

OPINION

By Dr. Stoyanka Nikolova Atanasova, Associate Professor at the Paisii Hilendarski Plovdiv University

of a PhD thesis for awarding of the educational and scientific degree "Doctor"

in: field of higher education 4. Natural Sciences, Mathematics and Informatics;

professional field 4.2. Chemical Sciences

scientific specialty: Organic Chemistry

Author: Yordanka Dimitrova Sapundzhieva

Title: Synthesis of quinoline derivatives with potential antibacterial activity

Scientific advisor: Assoc. Prof. Dr. Plamen Angelov

1. General description of the procedure and the doctoral candidate.

By a written order No. PD-22 of 04.02.2025 of the Rector of the Paisii Hilendarski Plovdiv University I am appointed as a member of the scientific jury for ensuring a procedure for the defense of a PhD thesis on the topic "Synthesis of quinoline derivatives with potential antibacterial activity" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural Sciences, Mathematics, and Informatics; professional field 4.2. Chemical Sciences, scientific specialty Organic Chemistry. The author of the dissertation is Yordanka Dimitrova Sapundzhieva - a part-time PhD student at the Department of Organic Chemistry, with scientific supervisor Assoc. Prof. Dr. Plamen Angelov from the Paisii Hilendarski Plovdiv University.

The submitted thesis and all accompanying documents are in complete accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the internal regulations of the Plovdiv University.

2. Brief biographical data of the PhD student

Yordanka Sapundzhieva was born on 08.12.1976 in the city of Sliven. She graduated from the Paisii Hilendarski University, Faculty of Chemistry, (MSc in Chemistry) and specialization in Food Chemistry, and later in Medical Chemistry (MSc). In the period 2021–2025, Ms. Sapundzhieva is studying as a PhD student in the scientific specialty "Organic Chemistry" at the Faculty of Chemistry of the Paisii Hilendarski Plovdiv University.

During her studies, the PhD student has fully completed the educational part of her individual plan. She successfully passed the exam in the specialty with an overall grade of very good 5.00.

She has attended courses to improve her English language skills and specialized courses related to her direct scientific interests, such as the qualification on "High-performance liquid chromatography"; a course at the Yuventutis Academy "Biofunctionalized heterocyclic compounds" with Assoc. Prof. Statkova; a course "Peptides - an alternative to conventional drugs" with Prof. Danalev; seminars with Prof. F. Alonso from the University of Alicante on the topic: "Organic stereochemistry", "Preparation of organometallic compounds for selective transformations in organic synthesis" and "Aspects of enantioselective synthesis and modern applications", in addition, a seminar in Organic Chemistry with Assoc. Prof. Angelov.

Last but not least, we should also mention the specialization that the doctoral student conducted under the Erasmus+ program with Prof. F. Alonso from the University of Alicante.

The PhD student regularly takes classes at the Department of Organic Chemistry at the Faculty of Chemistry of the Plovdiv University.

3. Relevance of the topic.

The presented work by Yordanka Sapundzhieva examines the synthesis of 2- and 4-quinolones with potential antibacterial activity. The introduction of the problem of the insufficient effectiveness of antibacterial agents and the increasing resistance to them is well-founded and relevant, emphasizing the importance of finding new synthetic approaches for creating alternative drugs. The main goal of the study, related to the development of new methods for the synthesis of quinoline derivatives with antibacterial activity, is clearly defined. More importantly, the PhD student presents specific examples, emphasizing the synthesis of 4-quinolone derivatives and their analogues associated with pathogens such as *Pseudomonas aeruginosa*, which gives applied value to the study and confirms the relevance of the topic.

4. Knowledge of the problem.

The PhD student has demonstrated detailed knowledge and deep understanding of the chosen research theme. The literature related to the research problem has been thoroughly reviewed in a dedicated chapter of the thesis, comprising 52 pages and including 137 references. The methods for construction of a quinoline ring, biological activity and methods for synthesis of 4-quinolones, as well as biological activity and methods for synthesis of 2-quinolones are considered. Examples of 4-quinolones of plant and bacterial origin are also considered. The author's comprehensive literature review on the topic demonstrates excellent information, analytical and in-depth insight into the

problem. The analysis of the synthesis methods known from the scientific literature is correct, complete and up-to-date and covers a wide period, but basically contains publications from the last 20 years. The skillful application of relevant methodological approaches in the pursuit of the chosen research goals is another indication of the knowledge of the PhD student.

5. Research methodology

Achieving the goal of the PhD thesis and the associated research tasks in the PhD thesis requires using traditional synthetic methods while also creating innovative ones, all in combination with suitable modern molecular spectroscopy techniques. In this respect, the methodological aspects of the PhD thesis are executed excellently. In order to fully characterize the compounds and prove their structure, ^1H - and ^{13}C -NMR techniques were used, including two-dimensional techniques (HSQC, HMBC, NOE correlations) and for most compounds, their HRMS spectrum was also applied. NMR techniques were used to study the influence of the substituent in establishing rotameric-tautomeric equilibria in CDCl_3 . Possible structures of the intermediates formed were proposed.

