REVIEW

by Assoc. Prof. Dr. Kameliya KirilovaAnichina-Zarkova,
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on the materials submitted for participation in the competition
for the academic position of **Associate Professor at the Institute of Organic Chemistry with Centre for Phytochemistry (IOCCP), Bulgarian Academy of Sciences (BAS)**
in the professional field 4.2. "Chemical Sciences",
scientific specialty "Organic Chemistry"

In the competition for "Associate Professor", announced in the State Gazette, issue 40 of 16.05.2025, and on the Internet page of IOCCF, BAS, as a candidate participates **Senior Assist. Prof. Dr. Neda Orlinova Anastasova,** IOCCP-BAS.

1. General presentation of the materials received

For participation in the announced competition only one candidate has submitted documents: Senior Assist. Prof. Dr. Neda Orlinova Anastasova, the Laboratory “Structural Organic Analysis”, IOCCP-BAS. The set of documents submitted by Senior Assist. Prof. Dr. Neda Anastasova complies with the Regulations for the Development of the Academic Staff of the IOCCP, and meets the criteria of the IOCCP-BAS for holding the academic position of Associate Professor.

A summary of the fulfilment of the minimum requirements, a Habilitation paper, a list and copies of the scientific works under the competition (17 in total, corresponding to the scientific specialty of the competition), as well as an abstract for obtaining the PhD degree and materials certifying the candidate's participation in scientific projects, conferences, etc. are attached.

Senior Assist. Prof. Dr. Neda Anastasova participates in the competition with a total of 17 scientific publications, distributed according to the rank of the scientific journals, expressed in quartiles (Q-factor), as follows: 9 publications in scientific journals with Q1; 5 publications in scientific journals with Q2; 1 publication in a scientific journal with Q3 and 4 publications in scientific journals with Q4. Five of these publications were submitted under group B of the indicators (equivalent to a Habilitation paper). The remaining 12 research papers are presented under group D of the indicators.

2. Brief biographical details of the candidate

Neda Anastasova completed her higher education at the University of Chemical Technology and Metallurgy (UCTM), where in 2011 she obtained a bachelor's degree with professional qualification as a chemical engineer in two specialties – "Organic Chemical Technologies" and "Engineering Ecology and Environmental Protection," and in 2012, she obtained a Master's degree in "Fine Organic Synthesis."

In 2013, she enrolled as a PhD student in the Department of Organic Synthesis and Fuels at UCTM, under the supervision of Assoc. Prof. Dr. Anelia Mavrova and Prof. Dr. Vladimir Bojinov. In 2017, Neda Anastasova defended her PhD thesis on "*Synthesis and Investigation of Hepatotoxicity and Antioxidant Activity of N,N'-Disubstituted Benzimidazole-2-thiones*" and obtained the educational and scientific degree of "Doctor" in the professional field 7.3 Pharmacy in the scientific specialty "Pharmaceutical Chemistry ."

She first worked as a chemist at the Laboratory „Structural Organic Analysis“ of the Institute of Organic Chemistry with Centre of Phytochemistry, BAS, initially as a student (January 2012 – February 2013) and later as a PhD student (March 2016 – September 2016). In 2016, she was appointed Assistant Professor at IOCCP-BAS, and in 2018, she was promoted to Senior Assistant Professor.

She is the recipient of several awards, including the award for "*Best Popular Science Article*" from BG Science magazine (October 1, 2022) and "*Outstanding Young Scientist in the Field of Organic Chemistry*" from the National Competition "Acad. Yukhnovsky" (07.06.2019). She is a member of the American Chemical Society (ACS), the European Federation of Medicinal Chemistry (EFMC) – Young Scientist Network, the Union of Chemists, the Union of Scientists, and the Bulgarian Toxicological Society.

3. General characteristics of the candidat's activity

Evaluation of the candidate's scientific and applied activity

In connection with her application for the academic position of Associate Professor, the Senior Assist. Prof. Dr. Neda Anastasova has submitted a report attesting to her compliance with the minimum national requirements for the position, as detailed below:

According to Indicator 1 from group A – 50 points (50 points required): PhD thesis on "*Synthesis and investigation of hepatotoxicity and antioxidant activity of new N,N'-disubstituted benzimidazole-2-thiones*" (2017) for the acquisition of a PhD degree in professional field 7.3 Pharmacy in the scientific specialty "Pharmaceutical Chemistry" at the University of Chemical Technology and Metallurgy.

