

OPINION

of

Assoc. Prof. Svetlana Fotkova Georgieva, Ph.D.

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, elected as an Internal Member of the Scientific Jury, according to Order No. R109-18/23.01.2024 of the Rector of the Medical University - Varna - Prof. Dr. Svetoslav Georgiev, Ph.D. and Protocol No. 1/30.01.2024 from the first meeting of the scientific jury.

Regarding the procedure for obtaining the educational and scientific degree of "Doctor" by Stella Toshkova Dragomanova, a doctoral student in the doctoral program "Pharmacology, including pharmacokinetics and chemotherapy", Field of higher education - 7. Healthcare and Sports, Professional direction - 7.3. "Pharmacy" with a dissertation on the topic: "Neuropharmacological study of myrtenal conjugates with amino-adamantanes". The scientific supervisor of the doctoral student is Assoc. Prof. Velichka Andonova, Ph.D., and the scientific consultant is Prof. Lyubka Tancheva, Ph.D.

The opinion on the materials submitted in the competition is based on the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulation for its Application, as well as the Regulation on the Conditions and Procedure for Obtaining Scientific Degrees and Occupying Academic Positions and the Quality Criteria for the Development of the Academic Staff of the Medical University - Varna.

The opinion is prepared based on the Decision of the Scientific Jury Meeting Protocol No. 1/30.01.2024. No procedural violations, conflicts of interest, or remarks on the submitted materials have been identified.

Biographical information: Stella Toshkova Dragomanova graduated from PMI-Varna in 1998 with a professional qualification as a "assistant pharmacist" and from the Faculty of Pharmacy at the Medical University - Sofia in 2004 with a professional qualification as a "master pharmacist". From 2000 to 2005, she worked as a pharmacist, and from 2005 to 2010 as a manager in an open-type pharmacy.

Stella Dragomanova began her academic career in 2010 when she was appointed as an assistant at the Department of Pharmacology and Clinical Pharmacology at the Faculty of Medicine at MU-Varna. From 2015 to 2024, Ms. Dragomanova worked successively as an assistant and senior assistant at the Department of Pharmacology, Toxicology, and

Pharmacotherapy at the Faculty of Pharmacy at MU-Varna. She teaches pharmacology and toxicology to students majoring in pharmacy and assistant pharmacists. Senior Assistant Dragomanova defended her dissertation on the topic "Pharmacological, Toxicological, and Neurobiological Studies of Myrtenal - a Bicyclic Monoterpene from Natural Origin" in the scientific specialty of pharmacology, direction 7.1. Medicine, in 2020. She obtained a specialization in "Clinical Pharmacy" in the healthcare system in 2017.

The dissertation consists of 220 standard typewritten pages and contains 41 figures, 5 appendices, and 7 tables. It cites 482 literary sources.

The aim and scientific hypothesis lie in the field of pharmacology, specifically focusing on the investigation of the *in vivo* potential neuropharmacological effects of synthetic myrtenal conjugates with amino-adamantane and the revelation of their basic neuroprotective mechanisms on an experimental model of Alzheimer's-type dementia in rats.

The dissertation entails numerous experiments related to studying the physicochemical parameters of myrtenal conjugates with amino-adamantane; *in silico* analysis for predicting their neurobiological effects and theoretical possibilities for their binding to biological targets. It is found that the physicochemical properties of synthetic myrtenal conjugates with amino-adamantane (MAC-197 and MAC-198) favor their passage through the blood-brain barrier, and software analysis of potential targets reveals their ability to bind to structures in the CNS and thus influence various neurotransmitter systems and regulatory processes.

The neuropharmacological effects of myrtenal derivatives on healthy experimental rats have been investigated, including the substances' effects on memory, learning, and exploratory activity following repeated administration. The results show that repeated intraperitoneal administration of the two compounds (for 11 days at a dose of 1 mg/kg) does not induce negative effects on memory processes in intact rats, no general toxic action is observed, and no macroscopic damage to internal organs (heart, liver, kidneys, stomach, gallbladder, and intestines) is registered after dissection.

