OPINION

by Dr. Stoyanka Nikolova Atanasova, Associate Professor at the "Paisii Hilendarski" Plovdiv University

on the materials submitted for participation in a competition
for the academic position of "**Associate Professor**"
at the **Institute of Organic Chemistry with the Center for Phytochemistry (IOHCF), BAS**

in the field of higher education 4. Natural Sciences, Mathematics, and Informatics:
Professional Field 4.2. Chemical Sciences (Organic Chemistry)

In the competition for "Associate Professor", announced in the State Gazette, issue 40 of 16.05.2025, and on the website of IOHCF, BAS, only Chief Assistant Dr. Neda Orlinova Anastasova, from IOHCF-BAS, participated as a candidate.

1. General presentation of the procedure and the candidate

The set of materials on paper submitted by Dr. Neda Anastasova is in accordance with the Regulations for the Development of the Academic Staff of the IOHCF, and meets the criteria of the IOHCF-BAS for occupying the academic position of "Associate Professor". To participate in the competition, the candidate Dr. Neda Anastasova, has attached a list of 17 scientific papers. A total of 17 scientific papers were accepted for review, which are outside the dissertation and are taken into account in the final assessment, as well as participation in 13 research projects. The distribution of scientific papers by the relevant Q factors is as follows: 5 articles as an equivalent number of articles for habilitation work and 12 publications in indicator D (according to Appendix 1 of the Regulations for the Development of the Academic Staff of the IOHCF), of which the distribution by quartiles is as follows: 4 publications in Q1, 5 in Q2, one in Q3 and two in Q4. According to the submitted publications, Dr. Anastasova has 210 independent citations, as a result of which the candidate has an H-index of 8.

The materials presented in the competition from the scientific production which Chief Assistant Professor Dr. Neda Anastasova is using for application, meet the criteria set out in the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB) and the Regulations for its implementation, as well as those of the Institute of Academic Staff Development-BAS for holding the academic position of "Associate Professor".

2. General characteristics of the candidate's activities.

The scientific works of Chief Assistant Professor Dr. Anastasova are in the field of organic, medicinal, and pharmaceutical chemistry. The publications submitted to the competition can be divided into the following thematic areas:

- **Design, synthesis and structural characterization of benzimidazole and indole arylhydrazones**

The Michael reaction was successfully applied to obtain ester precursors of benzimidazole derivatives, followed by hydrazinolysis and condensation with differently substituted hydroxy- and methoxybenzaldehydes. Subsequently, alternative methods for obtaining the ester precursors were also proposed. The goal of the synthesis is to obtain a large set of derivatives with varying substituents in the arylhydrazone part. This allows for comparative analysis and selection of leading pharmacophore fragments used as a basis for the design of compounds from different groups.

Based on the obtained spectral data from IR, NMR, HRMS, and X-ray methods, the structural characterization of the compounds was also carried out.

- **Assessment of the cytotoxicity of the compounds**

As a key step in the development of new pharmacologically active compounds, their safety profile was assessed. As a result of the measurements, compounds from each group with the lowest toxicity were distinguished, determined as promising for subsequent biological characterization.

- **Investigation of the neuroprotective properties of the compounds in various in vitro models**

Some of the disubstituted benzimidazole derivatives with established low toxicity were studied for neuroprotective activity.

A statistically significant neuroprotective effect was established for the compounds, confirmed also by microscopic examination for preservation of cell morphology. Some of the studied compounds were determined as more promising candidates for neuroprotective agents than the reference compounds.

- **In vitro studies on the radical-scavenging properties of the compounds**

Based on the obtained data, the knowledge about the structure-activity relationship related to the antioxidant effect of the substituents in the hydrazone side chains has been deepened and expanded. The key role of the catechol group in the manifestation of a strong antioxidant effect has been confirmed, both in systems with biologically relevant molecules - lecithin and deoxyribose, and against various free radicals.

- **In vivo study of scopolamine-induced dementia model in rats**

Behavioral and biochemical methods have been investigated to assess the effects on memory of a 3,4-dihydroxyl derivative in a model of dementia induced by scopolamine in rats, compared to a positive control, rivastigmine, and a negative control, scopolamine. Rivastigmine is a reversible acetylcholinesterase inhibitor that is clinically used to treat cognitive impairment and Alzheimer's disease, as well as various forms of dementia, including those associated with Parkinson's disease. Scopolamine, on the other hand, is a non-selective muscarinic cholinergic receptor antagonist that is known to cause memory and cognitive impairment, increased oxidative stress, neuroinflammation, adverse changes in monoaminergic neurotransmitter systems, mitochondrial dysfunction, apoptosis, and impaired BDNF signaling - pathological changes that accompany Alzheimer's disease.

Two tests were used to assess the compound's ability to enhance learning and memory and to delay the adverse effects of scopolamine - the step-through avoidance test and the Barnes maze. As a result of the experiments, the compound completely restored the latency to move in scopolamine-treated animals to the levels of the control group and demonstrated similar efficacy in the Barnes maze, showing better results than rivastigmine. In conclusion, the compound is identified as a promising candidate for further studies as a multi-targeted agent for the treatment of neurodegenerative diseases.

- **Quantum chemical calculations to elucidate the mechanism of action of the compounds**

To better understand the observed higher neuroprotective activity of some derivatives, quantum chemical calculations were performed to elucidate the most likely mechanisms of free radical scavenging, including hydrogen atom transfer, electron transfer, and radical adduct formation in aqueous (polar) medium and in benzene as a solvent (non-polar medium).

It is impressive that the habilitation report is very well prepared, it is meaningful, and reflects very well the essence of the candidate's scientific activity and her scientific contributions.

3. Critical remarks and recommendations

I have nothing noteworthy to say about Dr. Anastasova's scientific endeavors or the documents that were presented. The competition's documentation are really well-structured and produced, which makes it much easier to analyze them.

CONCLUSION

The documents and materials submitted by Chief Assistant Professor Neda Anastasova fully meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, the Regulations for the Implementation of ZRASRB of the Bulgarian Academy of Sciences and the Regulations of the Institute of the Faculty of Physical Culture and Physical Culture-BAS.

Dr. Anastasova has presented a sufficient number of scientific papers, published after the PhD defense and the competition for Chief Assistant Professor. Her works contain original scientific and applied contributions that have received international recognition and resonance, all of which have been published in journals published by international academic publishing houses.

The scientific qualification of Chief Assistant Professor Assoc. Prof. Neda Anastasova is unquestionable. The results achieved by Dr. Neda Anastasova in scientific research activities fully comply with the specific requirements of the Regulations of the Institute of Chemical and Biological Sciences-BAS for the implementation of the Law on the Application.

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Prepared by: .....

Assoc. Prof. Dr. Stoyanka Atanasova