

OPINION

by **Prof. Krastena Todorova Nikolova**, PhD – Medical University “Prof. Dr. Paraskev Stoyanov” – Varna,

on the dissertation submitted for the award 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**

Doctoral program: **Organic Chemistry**

Author: **Miglena Zlatkova Milusheva**

Title: *Synthesis of New Spasmolytic Agents Affecting Memory Functions in Experimental Animals*

Scientific supervisors:

Assoc. Prof. Stoyanka Nikolova Atanasova, PhD – Paisii Hilendarski University of Plovdiv

Assoc. Prof. Iliyana Stefanova-Kancheva, PhD – Medical University of Plovdiv

1. General information about the procedure and the PhD candidate

By Order No. RD-22-1184/15.05.2025 of the Rector of Paisii Hilendarski University of Plovdiv (PU), I have been appointed as a member of the scientific jury for the defense procedure of the dissertation titled “*Synthesis of New Spasmolytic Agents Affecting Memory Functions in Experimental Animals*”, submitted in fulfillment of the requirements for the award 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, doctoral program "Organic Chemistry", under the Department of Organic Chemistry, Faculty of Chemistry.

The author of the dissertation is Miglena Zlatkova Milusheva – a full-time doctoral student in the Department of Organic Chemistry at the Faculty of Chemistry, under the supervision of Assoc. Prof. Stoyanka Nikolova Atanasova, PhD (PU Plovdiv) and Assoc. Prof. Iliyana Stefanova-Kancheva, PhD (MU Plovdiv).

The materials submitted by Miglena Zlatkova Milusheva in printed form comply with Al. 36 (1) of the Regulations for the Development of the Academic Staff of PU and include the following documents:

- Application to the Rector of PU to initiate the dissertation defense procedure;
- Curriculum Vitae in European format;
- Minutes from the preliminary discussion;
- Opinions from the scientific supervisors;
- Dissertation thesis;
- Author's abstract of the dissertation in Bulgarian and English;
- Declaration of originality and authenticity of the submitted documents, contributions, and results related to the dissertation topic;
- Reference verifying compliance with the minimum national requirements and the internal rules of the Faculty of Chemistry at PU for awarding the doctoral degree;
- List of scientific publications related to the dissertation topic;
- Copies of the scientific publications.

The doctoral candidate has submitted three publications, all published in peer-reviewed international journals ranked in Q₁, with impact factors above 4. It is noteworthy that Miglena Milusheva has prepared the documentation for the procedure with precision and in full compliance with the requirements. All supporting documents are duly signed and provide reliable information. The materials are well-organized, and both the author's abstract and the dissertation are written in a clear and scientifically sound language, without any spelling or grammatical errors.

2. Brief biographical notes on the PhD candidate

Miglena Zlatkova Milusheva is a full-time doctoral student in Organic Chemistry at Paisii Hilendarski University of Plovdiv (discontinued with the right to defend as of 01.05.2025), actively engaged in research focused on the synthesis and pharmacological profiling of new spasmolytic agents. She holds a Master's degree in Food Chemistry and a Bachelor's degree in Medical Chemistry from the same university.

Currently, she holds the academic position of Assistant Professor at the Medical University of Plovdiv, where she conducts practical chemistry courses in both Bulgarian and English. Her professional background also includes experience as a technologist in the industrial sector and as a clinical laboratory assistant.

She is the recipient of the national award "*PhD Student of the Year 2024*" and has earned distinctions for scientific achievements. She has a C₁ level of proficiency in English and possesses a wide range of communication, organizational, and technical skills applicable to both academic and research environments.

3. Relevance of the Topic

In her dissertation, the doctoral candidate synthesizes and investigates newly obtained hybrid derivatives of anthranilic acid with potential spasmolytic and neurotropic activity. The topic is highly relevant, combining molecular, pharmacological, and physiological approaches for evaluating chemical compounds that influence both smooth muscle function and central (cognitive) mechanisms.

The aims and objectives of the research are clearly defined, scientifically substantiated, and logically applied and implemented. A total of 18 hybrid compounds were synthesized and characterized using spectroscopic techniques such as IR, ¹H-NMR, and ¹³C-NMR. The pharmacological profile was assessed through *ex vivo* experiments on isolated tissues from the corpus region of rat stomachs, in order to evaluate the spasmolytic activity and anti-inflammatory potential. *In vivo* studies were also conducted to assess memory functions in animal models.