6. Characteristics and evaluation of the thesis

The PhD thesis is presented on 144 standard pages and includes 12 tables, 100 schemes, and 45 figures. The bibliography covers a total of 155 literary sources. It is structured classically - Introduction; Literature review; Tasks; Results and discussion; Experimental part; Conclusions; Publications on the topic of the thesis, and literature references.

The dissertation is written consistently and clearly and contains original scientific research and results. Based on the extensive, orderly and very clear literature review, the research tasks in the dissertation are correctly formulated. The task of synthesis of 2- and 4-quinolones is clearly set, with the following tasks – synthesis of starting beta-ketoamides, enamination and acylation of beta-ketoamides with benzoyl chlorides, decarbamylation to benzoylated enamines and investigation of the possibility of intramolecular cyclocondensation to obtain 2-substituted 4-quinolones on the one hand and attempts at Knorr cyclization of beta-ketoamides to 2-quinolone derivatives. The possibility of obtaining the natural alkaloid Pseudan IX has been investigated and its antibacterial activity has been determined. The synthesis of a metabolite of *Pseudomonas aeruginosa* 2-benzyl-4(1H)-quinolone has also been proposed. The overall work is a continuation of the topic developed for years in the Department of Organic Chemistry by the doctoral student's scientific supervisor, Assoc. Prof. Angelov.

The thesis is very well-formed and structured, with the individual parts presented in chronological order. The PhD student has mastered and successfully applied in her work methods for syn-

thesis, isolation of compounds in pure form, characterization and confirming the structure of compounds using modern analysis techniques. This allows solving the tasks set in the thesis. The precisely conducted experiment and interpretation of the methods for studying the structure of compounds make a very good impression.

An impressive amount and quality of experimental work has been carried out. In the experimental part of the dissertation, all the developed synthetic methods are described in detail, with full spectral characteristics of the synthesized compounds. All the compounds have been fully characterized using various NMR techniques, mass spectrometry, elemental analysis, and melting points. Therefore, the educational tasks of the doctoral program have also been fulfilled.

7. Contributions and significance of the thesis.

The contributions of the doctoral student's dissertation work include: **scientific** - a set of 2-alkyl-4-quinolones, 2-alkyl-4-quinolone-3-carboxamides and their N-hydroxy derivatives, 1,2-dialkyl-4-quinolone-3-carboxamides and 2-quinolones were synthesized; the two tautomeric forms of 2-nonyl-4-quinolone-N-oxide were isolated and characterized; six natural 4-quinolones were synthesized; and **scientific and applied** - very good antibacterial activity of some of the obtained 4-quinolones was proven and activity against *S. Aureus* ATCC 25923 and moderate activity against *E. coli*, *Enterococcus faecalis*, and *Bacillus subtilis* was established.

8. Publications in connection with the thesis and personal contribution of the PhD student

Results from the thesis have been published in six separate peer-reviewed papers in journals with impact factor, as follows: one of them is published in a journal with Q1, one in Q2, and three of the papers are published in Q4. These papers fully correspond to the results presented in the thesis and the PhD student is the first or second author in all of them, which is a good indication of her own involvement and contribution. The PhD student presented results from her work at 3 scientific forums with two oral and three poster presentations.

9. Remarks and recommendations

I have no major remarks or recommendations.

10. Personal opinion.

I have known PhD student Sapundzhieva as my student since her studies in her Master's degree in the specialty "Medicinal Chemistry" and later as a PhD student in the Department. The impression I have of her is of a modest but strict, hardworking, innovative, and searching young researcher, who I hope will develop her potential in the future.

11. Recommendations for future use of the thesis contributions and results

The research conducted is in a relevant and interesting area of modern knowledge and in particular in the search for new more effective medicinal substances to combat socially significant diseases. Simultaneously with the accumulation of useful information, the PhD student has also carried out a huge amount and quality of experimental work, which could provide her with a basis for further continuation of research and growth in her scientific career.

CONCLUSION

The PhD thesis contains both scientific and applied scientific results that represent an original contribution to science and meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law, and the relevant Regulations of the Paisii Hilendarski Plovdiv University.

The thesis shows that the PhD student Yordanka 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.

Considering the relevance and significance of the topic of the dissertation, the abstract, the fulfillment of the set goal, the scientific and applied scientific results contained therein, which represent an original contribution to science, I kindly recommend to the 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, scientific specialty "Organic Chemistry".

28.03.2025 г.

.....
(Assoc. Prof. Stoyanka Nikolova, PhD)