

## STATEMENT

**By Prof. Albena Todorova Zlatareva, MD, PhD,**

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Faculty of Pharmacy, Medical University – Varna,

Determined as a member of the scientific jury on the basis of Art. 24 of Regulations for the Implementation of LDASRB and the Regulations of the MU – Varna, by order No. R-109-18/23.01.2024 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna,

Regarding a Ph.D. thesis for awarding the educational and scientific degree 'doctor' to Stela Toshkova Dragomanova – a doctoral student in an independent form of education in PhD program "Pharmacology, incl. pharmacokinetics and chemotherapy", professional direction 7.3. Pharmacy, field of higher education 7. Health care and sports, with a dissertation on the topic: "Neuropharmacological investigation of myrtenal conjugates with aminoadamantane", scientific supervisor: Assoc. Prof. Velichka Andonova, PhD, and scientific consultant: Prof. Lyubka Tancheva, PhD.

The statement has been prepared on the basis of the Decision of the Meeting of the Scientific Jury, Protocol No. 1/30.01.2024.

### ***Brief biographical data***

Stela Dragomanova graduated the specialty "assistant pharmacist" at the Medical College - Varna in 1998, and the specialty "Pharmacy" with the professional qualification "master pharmacist" at the Medical University - Sofia in 2004. From 2000 to 2005, she worked in an open pharmacy, respectively as a pharmacist and as a master pharmacist, and from 2005 to 2010, she was a manager of an open pharmacy. From 2010 to the present, she is an assistant in the departments of "Pharmacology and Clinical Pharmacology and Therapy" of the Faculty of Medicine at the Medical University of Varna, the Department of "Preclinical and Clinical Sciences", the Faculty of Pharmacy of the Medical University of Varna and the Department of "Pharmacology, Toxicology and Pharmacotherapy" of Faculty of Pharmacy of MU-Varna. In the period 2014-2020, she was an administrative assistant in the Department of "Pharmacology, Toxicology and Pharmacotherapy" of the Faculty of Pharmacy at the Medical University of Varna, and from 2021 she was appointed as a chief assistant. Stela Dragomanova is involved in the teaching of "Pharmacology" and "Toxicology" to students of "Pharmacy" and "Assistant Pharmacist", and "Pharmacology" to students of "Medicine" and "Dentistry". In 2015, the PhD student participated in training at Thracian University – Stara Zagora on "Protection and humane treatment of experimental animals used for scientific and educational purposes." In 2017, she acquired the specialty "Clinical Pharmacy" at Medical University – Sofia. In 2020, she defended her doctoral thesis in "Pharmacology", in scientific direction 7.1. Medicine, at the Institute of Neurobiology – Bulgarian Academy of Sciences. Stela Dragomanova started a second specialization in "Pharmacology and Pharmacotherapy" at the Medical University of Varna in 2022.

### ***Dissertation – structure, relevance, assessment of results and contributions***

The dissertation submitted for a statement is written in 219 standard typewritten pages. Contains: 3 pp. introduction, 48 pp. literature review, aim and objectives 2 pp., materials and methods 15 pp., results, discussion, summary of results and appendices 81 pp., conclusions 2 pp. and contributions 2 pp., bibliography – 49 pages. It is illustrated with 42 figures and 7 tables. The bibliographic reference covers 482 sources. The thesis work is devoted to the neuropharmacological study of two newly synthesized compounds, conjugates of the naturally occurring bicyclic monoterpene myrtenal with aminoadamantane, in healthy rodents and in those with an experimental model of Alzheimer's type dementia.

Since the identification of Alzheimer's disease, numerous scientific teams have tried to develop effective and safe therapeutic agents. At this stage, the available medicinal substances affect the symptomatic neurodegenerative changes and do not significantly affect the course of the disease process. Therefore, the discovery of biologically active substances with potential effects for the prevention and therapy of neurodegenerative damages is an important direction in science. The disease is characterized by a multifactorial etiology and pathogenesis, and a clinical picture that manifests differently in individual patients, which makes it difficult to find effective therapeutic products. On the other hand, there has been a huge scientific interest in the modification of natural substances in order to improve their potential pharmacological properties recently. In this sense, *the topic of the dissertation is extremely relevant*. This is well highlighted in the introduction, with emphasis also placed on the role of adamantane as a powerful and commonly used pharmacophore. The spectrum of myrtenal's biological effects and its so far synthesized compounds is outlined on the basis of experimental studies to date, and the fact that substantial data is lacking in the field of neuropharmacology is highlighted. The scientific information is well structured and summarized within the comprehensive literature review. The latter is the basis for an adequate formulation of the scientific goal and the resulting tasks for complex and targeted research and comparative characterization of the effects of myrtenal conjugates in healthy rodents and rodents with an experimental model of dementia.

