STATEMENT

by prof. Ginka Atanasova Antova, PhD,

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On dissertation for awarding the educational and scientific degree "Doctor of Philosophy" (PhD)

Area of higher education: 4. Natural sciences, mathematics and informatics

Professional field: 4.2. Chemistry sciences Doctoral program: Organic chemistry

Author: Miglena Zlatkova Milusheva

Thesis: Synthesis of novel antispasmodics affecting memory functions in experimental animals

Supervisors: Assoc. Prof. Stoyanka Nikolova Atanasova, PhD – University of Plovdiv "Paisii Hilendarski" and Assoc. Prof. Iliyana Dimitrova Stefanova-Kuncheva, PhD - Medical University - Plovdiv

1. General overview of the procedure and the PhD student

By order № RD-22-1134/15.05.2025 of the Rector of University of Plovdiv "Paisii Hilendarski" I was appointed a member of the scientific jury to provide a procedure for the defense of a dissertation on "*Synthesis of novel antispasmodics affecting memory functions in experimental animals*" for obtaining the educational and scientific degree "Doctor" in the area 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 Miglena Zlatkova Milusheva - PhD student in full-time form at the Department of Organic Chemistry with supervisors Assoc. Prof. Stoyanka Nikolova Atanasova, PhD – University of Plovdiv "Paisii Hilendarski" and Assoc. Prof. Iliyana Dimitrova Stefanova-Kuncheva, PhD - Medical University – Plovdiv.

The set of materials on electronic media presented by doctoral student Miglena Milusheva is in accordance with Article 36 (1) of the Regulations for Development of the Academic Staff of the University of Plovdiv and includes all the necessary documents.

The doctoral student has submitted a dissertation, an abstract and 3 publications published in scientific journals indexed in world-renowned databases (Web of Science and Scopus). The dissertation consists of 11 sections, presented on 171 standard pages, including an introduction, a literature review, goals and objectives, own research, an experimental part, conclusions, contributions, publications on the topic of the dissertation, participation in projects, presentations at scientific forums and literature (394 references).

Miglena Milusheva was enrolled as a full-time doctoral student from 1.03.2023, and from 01.05.2025 she was enrolled with the right to defend, during which period she worked as an assistant professor at the Medical University - Plovdiv.

2. Relevance of the topic

The relevance of the topic in Miglena Milusheva's dissertation is clearly justified in the introduction and literature review. The research focuses on functional disorders of the gastrointestinal tract, such as irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD). These condi-

tions affect millions of people worldwide and remain a significant clinical challenge due to their complex pathophysiology and limited therapeutic options. Existing antispasmodic drugs often show low efficacy or lead to side effects, creating a need for new pharmaceutical molecules with improved safety and efficacy profiles. The dissertation integrates various fields: organic synthesis of new hybrid derivatives of anthranilic acid, in silico modeling for drug potential assessment, biological evaluation of the obtained compounds, and pharmacological testing for antispasmodic, antiinflammatory, antimicrobial, and cognitive effects, highlighting its interdisciplinary nature. Particularly innovative is the approach involving hybrid molecules that combine pharmacophoric fragments to achieve synergistic effects and enhanced biological activity. The use of anthranilic acid as a structural foundation is motivated by its biological significance and presence in numerous bioactive molecules, reinforcing its potential for developing effective therapeutic agents. An additional focus on the impact of the compounds on memory functions in experimental animals adds another dimension to the relevance of the research, positioning the developed compounds within the context of multifunctional therapeutic agents. The dissertation topic is highly relevant as it addresses a real medical need, incorporates modern scientific approaches, and proposes innovative solutions with potentially high practical value.

3. Knowledge of the problem

The topic of the present dissertation is a natural continuation of previous research within the department on the synthesis of new compounds with potential biological activity. A thorough bibliographic review has been conducted, processing a large volume of information, which has been interpreted with understanding. The 394 cited literary sources, all in Latin script, demonstrate the doctoral candidate's strong familiarity with the literature. Thirty-five percent of the sources are from after 2015, indicating the use of contemporary scientific studies relevant to the dissertation topic. Based on the well-structured and up-to-date literature review and its conclusions, the research objectives and tasks in the dissertation have been correctly formulated.

4. Research methodology

The Experimental Section describes the reagents used, the methods for synthesizing the newly synthesized compounds, the chromatographic techniques for isolation and purification, the spectral methods for confirming the obtained substances, and the computational approaches used to establish the structure–biological activity relationship for each group of synthesized compounds. The dissertation provides a detailed examination of *ex vivo* methods for determining spasmolytic activity, *in vitro* and *ex vivo* methods for assessing anti-inflammatory activity, and *in vitro* techniques for evaluating the antimicrobial activity of the synthesized compounds. Additionally, *in vivo* methods are described for assessing the effects of the new molecules on motor activity and cognitive abilities in rats following treatment.

The selected procedures for synthesizing new compounds, along with the methods for their characterization and the experimental designs for studying their biological activity, enable the achievement of the research objectives and provide answers to the dissertation's key questions. The majority of the research was conducted at the Faculty of Chemistry at University of Plovdiv "Paisii Hilendarski". The experimental determination of biological activity was carried out at the Medical University of Plovdiv, while antimicrobial studies were conducted at the University of Food Technologies-Plovdiv. NMR measurements were performed at Sofia University "St. Kliment Ohridski".