According to Indicator 4 from group C – 100 points (100 points required): A total of 5 publications (Q1) as an equivalent of a Habilitation paper are presented, published in international journals (*ACS Chemical Neuroscience, Molecules, Antioxidants* and *Neural Regeneration Research*) in the period 2021-2025, in 4 of which Dr. Anastasova is the first and corresponding author.

According to Indicator 7 from Group D – 239 points (220 points required): 12 publications (4-Q1, 5-Q2, 1-Q3, and 2-Q4) have been published in journals indexed in internationally recognized databases. Dr. Anastasova is the first author of five of these publications.

According to Indicator 11 from group E – 420 points from 210 citations (70 points required).

In accordance with the requirements of the Regulations for the Development of Academic Staff at IOCCP-BAS for the academic position of "Associate Professor", the candidate's h-index is 9 according to the information in the Scopus scientific database, and after excluding self-citations, the h-index is 8 (required ≥ 5).

The total number of points across all indicators is 809, demonstrating that Dr. Neda Anastasova significantly surpasses the minimum requirements for the academic position of Associate Professor (440 points), in accordance with the Regulations of the IOCCP-BAS

In addition, Dr. Anastasova presented data on 52 participations in 41 scientific forums, as well as on 3 specialized training courses abroad, presented by her under the name "scientific expeditions."

To date, Dr. Anastasova has led five research projects, three of which were funded by the Scientific Research Fund and two by the Ministry of Education and Science under the National Program “Young Scientists and Postdoctoral fellows”. In addition, she has participated as a team member in eight other research projects.

In parallel with her active research activities, Dr. Anastasova has also participated in the implementation of industrial projects, including the development of a specialized electronic library for the study of artistic materials, as well as the identification and preservation of Bulgarian cultural heritage using infrared spectroscopy (libra.orgchm.bas.bg). She is also a co-founder of a company engaged in the production and trade of organic cosmetics. These activities demonstrate her ability to combine fundamental scientific research with applied solutions.

Assessment of teaching and educational activities

Dr. Anastasova was the scientific supervisor of a bachelor’s thesis defended at UCTM and the co-supervisor of two master’s theses defended at UCTM and at the Faculty of Pharmacy, Medical University of Sofia, respectively. Furthermore, she has served as the primary supervisor

for two master's students and as a mentor in organic synthesis for two additional master's students as part of the Ministry of Education and Science's "Student Internships" project.

As a PhD student, she participated in conducting laboratory exercises in the discipline "Technology of Organic Synthesis" at the Department of Organic Synthesis and Fuels, UCTM.

Contributions (scientific, scientific-applied, applied)

The research presented by the candidate for the academic position of Associate Professor is in the field of organic and pharmaceutical chemistry. It primarily focuses on the design, synthesis, and structural characterization of novel benzimidazole (publications C4, C5, D1–D3, D6, D8, D10–D12) and indole (publications C1–C3, D5) compounds, supported by theoretical analysis of molecular geometry and electronic structure. Furthermore, the studies include evaluation of their cytotoxic activity (publications D6, D10), neuroprotective activity (publications C1–C4, D2, D5, D8), antioxidant activity (publications D3, D11, D12), as well as their ability to inhibit deoxyribonuclease I (publication D4) and MAOB (publications D4, C5) in various in vitro models.

A significant contribution to Dr. Anastasova's research is the synthesis and structural characterization of a large number of compounds (40 benzimidazole and indole compounds are described in the Habilitation paper alone). The series of N,N'-disubstituted benzimidazole-2-thione arylhydrazones (publication B4), referred to in the habilitation paper as Series I (BIM1), was used to determine leading pharmacophore fragments for neuroprotective activity, which served as the basis for the design of the series of N-substituted benzimidazole arylhydrazones (Series II (BIM2), publication C5); arylhydrazones of indole-3-propionic acid (Series III (IPA) publication C3); arylhydrazones of 5-methoxyindole carboxylic acid (Series IV (5MICA), publications C3, C2), as well as arylhydrazones of indole-3-acetic acid (Series V (IAA) publication C1).

Another contribution to her scientific research work is the comprehensive toxicological assessment of newly synthesized benzimidazole and indole arylhydrazones, including *in vitro* studies on the SH-SY5Y neuroblastoma cell line, as well as on isolated synaptosomes, erythrocytes, mitochondria, and microsomes from rat brain. The IC_{50} values were used as a comparison parameter and, based on them, conclusions were drawn about how the substituents in the structure of the compounds affect their toxicity. The arylhydrazones exhibiting the most favorable safety profiles were selected as priorities for further investigation.

