OPINION

by Dr. Antoaneta Borissova Trendafilova, Professor at the Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences (BAS),

on the materials submitted for participation in the competition for the academic position of *Associate Professor* at the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS, in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.2. Chemical Sciences, scientific specialty *Organic Chemistry*.

1. General presentation of the procedure and the candidate

In the competition for *Associate Professor*, announced in the State Gazette, issue 40 of May 16, 2025, and on the website of the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS, the sole candidate is Dr. Neda Orlinova Anastassova, Chief Assistant Professor at the IOCCP, BAS. The set of materials submitted by Dr. Neda Anastassova in hard copy complies with the Regulations for the Development of the Academic Staff of the IOCCP and meets the criteria of IOCCP–BAS for holding the academic position of *Associate Professor*. The application includes: a reference for meeting the minimum requirements, a habilitation reference for scientific contributions, a list and copies of scientific publications submitted for the competition (17 in total, corresponding to the scientific field of the competition), as well as the PhD dissertation abstract and a list of recorded citations. The submitted materials are well-prepared and clearly present the candidate's previous scientific work and achievements. In addition to the required documents, information on participation in research projects, scientific conferences, and a list of received awards has also been provided.

Dr. Neda Anastassova completed a Master's program in Fine Organic Synthesis in 2012 at the University of Chemical Technology and Metallurgy (UCTM), Sofia. In 2017, she successfully defended her doctoral dissertation at UCTM, Sofia, and was awarded the educational and scientific degree *Doctor*. In the period 2012–2013, she worked as a chemist in the "Structural Organic Analysis" laboratory at IOCCP–BAS. After a short interruption, since 2016 she has continued her career at IOCCP–BAS, consecutively holding the positions of Chemist, Assistant Professor, and Chief Assistant Professor in the "Structural Organic Analysis" laboratory. The results of her scientific research have been published in a total of 25 articles, which have received 230 citations. She has served as principal investigator in numerous research projects and is the recipient of six scientific awards.

2. General characteristics of the candidate's activity

Evaluation of the candidate's scientific and applied research work

For participation in the present competition, Dr. Neda Anastassova has submitted a report on meeting the minimum requirements for holding the academic position of *Associate Professor* at IOCCP–BAS, as follows:

- **Group A – 50 points (required: 50 points):** Doctoral dissertation on the topic *"Synthesis and study of hepatotoxicity and antioxidant activity of N,N'-disubstituted benzimidazole-2-thiones"* for the award of the educational and scientific degree *Doctor* in professional field 4.2. Chemical Sciences, scientific specialty 7.3. Pharmacy (Pharmaceutical Chemistry), at UCTM, Sofia.
- **Indicator 4, Group V – 125 points (required: 100 points):** A total of 5 publications in Q1 journals (ACS Chem. Neurosci., Molecules, Antioxidants, and Neural Regen. Res.), published in the period 2021–2025, where Dr. Anastassova is first or corresponding author.

- **Indicators 7 and 8, Group G – 239 points (required: 220 points):** A total of 8 publications (4 – Q1, 5 – Q2, 1 – Q3, and 2 – Q4) in journals referenced and indexed in internationally recognized databases. Dr. Anastassova is first or corresponding author in 5 of them.
- **Indicator 12, Group D – 420 points (required: 70 points):** Particularly impressive is the number of citations (210) to the scientific publications of Dr. Anastassova included in the competition for Associate Professor, as recorded in the Scopus and/or Web of Science databases.

In accordance with the requirements of the IOCCP regulations for the development of academic staff for the academic position of *Associate Professor*, the candidate's **h-index is 9** (minimum required ≥ 5), according to the Web of Science database, excluding self-citations.
The total number of points from all indicators is **834**, by which Dr. Neda Anastassova significantly exceeds the minimum requirements for holding the academic position of *Associate Professor* (minimum total: 440), as stipulated by the IOCCP–BAS regulations.

Evaluation of the candidate's teaching and pedagogical activity

Dr. Neda Anastassova participates in the training of students in the programs in Organic Synthesis. She is co-supervisor of one diploma student, direct supervisor of two Master's students, and mentor of two undergraduate students under the Ministry of Education and Science project *"Student Internships."*

Evaluation of the candidate's scientific and applied research activity

The main research areas of Chief Assistant Professor Dr. Neda Anastassova lie in the field of organic and pharmaceutical chemistry and are focused on the development of new potential multitarget agents for the treatment of neurodegenerative diseases. This interdisciplinary research area is topical, modern, and socially significant, given the increasing number of people worldwide affected by neurodegenerative disorders in recent years and the urgent need for the development of more effective therapeutic agents against these diseases. The principal scientific contributions result from interdisciplinary studies and can be summarized in five thematic directions: Design, synthesis, spectral, and structural investigations of benzimidazole and indole hybrids; Study of the pharmacological activity of the compounds in various *in vitro* models; Investigation of the radical-scavenging properties of the compounds in different *in vitro* models; Performance of *in vivo* studies in a rat model of dementia; Quantum chemical calculations to elucidate the mechanism of action.