During the research process, the doctoral candidate mastered new synthesis techniques, as well as methods for characterizing the newly obtained compounds. She independently conducted a significant portion of the research reflected in the dissertation. Therefore, I consider that the educational objective of the dissertation has also been successfully fulfilled.

5. Characteristics and evaluation of the dissertation and contributions

The dissertation is well-formatted and structured, with individual chapters presented in chronological order. It includes 10 tables, 32 figures, and 13 schemes. A substantial amount of work has been carried out, and the obtained experimental results are presented in 7 tables, illustrated with 24 well-designed figures and 3 schemes. The characterization studies of the newly synthesized compounds were conducted using modern scientific equipment, ensuring objective results. The obtained data have been correctly discussed, demonstrating that the doctoral candidate has acquired the skills necessary for presenting research findings. As a result of the conducted studies, 7 summarized conclusions have been drawn, along with 3 scientific and 3 applied scientific contributions, which hold practical significance and will contribute to the development of a new direction in medicine and pharmacology. This approach promises increased efficacy and reduced side effects compared to traditional medications. The conclusions and contributions fully align with the obtained results.

I accept the contributions presented by the doctoral candidate and their classification into scientific and scientific-applied categories. The main contributions of the dissertation are as follows:

- ✓ For the first time, using the proposed synthetic method, 18 hybrid compounds of anthranilic acid have been obtained, 16 of which represent new structures that have not been previously published in scientific literature.
- ✓ Relationships between molecular structure and biological activity have been established for the synthesized compounds, providing a foundation for future rational design of biologically active molecules.
- ✓ For the first time, the spasmolytic activity of all 18 synthesized compounds has been measured, with five of them demonstrating superior activity compared to the known spasmolytic mebeverine. Additionally, the theoretical impact of different substituents on spasmolytic activity has been investigated.
- ✓ The anti-inflammatory activity of all newly synthesized compounds has been evaluated using two methods—*in vitro* and *ex vivo*.
- ✓ Compound 4 has shown strong antimicrobial activity against pathogenic fungi and moderate activity against certain yeast species.
- ✓ A hybrid compound has been identified as a spasmolytic with anti-inflammatory properties, also influencing memory functions in experimental animals, demonstrating its potential as a multifunctional therapeutic agent.

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

The list of publications by Miglena Milusheva related to her dissertation includes three articles published in journals indexed in Web of Science and Scopus, all ranked in quartile Q1: (1 in *International Journal of Molecular Sciences* (IF = 4.9); 1 in *Pharmaceuticals* (IF = 4.3) and 1 in *Molecules* (IF = 4.2)). Miglena Milusheva's personal contribution to these scientific works is evident, as she is listed as the first author in all three publications. This strongly indicates her signifi-

cant role in their development. The publications, dated 2023–2024, reflect the core findings of her dissertation.

The quality and recognition of her research within the scientific community are further demonstrated by 22 independent citations of publication N_{21} and 27 citations of publication N_{22} in Scopus, all from international authors.

Her research findings have been presented at 10 international and 8 national scientific conferences, showcasing their broad dissemination within the chemical research community. Among these presentations, she was the first author in 11 and the second author in 5, further highlighting her active involvement.

Her publication activity exceeds the minimum national requirements and the regulations of University of Plovdiv for obtaining a PhD degree. She has submitted three publications in indexed Q1 journals, accumulating 75 points, far surpassing the required 30 points.

Additionally, she has mastered modern research techniques, including: Computational modeling; Spectroscopic analyses (NMR, FT-IR); Chromatography; Biological testing. She has independently conducted a significant portion of the research reflected in her dissertation and actively participated in interpreting and disseminating the experimental results. Therefore, I consider that the execution of the research tasks and the presentation of the findings in the dissertation are entirely her own work.

7. Abstract on dissertation

The abstract, which is 36 pages, corresponds to the content of the dissertation and is an abbreviated version of its essence, fully and accurately reflects the results achieved in the dissertation, and their analysis. In terms of structure, content and volume, it meets the requirements of the relevant regulations. It is also presented in English (36 pages).

8. Recommendations for future use of dissertation contributions and results

The presented dissertation contains a sufficient amount of material and is written in accordance with the requirements for such academic works. Overall, I have no critical remarks or recommendations regarding the conducted research and the presented materials. However, I have noticed a few technical errors, which are purely editorial in nature and do not diminish the scientific value of the dissertation. I strongly recommend that Miglena Milusheva continue her dedicated and indepth work in the field of synthesis of new biologically active compounds and the investigation of their pharmacological potential.

CONCLUSION

The dissertation contains scientific and scientific-applied *results that represent an original contribution to the science* and volume of work and the number of publications presented **fully meets** all the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDARB), the Regulations for application of ASDARB, and the Regulations of University of Plovdiv "Paisii Hilendarski".

The dissertation shows that the doctoral student Miglena Zlatkova Milusheva **has** in-depth theoretical knowledge and professional skills in the doctoral program "Organic Chemistry", **demonstrating** qualities and skills for independent research.

Due to the above, I confidently give my *positive assessment* of the study presented in the dissertation and *I propose to the esteemed scientific jury to award the educational and scientific degree "Doctor"* to Miglena Zlatkova Milusheva in area of higher education: 4. Natural sciences, mathematics and informatics, in professional field 4.2. Chemical sciences, in the doctoral program "Organic chemistry".

12.06. 2025

Statement prepared by: Prof. Ginka Antova, PhD