The presented dissertation is of both scientific and practical value, distinguished by methodological precision, interdisciplinary scope, and convincing experimental results. As a lecturer in biophysics, I find the topic to be entirely in line with contemporary scientific trends, and the doctoral candidate demonstrates multidisciplinary knowledge and a comprehensive approach in interpreting the obtained results. The relevance of the research is further evidenced by the high number of citations (21) of the candidate's publications in the Web of Science and Scopus databases.

4. Familiarity with the Problem

The doctoral candidate demonstrates a thorough understanding of the scientific problem under investigation, from both chemical and biophysical perspectives. The introduction and literature review sections of the dissertation reflect a solid awareness of current concepts regarding the role of smooth muscle, mediator systems, and neuroinflammatory processes in the pathogenesis of functional gastrointestinal disorders and associated cognitive dysfunctions.

Particularly noteworthy is the discussion of the complex regulatory interactions between the gastrointestinal tract and the central nervous system. This aspect highlights the potential for establishing interdisciplinary connections between biophysics and neurogastroenterology.

The candidate pays special attention to multicomponent regulatory mechanisms involving transmembrane signaling and the effects of small organic molecules on physiological and biophysical parameters of tissues. A substantial number of contemporary scientific sources (394 in total) were consulted, all in English, the majority of which have been published within the last 10 years.

5. Research Methodology

The methods employed in the dissertation are modern and entirely appropriate to the stated aims and objectives. The study is structured across three main levels of investigation: *in silico*, *ex vivo*, and *in vivo*.

Through *in silico* analysis, a preliminary selection of molecules was conducted using molecular docking. The toxicological profiles and ADME (Absorption, Distribution, Metabolism, and Excretion) parameters were predicted to evaluate the pharmacokinetic properties of the newly synthesized compounds before they were physically obtained.

Synthetic methods were employed, applying appropriate spectroscopic and chromatographic techniques to confirm the structural purity and identify the newly synthesized molecules. The *ex vivo* tests on isolated smooth muscle tissue demonstrate a precise analysis of the compounds' mechanism of action. The *in vivo* studies evaluate both the anti-inflammatory effects and the influence on memory-related cellular functions in experimental animals.

The applied behavioral tests and physiological models reveal a deep understanding of the interactions between the peripheral and central nervous systems. The methodologies used are scientifically sound, interdisciplinary, and well-suited for comprehensive preclinical evaluation of the pharmacological potential of the synthesized compounds. They reflect a solid knowledge of biophysical mechanisms of action and adherence to established experimental practices.

6. Characteristics and Evaluation of the Dissertation and Its Contributions

The dissertation of Miglena Zlatkova Milusheva is comprehensive and well-structured, following a logically consistent scientific sequence. Each part of the thesis demonstrates solid command of the subject matter, precise application of methodologies, and clearly articulated conclusions. The work is divided into several main chapters that cover all stages of the research – from the theoretical framework to the practical experiments and analytical interpretation.

In the introduction, the candidate highlights the scientific and societal relevance of the topic. She underscores the need for the development of new spasmolytic agents with improved efficacy and reduced side effects for the treatment of functional gastrointestinal disorders. The connection between peripheral gastrointestinal processes and the central nervous system is well justified, with emphasis placed on the potential to identify compounds exhibiting simultaneous spasmolytic, anti-inflammatory, and neurotropic activity.

The literature review is extensive and in-depth. The author demonstrates excellent awareness of the pathophysiology of irritable bowel syndrome and inflammatory bowel diseases, the role of neuroimmune regulation and the gut microbiota, as well as the mechanisms of currently available pharmacological agents. Anthranilic acid is systematically discussed as a pharmacophoric unit, including its derivatives with various biological activities. Special attention is given to the concept of hybrid molecules and the potential of nonsteroidal anti-inflammatory drugs (NSAIDs), including their combination with other pharmacophores.

Relevant preclinical models for pharmacological evaluation are described—*in vitro*, *ex vivo*, and *in vivo*—along with the ethical and regulatory aspects of animal experimentation. The references used are up to date, with numerous sources from recent years, which enhances the contemporary scientific value of the discussion.

The objectives and tasks are clearly and precisely defined. The main goal is the synthesis of new hybrid derivatives of anthranilic acid and the evaluation of their pharmacological activity—spasmolytic, anti-inflammatory, antimicrobial, and effects on memory functions. The outlined tasks cover all necessary stages: from design and *in silico* modeling, through synthesis and structural characterization, to comprehensive biological evaluation.

