STATEMENT

by

Prof. Dr. Reni Emil Kalfin, PhD

on the dissertation for the award of the Educational and Scientific Degree **'Doctor'**

Professional Field **4.3 "Biological Sciences"**

Doctoral Program **"Biophysics"**

Author: Vera Nikolaeva Gledacheva

Form of Doctoral Training: Independent Study

Department: Medical Physics and Biophysics, Medical University - Plovdiv

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

Scientific Supervisors:

Assoc. Prof. Iliana Stefanova-Kancheva, PhD – Medical University of Plovdiv
Assoc. Prof. Stoyanka Atanasova, PhD - Plovdiv University "Paisiy Hilendarski"

1. General presentation of the procedure and PhD student

The materials presented by the PhD student Vera Gledacheva on electronic and paper medium are in accordance with the Article 70 (1) of Section I. Acquisition of Educational and Scientific Degree "DOCTOR" at the Medical University – Plovdiv and Regulation of MU-Plovdiv dated January 28, 2021.

Regarding the dissertation, the doctoral student has attached two articles in refereed scientific journals and 5 full-text publications in non-refereed journals and proceedings of scientific forums. It is worth noting the fact that the total number of credits received by Vera Gledacheva exceeds twice the mandatory doctoral minimum.

Vera Nikolaeva Gledacheva was born on January 21, 1986 in the city of Plovdiv. In 2004, she graduated in a class with specialized teaching in English and mathematics at the "Saint Kliment Ohridski" secondary school in the same city. Vera holds a Bachelor's Degree in "Engineering Physics" (2008) and a Master's Degree in "Medical and Radiation Physics" (2017). Gledacheva obtained both those degrees at the Paisiy Hilendarski University of Plovdiv. She worked successively as a junior inspector in the Regional Health Inspectorate in the city of Plovdiv and as a medical physicist at the Department of Imaging Diagnostics at the St. George University Hospital for Active Treatment in Plovdiv. Since September 2017, Vera is an assistant in the Department of "Medical Physics and Biophysics" at the Faculty of Pharmacy, Medical University in Plovdiv.

Vera Gledacheva participated in the National Scientific Program "Young Scientists and Postdoctoral Students". She is a team member in three national research contracts and two scientific projects of Medical University-Plovdiv. Currently Vera is a specialist in the Department of Medical Physics and Biophysics, and she is about to acquire a "Biophysics" specialty. The PhD student is fluent in written and spoken English. She is a member of the Bulgarian Nuclear Society and the Union of Scientists in Bulgaria.

On December 1st 2021, Vera Gledacheva was enrolled in a self-study doctoral course, and on April 26th 2024, she was dismissed with the right of defense.

2. Relevance of the topic

The drug papaverine is a muscle relaxant that reduces the tone of smooth muscles in the walls of blood vessels, the gastrointestinal tract, uterus, biliary and urinary tracts. It is used to affect colics of the gut and stomach, biliary and urinary tracts, spasms of central and peripheral blood vessels. However, despite its proven medicinal properties, the systemic use of papaverine is a prerequisite for the manifestation of undesirable side effects such as arterial hypotension, tachycardia, loss of appetite, drowsiness, dizziness, overactivity of the sweat glands and potential liver toxicity. In this aspect, the search for new effective muscle relaxants with good biological tolerance on one hand and the creation of a complex approach including different methods for evaluating their bioactivity on the other is a challenge for modern medical biological science. Everything said so far makes the topic of the dissertation relevant in a scientific and scientific-applied sense and justifies the need for the conducted research.

3. Understanding of the problem

From the literature review, it can be seen that the doctoral student Vera Gledacheva is very well acquainted with the state of the researched problem, the subject of her dissertation work. The literature review of the dissertation consists of three sections. At the beginning, Vera presents historical data and contemporary aspects regarding the chemistry of isoquinoline derivatives, paying special attention to isoquinoline derivatives with biological activity.

The following sections of the review are devoted to the structure of the gastrointestinal musculature and the bioelectrical activity of smooth muscle cells. The main neurotransmitters in the gastrointestinal tract and the influence of pH on the contractile activity of visceral smooth muscles are reviewed.

The literature review is generally written clearly and concisely, with competent handling of scientific terminology. The PhD student analyzes the problem of obtaining new isoquinoline compounds and their precursors, improving the known classic synthesis methods and searching for suitable experimental models for studying the biological action of the newly synthesized molecules. In conclusion, Vera Gledacheva with understanding evaluates the literary data, points out both established dependencies and also unresolved issues that condition the set goals and tasks of the dissertation work.

The aim of the dissertation work is to study the biological action of newly synthesized N-containing heterocyclic compounds and their precursors as analogues of papaverine and to investigate their potential biological activity. The five tasks set to realize the purpose of the research are specific and correctly formulated, and their implementation guarantees the intended goal.

4. Methodology of the study

The experimental methods are described in 18 pages. An excellent impression is made by the use of biochemical, isometric, histological, in silico and behavioral

methods, which fully correspond to the formulated tasks. The experiments are very well planned and conducted, and the obtained results have a good informative value and the possibility of evaluating and analyzing the obtained data.