Dr. Anastasova's research on the activity of arylhydrazone derivatives from the five series mentioned above in several established models of induced oxidative stress is of considerable scientific interest: a model of H_2O_2-induced oxidative stress in SH-SY5Y cells; a model of 6-

OHDA-induced neurotoxicity in synaptosomes, as well as a model of t-BuOOH-induced oxidative stress in isolated mitochondria from rat brain. The activity of the compounds in the different models has been confirmed.

The 2,3-dihydroxy and 4-hydroxy-3-methoxy groups have been identified as key structural motifs likely responsible for the pronounced neuroprotective activity of the tested compounds. An interesting observation is that while the 2,3-dihydroxy group serves as the main structural motif responsible for the neuroprotective activity in disubstituted benzimidazolyl arylhydrazones (Series I, publication C4) and in the indole derivatives of Series III (publication C3) and Series V (publication C1), in monosubstituted benzimidazoles (Series II, publication C5) the strongest effect is observed for the derivative containing a 2-hydroxy-4-methoxyphenyl substituent. Extensive quantum-chemical calculations were performed to provide a rationale for the observed activity (publication D2).

In addition, the inhibitory activity of benzimidazole and indole arylhydrazones against the human recombinant enzyme hMAO-B was evaluated as part of their multitarget neuroprotective potential. All tested compounds exhibited statistically significant inhibitory activity; however, the 2,3-dihydroxy derivative from Series I (publication C4), the 2-hydroxy-4-methoxy analogue from Series II (publication C5), and the indole-3-acetic acid arylhydrazones containing 2,3-dihydroxy, 2-hydroxy-4-methoxy, and 3,5-dimethoxy-4-hydroxy substituents on the benzene ring (publication C1) demonstrated the strongest effects. Molecular docking studies elucidated the structural factors underlying their high affinity, including favorable geometry, the formation of stabilizing hydrogen bonds, and interactions within the “aromatic cage” of MAO-B (publication C5)

With respect to indole-3-acetic acid arylhydrazones (Series V), all compounds were found to inhibit the MAO-B enzyme in the submicromolar range (IC_{50} = 0.130–0.493 μM), with three of them – bearing 2,3-dihydroxyphenyl, 2-hydroxy-4-methoxyphenyl, and 3,5-dimethoxy-4-hydroxyphenyl moieties — showing selectivity for MAO-B over MAO-A (publication C1). This is significant, as selective MAO-B inhibition may contribute to the treatment of Parkinson’s and Alzheimer’s diseases while minimizing side effects associated with elevated serotonin and norepinephrine levels. In this context, the identification of these three selective MAO-B inhibitors represents an important contribution to Dr. Anastasova’s research, highlighting the potential of indole-3-propionic acid hydrazone derivatives as novel neuroprotective drug candidates.

It is well established that nervous tissue is particularly susceptible to oxidative damage due to its high oxygen consumption and the abundance of polyunsaturated fatty acids. In patients with early-stage Parkinson’s disease, an accumulation of lipid peroxidation markers

such as malondialdehyde and lipid hydroperoxides has been observed, underscoring the need for therapies that address not only dopaminergic deficiency but also the restoration of redox homeostasis. In this context, the antioxidant activity of newly synthesized benzimidazole and indole derivatives has been investigated in several in vitro models. The key role of the catechol group in exerting a strong antioxidant effect has been confirmed, both in systems containing biologically relevant molecules – lecithin and deoxyribose – and in assays against various free radicals. The scientific contribution of these studies consists in expanding the knowledge of the structure-activity relationship in antioxidant derivatives and in establishing catechol fragments as a leading factor in the antioxidant activity of compounds.

To explain the observed higher neuroprotective activity of the 2-hydroxy-4-methoxy derivative compared to its 2,3-dihydroxy analogue from the series of N-substituted benzimidazole arylhydrazones (Series II), quantum-chemical calculations (publication D2) were performed to elucidate the most probable mechanisms of free radical scavenging: hydrogen atom transfer (fHAT), single electron transfer (SET), and radical adduct formation (RAF), both in polar (water) and nonpolar (benzene) media. The results show that the fHAT mechanism is the main pathway for the elimination of both •OH and •OCH_3 and •OOH radicals. NBO analyses show that the spin density of the radicals of the compounds formed after the abstraction of a hydrogen atom from the amide group is delocalized over the aromatic ring and the hydrazone chain. The higher degree of delocalization observed in the radical of the compound containing a 2-hydroxy-4-methoxyphenyl residue (R2eN12) is consistent with its theoretically predicted better radical-scavenging activity, as well as with the experimentally established higher neuroprotective activity. This is evidence of both the reliability of the results obtained and the correct choice of theoretical methods and models.