Undoubtedly, the most significant contribution of Chief Assistant Professor Neda Anastassova lies in the design and synthesis of new compounds affecting different mechanisms in the pathogenesis of neurodegeneration. Five series comprising a total of 40 new arylhydrazone derivatives have been synthesized: derivatives of 1,3-disubstituted benzimidazole-2-thione, 1-substituted benzimidazole, indole-3-propionic acid, 5-methoxyindole-2-carboxylic acid, and indole-3-acetic acid, containing various combinations of hydroxyl and methoxy substituents in the arylhydrazone fragment. Structural characterization of the compounds was carried out using infrared (IR) spectroscopy, nuclear magnetic resonance (^{1}H and ^{13}C NMR), and high-resolution mass spectrometry (HRMS), while two derivatives were further studied by X-ray crystallographic analysis. Quantum chemical calculations were performed for several of the synthesized compounds to determine the most probable molecular geometry and the likely mechanisms of radical scavenging. Moreover, DFT methods were applied to explain the duplicated signals observed in the NMR spectra of some compounds, suggesting the presence of conformers. These contributions are of original character.

The conducted interdisciplinary studies enabled a more thorough pharmacological evaluation of the synthesized compounds, leading to the formulation of the following major contributions:

- A toxicological assessment of the newly synthesized compounds was carried out, and priority candidates with the most favorable safety profile and low cytotoxicity were identified for further biological evaluation.
- A relationship was established between the structure of the synthesized molecules and their effectiveness under conditions of induced oxidative stress, a key pathogenic mechanism in neurodegenerative diseases, through studies on a cellular model of hydrogen-peroxide–induced oxidative stress in the human neuroblastoma cell line SH-SY5Y.
- Through studies in a model of induced neurotoxicity with 6-hydroxydopamine (6-OHDA) in isolated synaptosomes from rat brain, the neuroprotective potential of the tested compounds was further evaluated.
- The strongest neuroprotective activity was identified for the 2,3-dihydroxy derivative 5a in a model of t-BuOOH–induced oxidative stress in isolated mitochondria from rat brain.
- Compound 4b was determined as the lead structure for more in-depth *in vivo* studies, based on permeability assessments across the blood–brain barrier.
- A structure–activity relationship was established between the benzimidazole and indole hybrids and their radical-scavenging properties through various *in vitro* models.
- Compound 4b, containing a 3,4-dihydroxy substituent, tested *in vivo* in a rat model of Alzheimer's-type dementia, was identified as a promising candidate for further in-depth studies as a multitarget agent for the treatment of neurodegenerative diseases. This contribution is not only original but also of applied scientific significance.

The candidate's contribution to the conducted research is indisputable, as in 10 of the submitted scientific publications Chief Assistant Professor Dr. Neda Anastassova is first and/or corresponding author. The candidate has undoubtedly developed the necessary scientific competence and skills for the design, synthesis, and characterization of benzimidazole and indole compounds, as well as for the analysis and interpretation of the obtained results, both in the field of organic synthesis and in the areas of pharmacology and medicine. This acquired expertise has also defined the candidate's future research directions as a continuation and expansion of her current field of studies, namely: the synthesis of new potential multitarget compounds through rational design; investigation of synergistic effects; extension of *in vitro* studies to new cellular models for the evaluation of efficacy and mechanism of action; new *in vivo* experiments with selected compounds and nanoparticles in models of Parkinson's and Alzheimer's disease to monitor behavioral and oxidative status effects; as well as the development of novel antitumor agents, including hybrid molecules and nanoparticles with potential synergistic activity.

3. Critical remarks and recommendations

I have no critical remarks concerning the materials submitted by Chief Assistant Professor Dr. Neda Anastassova.

CONCLUSION

The documents and materials submitted by Chief Assistant Professor Dr. Neda Anastassova meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its Implementation, the Regulations of the Bulgarian Academy of Sciences (BAS) for the Implementation of LDASRB, and the

Regulations of IOCCP–BAS. The candidate in the competition has presented a significant number of scientific works published after those included in her PhD dissertation defense. Her research contains original scientific and applied contributions, which have gained international recognition, with a representative part of them published in journals and scientific proceedings issued by international academic publishers. The scientific qualification of Chief Assistant Professor Dr. Neda Anastassova is beyond doubt.

The results achieved by Chief Assistant Professor Dr. Neda Anastassova in her research activity fully comply with the specific requirements of the IOCCP–BAS Regulations for the implementation of LDASRB.

After reviewing the materials and scientific works submitted for the competition, analyzing their significance and the scientific and applied contributions contained therein, I find it justified to give my positive evaluation and to recommend to the Scientific Jury to prepare a report-proposal to the Scientific Council of IOCCP–BAS for the election of Chief Assistant Professor Dr. Neda Anastassova to the academic position of *Associate Professor* at IOCCP–BAS in the professional field 4.2. Chemical Sciences, scientific specialty *Organic Chemistry*.

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(Prof. Dr Antoaneta Trendafilova)