The section entitled “Own Research” demonstrates a high level of experimental work. The doctoral candidate begins with *in silico* screening of potential hybrid compounds based on pharmacophore modulation, molecular docking, and prediction of ADME and toxicological

properties. This is followed by the synthesis of selected compounds using classical and modified organic methods, followed by spectral (NMR, IR) and chromatographic characterization. The biological part includes *ex vivo* tests on isolated smooth muscle from laboratory animals to assess spasmolytic activity and interactions with pharmacological antagonists. *In vitro* tests evaluate anti-inflammatory and antimicrobial effects, while *in vivo* models are used to study cognitive functions via established behavioral tests. The combination of methodologies allows for an interdisciplinary interpretation—from chemical structure to functional physiological effect.

In the concluding section, the dissertation presents clearly formulated conclusions and original contributions that summarize the achievements of the doctoral candidate. The research successfully identifies new compounds with a superior spasmolytic profile compared to mebeverine, and anti-inflammatory activity has also been established.

These results not only confirm the hypotheses proposed by the doctoral candidate at the beginning of the study but also open up prospects for the development of new molecules with combined therapeutic effects.

7. Contributions and Significance of the Dissertation for Science and Practice

The practical significance of the dissertation lies in several key areas:

- The proposed compounds represent viable candidates for the development of new spasmolytic agents with an improved profile compared to the reference substance mebeverine.
- Some of the compounds exhibit additional pharmacological effects—anti-inflammatory and cognitive-protective—which makes them potentially applicable in complex clinical conditions such as irritable bowel syndrome and inflammatory bowel diseases, where psychosomatic and inflammatory components are closely intertwined.
- The proposed methodology can serve as a model for future studies in the field of drug design. The dissertation demonstrates both scientific novelty and practical applicability, making it a valuable contribution to contemporary pharmaceutical and biophysical science.

8. Evaluation of Publications and the Doctoral Candidate's Personal Contribution

Miglena Milusheva's publication activity related to the topic of the dissertation is impressive and fully meets—indeed exceeds—the established academic criteria for the defense of a doctoral degree. Three scientific articles on the dissertation topic have been published in international journals with impact factors, indexed in Scopus and Web of Science (Q1 – *Biomedicines*, *IJMS*, *Molecules*, *Pharmaceuticals*). The doctoral candidate is listed as first author in all three publications, which clearly indicates her active and leading role in the development, execution, and analysis of the experimental studies.

In the published papers, the candidate demonstrates not only strong experimental skills but also the ability to independently interpret results and formulate well-reasoned scientific conclusions. She has actively participated in 8 national and 10 international scientific events, including oral and poster presentations at conferences and symposia. Particularly noteworthy is her involvement in 5 research projects.

Her personal contribution stands out clearly in the synthesis and selection of the molecules, the organization and execution of the biological experiments, and in the preparation and publication of the results. Her work reflects a high degree of independence, scientific maturity, and critical thinking—qualities that are essential for a successful researcher and future academic supervisor.

9. Author's Abstract

The author's abstract accompanying the dissertation is prepared in accordance with the formal requirements and presents a concise summary of the research program and the theoretical foundations of the dissertation.

10. Recommendations for Future Use of the Dissertation's Contributions and Results

I have no remarks regarding the content of the submitted dissertation. Given the topicality of the subject and the extensive experimental material, I recommend that the work be developed into a monograph in the future.

CONCLUSION

The dissertation contains scientific, applied-scientific, and practical results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, and the internal regulations of Paisii Hilendarski University of Plovdiv.

The dissertation demonstrates that the doctoral candidate, Miglena Milusheva, possesses in-depth theoretical knowledge and professional skills in the scientific field of Organic Chemistry, and shows the ability to independently conduct scientific research.

In view of the above, I confidently give a positive assessment of the research presented in the reviewed dissertation, author's abstract, achieved results, and contributions, and I propose to the esteemed scientific jury that the educational and scientific degree "**Doctor**" be awarded to **Miglena Milusheva** in the field of higher education: *4. Natural Sciences, Mathematics and Informatics*, professional field *4.2. Chemical Sciences*, doctoral program *Organic Chemistry*.

10.06.2025

Prepared by:

(Prof. Krastena Nikolova)