The study was conducted on sexually mature male Wistar rats. It is worth noting the fact that in general this is a laborious experimental process and that over 190 rats were used in the studies related to the PhD thesis. Appropriate statistical methods were selected for processing the obtained experimental data.

5. Characterization and evaluation of the dissertation and contributions

The work is written on 143 pages according to the standard scheme and includes all the sections recommended for obtaining the educational and scientific degree "Doctor" at the Medical University - Plovdiv. The dissertation is properly constructed, very well written and richly illustrated with 46 figures, 18 tables and 17 diagrams. The bibliography consists of 211 alphabetically arranged literary sources, all in Latin. The cited literary sources meet the tasks of the dissertation, they are selected and described correctly.

Section five is the most important of the dissertation work and is dedicated to the own results, which are described in detail. The PhD student has considered each own results chapter to be followed by a discussion, rather than separating it into a general Discussion section. A very good impression is made by the two-page summary of the results and a brief discussion at the end of this section.

The fifth section of the work contains a lot of original data obtained on the basis of the used set of various methods and techniques. The obtained results are processed statistically, clearly documenting the information from the conducted research and giving adequate answers with theoretical and scientific-applied significance. Based on the data obtained from the conducted research, certain patterns of important scientific and applied results have been established. A valuable result of the dissertation work is the conducted complex model study, combining in silico, ex vivo, in vitro and in vivo methods, which allows unifying the screening of new or unexplored model and/or real isoquinolines.

The analyzes made are a logical prerequisite for the credibility of the discussion and the formation of scientifically sound conclusions that well summarize the results of the research conducted by the PhD student.

6. Contributions and significance of the thesis for science and practice

In her dissertation, Vela Gledacheva reached significant results that could be summarized as follows:

□ Original data were obtained regarding the improvement of memory functions after administration of the compound IQP, a substance that could be used to slow the progression of neurodegenerative diseases;

□ Stereoselective synthesis was applied for the first time to obtain 3-substituted isoquinolines or their precursors from starting L-alanine;

□ The original data obtained by Vera Gledacheva characterize the compound IQP as a powerful antispasmodic substance with myotropic action, which makes it a potential competitor of the drug papaverine;

□ Of important scientific and applied significance is the complex approach used in the dissertation for the screening of new or unexplored isoquinolines, including in silico, ex vivo, in vitro and in vivo methods.

7. Assessment of publications and PhD student's personal contribution

The results of the PhD student Vera Gledacheva in connection with her thesis are summarized in 7 published scientific articles. Two of the publications are open access, respectively in the journals *"Applied Sciences"* with quartile Q2 and in the journal *"Folia Medica"* with quartile Q3. The doctoral student is first author in six of the articles. This fact unequivocally shows that the contributions of the dissertation work noted by the doctoral student are to a significant extent her work, obtained with the support of the scientific supervisors.

8. Abstract of the thesis

The abstract is written on 52 standard pages and fully corresponds to the content of the dissertation work. The abstract is very well laid out and richly illustrated with 20 figures, 7 tables and 4 diagrams. Included are the aim and objectives of the study, all main results and their discussion, conclusions, contributions and published research papers related to the dissertation. In addition, a list of PhD student Vera Gledacheva's participation in international and national scientific forums is presented. The intra-university projects that financially support the development of the PhD thesis are listed. In conclusion, the dissertation's abstract fully meets the requirements regarding content and quality.

9. Personal Impressions

I know the doctoral student from the three-day training of the research team from the scientific group "Biomaterials and Nanostructures for Drug Delivery" in connection with the implementation of Contract No. BG-RRP-2.004-0007-C01 "Program for Strategic Research and Innovation for the Development of MU - Plovdiv", held during the period 7-9 February 2024 by a team from the Institute of Neurobiology at the Bulgarian Academy of Sciences. Vera Gledacheva took an active part in this training and performed excellently.

CONCLUSION

The dissertation work of PhD student Vera Gledacheva is dedicated to a current problem for pharmacology and medical practice. In the performance of the tasks, the doctoral student shows a very good knowledge of the literature on the problem, of the experimental setups and methods, excellent qualities for independent research, maturity in the presentation and interpretation of the results and in-depth professional skills in the scientific specialty "Biophysics". During the development of the dissertation, Vera

Gledacheva obtained original experimental data, which have not only scientific value, but are of interest for medical practice.

The presented results and scientific publications in connection with the dissertation work fully meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application, and the Regulations of the Medical University - Plovdiv.

Given the well-conducted scientific research, the sufficient amount of material, the overall layout of the thesis and the scientific publications in connection with the dissertation work, I give my positive assessment and with conviction I do propose to the honorable members of the Scientific Jury to award the Educational and Scientific Degree "Doctor" to Vera Nikolaeva Gledacheva in the doctoral program "Biophysics", professional direction 4.3. "Biological Sciences".

23rd of February, 2024 **REVIEWER:**

Sofia Prof. Dr. Reni Kalfin, PhD