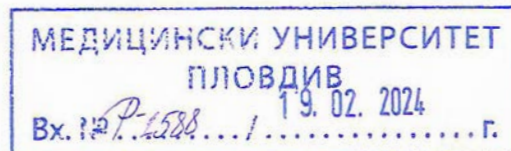


REVIEW



by Prof. Krastena Todorova Nikolova, PhD,
Head of the Department "Physics and Biophysics", Medical University - Varna "Prof.
Dr. Paraskev Stoyanov", Faculty of Pharmacy,
Member of the scientific jury according to the order of the rector of MU-Plovdiv R-
385 dated 29.01.2024.

About: dissertation work for awarding the educational and scientific degree "Doctor",
Professional direction: 4.3. Biological Sciences, Department of Higher Education 4.
Natural Sciences, PhD Program: "Biophysics"

Topic: "BIOLOGICAL ACTIVITY OF NEW MOLECULE ANALOGUES OF PAPAVERINE"

Author: Vera Nikolaeva Gledacheva,

Form of doctoral studies: independent preparation for the Faculty of Pharmacy;
Department of Physics and Biophysics

Scientific supervisors: Associate Professor Iliyana Stefanova-Kancheva, db.
Associate Professor Stoyanka Atanasova, Ph.D

Scientific consultant: Ch. assistant professor Valeri Slavchev, Ph.D

General presentation of the procedure:

I present this review as an external member of the Scientific Jury appointed by Order No. R-385 of 29.01.2024 of the Rector of the Medical University - Plovdiv, to ensure a procedure for the defence of a dissertation on the topic "Biological activity of new molecule analogues of papaverine."

The presented dissertation fully complies with the Law on the Development of the Academic Staff of the Republic of Bulgaria, the Regulations for the Terms and Conditions for the Acquisition of Scientific Degrees, and the Occupancy of Academic Positions at the Medical University - Plovdiv.

Biographical data of the doctoral candidate:

Vera Gledacheva graduated in "Engineering Physics" at the University of Plovdiv in 2004. In 2017, she earned a Master's in medical and radiation physics and technology at

the University of Plovdiv. Her professional biography began in 2010 with her appointment as "Junior Inspector" in the RZI - Plovdiv, Department of " Radiation Control." From 2011 to 2017, she was a medical physicist at the Department of Imaging Diagnostics at St. George Medical Center. From September 2017, she was an assistant at the Department of Physics and Biophysics at the Medical University of Plovdiv.

Relevance of the topic and appropriateness of the set goals and tasks:

The dissertation aims to apply new methods for synthesizing N-containing heterocyclic compounds and their precursors, such as papaverine analogs, and to investigate their potential biological activity. The specific goals and tasks the doctoral student sets for herself refer to selecting methods for selective synthesis, spectral characterization, and evaluation of biological activity through *in silico* methods of isoquinoline precursors papaverine analogs. The influence of the newly synthesized substances on the motor activity of laboratory animals is investigated, and the intensity and type of cellular and receptor mechanisms inducing contractile effects under the action of the molecules on smooth muscles from a rat stomach are determined.

The actuality of the problem is also confirmed by the literature cited in the dissertation. The sources cited in the dissertation are 72% after the year 2000.

Knowing the problem:

The doctoral student has in-depth scientific knowledge on the topic being developed. She is aware of the issues related to the topic and the need to create the dissertation work. Her theoretical reasoning results from the detailed analysis of many bibliographic sources - 211, all Latin. The studied material is evaluated critically and creatively, which allows her to make a competent analysis. As Gledacheva demonstrates excellent knowledge of the subject and the ability to analyze literary data. This makes me believe the doctoral student is well-informed about the issues under consideration.

Research methodology:

The methods used enable the realization of the set goal and are adequate for solving specific tasks. Instrumental methods such as liquid chromatography with mass detection, nuclear magnetic resonance, infrared spectroscopy, and determination of

melting temperatures by calorimetric methods are used to characterize the newly synthesized molecules.

Tests for active learning (Shuttle-box), passive learning (Step-through), and (Step-down), and a test for horizontal and vertical motor activity (Activity cage) were made in automatic devices for experimental pharmacology (Ugo Basile, Italy). In addition to the mentioned tests, some methods from biophysics were used, allowing:

- Recording bioelectrical activity of isolated smooth muscles using a single sucrose gap;
- Isometric recording of the mechanical activity of isolated smooth muscles.
- Immunohistochemistry of paraffin sections.
- Some software products were used, allowing:
- Theoretical calculation of pharmacokinetic processes.
- Theoretical determination of the toxicity of newly synthesized compounds.

Feature and rating:

The dissertation meets the requirements for developing a doctoral thesis and is presented in 146 pages. The content of the dissertation includes an introduction, three heads, conclusions, a self-assessment of contributions, and a bibliography. The text is richly illustrated with 46 figures and 18 tables.

The development content presents the possibility for the author to carry out research activities, form conclusions, and substantiate proposals with scientific and applied value.