All experimental materials and methods used are detailed, all types of experimental protocols, chemicals and reagents are comprehensively described. Properly selected and precisely characterized studies prove the broad scale and multidisciplinary nature of the present work.

The physicochemical properties of newly synthesized aminoadamantane myrtenal conjugates (MAC-197 and MAC-198) investigation reveals their ability to cross the blood-brain barrier, and software exploration of potential targets reveals their affinity to bind to structures in the CNS, thereby to influence various neurotransmitter systems and regulatory processes. Additionally, the stability of the used solutions of the two compounds was investigated, and a reasoned choice of the required minimum amount of co-solvent was made.

In "Results and Discussion" section the different sets of scientific data obtained from the experiments with myrtenal conjugates in healthy rodents and rodents with scopolamine-induced damage are presented in detail and precision. Repeated intraperitoneal administration of both compounds (11 days, at a dose of 1 mg/kg) did not cause negative effects on memory processes in

intact rats, no general toxic effects were found, and no macroscopic damage to the internal organs was recorded after dissection. The anticholinesterase potential of the derivatives, established in the docking studies, was confirmed *in vivo* in intact rats, and it was most strongly expressed with MAC-197 in the hippocampal region. Synthetic conjugates significantly improve impaired short-term and long-term memory in demented rodents, which is related to their anticholinesterase activity in the cerebral cortex, and in the case of MAC-198 – in the hippocampus. In addition, myrtenal analogs demonstrate neuromodulatory properties.

The doctoral student has made maximum efforts to display all the obtained results in a magnificent way - the tables and figures are accurate and easy for the reader to understand, which is especially important when comparing the many monitored parameters. The discussion follows the course of the obtained experimental results. The style is clear and specific, confirming the dissertant's ability to present well-formulated conclusions and contributions. The skillful shaping of the overall picture of the obtained data into two main groups, the originally presented conclusions of undoubted scientific and scientific-practical value is impressive.

For the first time, the neuropharmacological effects of newly synthesized conjugates of myrtenal with aminoadamantane have been investigated. In rats with an experimental model of dementia, the neuroprotective potential of the aminoadamantane derivatives was established for the first time, due to their complex mechanisms of action – anticholinesterase, antioxidant and neuromodulatory. The potential of the newly synthesized substances to affect diseases associated with an imbalance of cholinergic, noradrenergic and serotonergic neurotransmission has been revealed. A comparative analysis of the effects of myrtenal derivatives in healthy and demented rats on behavioral indicators and biochemical data was performed. It was found that their specific neuroprotective properties were more pronounced in rodents with memory impairments compared to healthy ones. Original data obtained for the first time in this study reveal the greater efficacy of synthetic conjugates of myrtenal with aminoadamantane, compared to the referent natural product.

#### ***Evaluation of the preparation of Stela Dragomanova as a doctoral student***

Stela Dragomanova, MPharm, has collected the maximum number of credit points. The doctoral student has participated in 5 scientific forums on the topic of the dissertation, being the first author in all of them. Three of the scientific announcements were made in our country, two – abroad. For the Ninth Pharmaceutical Business Forum, Varna, 2023, participation Dragomanova won first place for a doctoral poster. The presented publications in connection with the topic of the dissertation are two, published in refereed and indexed scientific journals. The preparation of the doctoral student in the Doctoral School and in the development and reporting of the results of the dissertation topic at scientific forums can be assessed as excellent. The indicated scientometric data confirm the relevance of the problems considered in the dissertation work of Stela Dragomanova and their scientific value.

***The abstract*** meets the requirements and fully covers the dissertation work.

## CONCLUSION

The dissertation shows that the doctoral student Stela Dragomanova possesses the necessary theoretical and professional knowledge and skills for independent conducting of research work on a given scientific problem. The contribution nature of the dissertation work is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations of the Medical University – Varna for the application of the LDASRB.

Given all of the above, I give my *positive assessment of the conducted research and propose to the honorable jury to award the educational and scientific degree "doctor"* to chief assist. Stela Toshkova Dragomanova in a doctoral program in the specialty "Pharmacology, incl. Pharmacokinetics and Chemotherapy", Department of Higher Education 7. Health Care and Sports, professional direction 7.3. "Pharmacy".

23.02.2024

Prepared by:

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Prof. Albena Zlatareva, MD, PhD