The biochemical mechanisms of influence on brain structures related to memory have been determined by determining antioxidant activity - determining LPO, GSH, CAT, SOD, GPx, changes in acetylcholinesterase activity. And the neuromodulatory properties of the compounds (content of NA, 5-HT).

The potential preventive effects of myrtenal derivatives on rats with experimental Alzheimer's-type dementia have been studied by investigating the substances' memory-recovering abilities on demented rats with scopolamine-induced brain damage, and the

biochemical mechanisms of the neuroprotective action of myrtenal derivatives on demented rats with scopolamine-induced memory impairments have been determined. The results of molecular modeling show that brain AChE is a plausible target for amino-adamantane conjugates of myrtenal. Their anti-cholinesterase potential, established in docking studies, is confirmed *in vivo* in intact rats, with the strongest expression observed in the hippocampal area for MAC-197. The synthetic derivatives significantly improve the impaired short-term and long-term memory of demented rodents, which is associated with their anti-cholinesterase activity in the cerebral cortex and, in the case of MAC-198, also in the hippocampus.

The strength of the neuroprotective effects of synthetic amino-adamantane conjugates with natural myrtenal in healthy and dementia-induced experimental rodents has been compared. It can be summarized that the myrtenal analogs demonstrate pronounced neuromodulatory properties in both brain structures of intact rodents associated with memory (hippocampus and cortex), causing specific changes in the levels of biogenic amines - MAC-198 decreases the levels of NA and 5-HT, while MAC-197 significantly increases them. These differences are maintained in dementia-induced rodents - MAC-197 increases the content of NA in the cerebral cortex and hippocampus, and of 5-HT in the hippocampus, while MAC-198 decreases the concentration of 5-HT in the cortex and hippocampus, and of NA in the hippocampus.

It is noteworthy that the reported antioxidant properties of the myrtenal analogs, more prominently expressed than those of the natural reference, manage to correct the oxidative stress induced by scopolamine in the cerebral cortex of dementia-induced rodents. The two new substances suppress the increased catalase activity, with MAC-197 decreasing the content of MDA, and MAC-198 increasing the levels of total glutathione.

Both myrtenal conjugates with amino-adamantane, MAC-197 and MAC-198, exhibit more pronounced effects on the memory impaired by scopolamine in rodents, as well as more pronounced anticholinesterase, antioxidant, and neuromodulatory properties compared to the natural reference.

It is also important to note that the complex neuroprotective mechanisms of the two studied substances (anticholinesterase, neuromodulatory, and antioxidant) are more clearly manifested in rodents with scopolamine-induced dementia compared to intact rodents.

Five scientifically-theoretical and scientifically-applied contributions are clearly and precisely defined.

Bibliometrics: The results of the doctoral work have been published in two scientific journals with impact factors. The doctoral student has presented 5 scientific communications on the topic of the dissertation.

The abstract fully covers the dissertation.

Conclusion:

The work is written in a good scientific language, with almost no typographical or grammatical errors. The dissertation addresses an actual topic from both theoretical and practical perspectives. The set goals and objectives have been successfully achieved, and the doctoral student has mastered a number of contemporary analytical and synthetic methods.

The dissertation demonstrates that the doctoral student Stella Dragomanova possesses the necessary theoretical and professional knowledge and skills to independently conduct scientific research on a given scientific problem. The contribution made by the dissertation is in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulation for its Application, and the Regulation of MU - Varna.

Given the above, I provide my **positive assessment** of the conducted research and **recommend to the esteemed jury to award the educational and scientific degree of "Doctor"** to Chief Assoc. Prof. Stella Toshkova Dragomanova in the doctoral program specializing in Pharmacology, including Pharmacokinetics and Chemotherapy, Field of Higher Education - 7. Healthcare and Sports, Professional Direction - 7.3. Pharmacy.

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

19.02.2024

City of Varna

With respect:.....

(Assoc. Prof. Svetlana Fotkova Georgieva, Ph.D.)