The study's purpose, tasks, and methodology are presented coherently and logically. The author's thesis is well formulated. The set goals and tasks are up-to-date, correctly, precisely, clearly defined, and aimed at solving the main research problem. Their implementation provides new solutions, expands knowledge, and enriches practice in the scientific field. The materials submitted by the doctoral student testify to a fully completed and competently written dissertation work.

The literature review covers 30 pages, is well-structured and analytical, and contains sufficient information related to the subject of the dissertation work. The scientific data is structured in the following directions: Chemistry of isoquinoline derivatives; General

information about the structure, organization, and innervation of the gastrointestinal muscles; Influence of pH on smooth muscle relaxation and contraction. The presented overview well reflects the multidisciplinary nature of the proposed dissertation. The overview is illustrated with ten diagrams and eight figures. The summary made at the end of the chapter is concise and outlines the skeleton of the present dissertation. The goal is defined precisely and clearly. The tasks to achieve it are precisely defined and are 5 in number.

Materials and methods are described in detail. They are presented on 18 pages and are illustrated in 2 figures and one table.

The discussion is diverse, given the multidisciplinary nature of the research. The results reflect the set tasks and fully correspond to the set goals in developing the dissertation work. Comparative examinations of the results with those obtained by other authors have been made.

In her research, the PhD student applies a combined approach, including *in silico*, *ex-vivo*, *in vitro*, and *in vivo* methods, enabling the successful screening of new models or actual isoquinoline molecules. The proposed set of methods expands the knowledge of their specific action to the potential innovative application in pharmacology. The dissertation used a stereoselective method to synthesize isoquinolines and their precursors from starting natural amino acids. Seven compounds were synthesized, isolated, and spectrally characterized, of which four were obtained for the first time. Software methods were applied to analyze biological activity, solubility, bioavailability, synthetic accessibility, toxicity, possible carcinogenicity, and mutagenicity for each of the compounds.

The discussion in the Results and Discussion section is analytical, multi-directional, and focused on the main aim and objectives set in the dissertation. Comparative examinations of the results obtained during the experiment were made. The conclusions proposed by the author are 6 in number and accurately reflect the goal and tasks presented at the beginning.

Contributions and significance of the development of science and practice.

The developed dissertation has a significant contribution in theoretical and scientific-applied aspects. I agree with the author's assessment regarding the contributions of the dissertation work.

I accept the following contributions offered by the PhD student:

- For the first time, a stereoselective synthesis was applied to obtain 3-substituted isoquinolines or their precursors from starting L-alanine.
- A successful complex modeling method has been developed, combining *in silico*, *ex vivo*, *in vitro*, and *in vivo methods*, enabling the unification of the screening of new molecules.
- The IQP compound could slow the progression of neurodegenerative diseases.

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Assessment of dissertation publications:

The doctoral student presents two publications on the topic of the dissertation work in the Web of Science and Scopus databases. One is in a journal with SJR, and the other is with Q₂ and IF. The two articles are co-authored, and the doctoral student is in the first place in both. On the dissertation topic, the author presents five more full-text reports at national and international conferences. Research results have been reported at four

national and eight international scientific meetings. In terms of number and quality, the scientific works presented are sufficient for awarding the scientific degree to the ESD "doctor."

Assistant Gledacheva participates in two scientific projects in which she is a researcher.

Personal participation of the doctoral student:

The personal participation of the doctoral student in the conducted research is indisputable, and the formulated contributions and results are entirely her merit.

Abstract:

The structure of the abstract meets the requirements and correctly reflects the results obtained in the dissertation work.

Personal impressions:

I have known the PhD student for more than five years. She is a well-organized, precise person, an accomplished researcher, able to evaluate and analyze obtained results of a scientific experiment, having potential for academic development and scientific work.

Critical notes and recommendations:

I have no critical remarks about the final version of the dissertation. As. Vera Gledacheva complied with all the necessary comments and recommendations made during the dissertation project presentation to the expanded departmental council in 2023.

I have the following two clarifying questions:

1. What do the characters *, **, #, etc. used mean? in figures 43 to 46 in the dissertation?

2. The PhD student in the Materials and Methods section indicates the use of the Turkey-Kramer Multiple Comparisons test. Have such comparisons been made when considering the concentration-effect dependence of influence for the various substances represented in Figures 23 to 26?

CONCLUSION

Vera Gledacheva's dissertation is an independent, in-depth study of a formulated problem, the development of which is relevant from a theoretical and applied point of view. The formulated goal of the development has been achieved, as the set research tasks have been solved. The author possesses deep theoretical knowledge and demonstrates a broad, creative research style and scientifically sound approach.

The dissertation contains scientific, scientific-applied, and applied results, which 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, the regulations for the implementation of the law on the development of the academic staff in the Republic of Bulgaria and correspond to the rules of MU-Plovdiv. The presented materials fully conform to the specific requirements of MU-Plovdiv.

Due to the above, I convincingly give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract achieved results and contributions, and I propose to the honourable scientific jury to award the educational and scientific degree "doctor" to Vera Gledacheva in the doctoral program in "Biophysics".

18.02.2024

Varna

Reviewer:



Заличено на основание
Чл.5 §1, 6."В" Регламент (ЕС)2016/679

(prof. Krastena Nikolova, PhD)

MU - Varna