quinoline derivatives based on β-ketoamides were investigated. The starting β-ketoamides were obtained by an original method developed in the research group of Assoc. Prof. Angelov, and its application in a broader context was demonstrated. This contributes to expanding the synthetic possibilities in the field of quinolones.

2. **A new strategy for the synthesis of 2-alkyl-4-quinolones and the discovery of tautomeric forms of a bacterial toxin** – A method for the synthesis of 2-alkyl-4-quinolones, 2-alkyl-4-quinolone-3-carboxamides and N-hydroxy derivatives by reductive cyclocondensation was developed for the first time. This approach allowed the isolation and characterization of the two tautomeric forms of the bacterial toxin 2-nonyl-4-quinolone-N-oxide (NQNO), which would be of great importance for understanding its biological activity.

3. **Innovative method for the synthesis of 1,2-dialkyl-4-quinolone-3-carboxamides** – A new method based on intramolecular aromatic nucleophilic substitution in ortho-fluorobenzoylated enamine intermediates has been developed, which allows for the efficient synthesis of 1,2-dialkyl-4-quinolone-3-carboxamides as compounds with potential biological activity.

4. **Synthesis of natural and novel 4-quinolone analogues with potential for significant biological activity** – The developed new methods have been successfully applied to the synthesis of six natural 4-quinolones, including metabolites of P. aeruginosa and plant alkaloids, as well as 37 new structural analogues with modifications at key ring positions.

5. **Discovery of unexpected reactivity in Knorr cyclization** – Studies on the behavior of γ-aminophenyl-functionalized β-ketoamides under Knorr cyclization conditions have provided new

mechanistic insights into the reactivity of β-ketoamides, as they demonstrate the occurrence of competing reactions to give cyclic products, including indole structures. The latter have been shown to undergo subsequent spirocyclization..

6. **Detection of substances with potential antibacterial activity** – Biological studies of newly synthesized 4-quinolones identified several substances with high activity against S. aureus, as well as one compound with broad-spectrum activity against Gram-positive and Gram-negative bacteria.

In conclusion, this dissertation makes a noteworthy contribution to the advancement of methods for synthesizing biologically active quinolones. The new strategies introduced, the identification of tautomeric forms of a bacterial toxin, the synthesis of both natural and novel analogues, and the discovery of compounds with antibacterial activity offer significant scientific and practical outcomes that hold promise for future investigations in medicine and chemistry.

8. Assessment of dissertation publications

The dissertation work includes six scientific reports published in specialized and multidisciplinary international journals: Molecules (Q1), Beilstein J. Org. Chem. (Q2), MolBank (Q4), and ChemRxiv. The acceptance of these articles in prestigious journals esteemed by the global scientific community reinforces my overall impression of the high quality and reliability of the dissertation material. Doctoral student Sapundzhieva is the lead author on three of the publications. At the time of this review, no citations from other authors were found, which we consider normal due to the brief publication period of these scientific works.

9. Personal participation of the doctoral student

Given my knowledge of the nature of the synthetic work, I have no doubt about the personal contribution of the doctoral student to the research conducted. This is also proven by the attached scientific publications, in which PhD student Sapundzhieva is the first author.

10. Abstract (Short thesis)

The shortened version of the PhD thesis is clearly articulated and succinct, demonstrating the progression of the research detailed in the dissertation and logically culminating in the presentation of the doctoral student's key achievements. The appropriate chemical terminology is employed, and the transformations discussed, along with the results obtained, are effectively supported by relevant diagrams, figures, and tables. A noteworthy critique of the abstract is the absence of a literature citation list, which is a necessary component of any independent scientific work.

11. Critical remarks and recommendations

I don't have any major comments about the dissertation, but I do have a few questions:

• Why was C. albicans selected as the sole test fungal strain?

• What factors do you believe contribute to the varied behavior of the rotameric-tautomeric mixtures in different solvents?

• Why were compounds 32 and 33 unable to be separated preparatively? Have alternative separation conditions been tested?

• Spiro compounds could also be interesting from a biological perspective. Why were they not investigated?

12. Personal impressions

I don't know the PhD student personally.

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

The results offer several opportunities for future development, considering both synthetic and biological aspects. The synthetic techniques developed allow for the creation of structurally diverse 4- and 2-quinolones. Furthermore, the isolated and verified unexpected products can aid in formulating methods for synthesizing corresponding structural analogues.

CONCLUSION

The dissertation contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the relevant Regulations of the University of Paisiy Hilendarski.

The dissertation shows that doctoral student Yordanka Dimitrova Sapundzhieva possesses in-depth theoretical knowledge and professional skills in the scientific field 4.2. Chemical Sciences, demonstrating qualities and skills for independent conduct of scientific research.

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, PhD Program in Organic Chemistry.

03 April 2025 г. Reviewer: .....

Prof. Milen Georgiev Bogdanov, PhD