A significant scientific and applied contribution by Dr. Neda Anastasova is the discovery of N'-(3,4-dihydroxybenzylidene)-5-methoxy-1*H*-indole-2-carboxamide as a lead compound for the development of derivatives with combined neuroprotective and antioxidant properties. This indole derivative exhibits a favorable safety profile and potent neuroprotective effects, surpassing reference compounds such as indole-3-propionic acid, melatonin, and rasagiline, as well as remarkable antioxidant capacity, inhibiting lipid peroxidation and deoxyribose degradation, with higher efficacy than melatonin and trolox (Publication C3). Furthermore, it significantly inhibits the activity of human recombinant MAO-B, which is supported by molecular docking studies confirming its optimal fit into the flat hydrophobic cavity of the enzyme. The compound's ability to capture hypochlorite ions using luminol-enhanced chemiluminescence and its potential to modulate oxidative damage induced by iron on the biologically significant molecules lecithin and deoxyribose to assess its possible antioxidant and

pro-oxidant effects. (Publication D5). The 3,4-dihydroxy-substituted derivative demonstrates excellent radical scavenging properties in all model systems and, moreover, is not prone to causing a pro-oxidant effect or subsequent toxicity under conditions of iron-induced oxidative stress. Publication C2 is devoted to *in vivo* studies of N'-(3,4-dihydroxybenzylidene)-5-methoxy-1*H*-indole-2-carbohydraside in a model of scopolamine-induced Alzheimer's-type dementia in rats. The compound exhibits beneficial effects on different types of memory, as assessed by the step-through and the Barnes maze tasks, and effectively restores scopolamine-induced reductions in brain-derived neurotrophic factor and acetylcholine levels, while normalizing the scopolamine-enhanced activity of acetylcholinesterase in the hippocampus. Owing to its complex pharmacological profile, good tolerability, and ability to cross the blood–brain barrier, the compound emerges as a promising candidate for further investigation as a multitarget agent for the treatment of neurodegenerative diseases.

The significance of the research conducted by Senior Assist. Prof. Dr. Neda Anastasova is supported by a significant number of citations of her scientific results in scientific publications referenced in WoS or Scopus for the period 2019-2025 – 210 in total.

4. Evaluation of the personal contribution of the candidate

Given the interdisciplinary nature of the research presented in Dr. Neda Anastasova's scientific papers, several co-authors are involved. Of the 17 publications submitted for the competition (with a cumulative impact factor of 57.715) under indicators C and D, Dr. Anastasova is listed as first and/or corresponding author in 9 publications (publication C1, C3–C5, D3, D5, D8, D10, and D12). In publication C2, it is explicitly stated that Neda Anastasova and the first author Polina Petkova-Kirova contributed equally to the work; therefore, the number of publications in which Dr. Anastasova is recognized as first author can be considered 10 (59%).

The contribution of the candidate to the research conducted can be assessed as significant, and in some cases as leading, which is evidenced by the first position of Senior Assistant Professor Dr. Neda Anastasova in the authoring team and her role as corresponding author, as well as the contributions declared by the co-authors themselves in some of the publications.

5. Critical comments and recommendations

I have no critical comments on the presented materials and scientific research.

6. Personal impressions

I have known Dr. Anastasova since she was a PhD student in the Department of Organic Synthesis and Fuels at UCTM. I have an excellent impression of her. She is ambitious,

dedicated to her work, and has a wide range of interests and ideas for development and improvement.

CONCLUSION

The documents and materials, presented by Senior Assist. Prof. Dr. Neda Anastasova comply with all the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Low, the Regulations for the Implementation of the Low at BAS and the Regulations of the IOCCP-BAS.

The candidate in the competition has presented a sufficient number of scientific papers published after the materials used in the defense of the PhD degree. In terms of volume and quality, the scientometric indicators of Senior Assistant Professor Dr. Neda Anastasova meet and exceed the recommended requirements for the academic position of "Associate Professor." The candidate's research contains original scientific and applied scientific contributions that have received international recognition. The personal contribution to research and the scientific competence acquired by Senior Assistant Professor Dr. Neda Anastasova are undeniable.

After reviewing the materials and scientific works presented in the competition, analysing their significance and the scientific and applied contributions contained in them, I confidently give my positive assessment of the candidate and recommend the members of the Scientific Jury and the Scientific Council of IOCCP-BAS to support the election of Senior Assist. Prof. Dr. Neda Anastasova to the academic position of Associate Professor in the professional field 4.2 "Chemical Sciences", scientific specialty "Organic Chemistry".

17.09.2025 Reviewer:

(Assoc. Prof. Kameliya Anichina-Zarkova)