SCIENTIFIC OPINION

Subject: Competition for the academic position of “Associate Professor” in “Organic chemistry”, professional field 4.2. Chemical sciences; Higher education field 4. Natural Sciences, Mathematics and Computer Science, for the needs of Laboratory "Structural Organic Analysis" at the Institute of Organic Chemistry with the Center for Phytochemistry, Bulgarian Academy of Sciences, announced in State Gazette No. 40/16.05.2025, and on the website of the Institute of Organic Chemistry with the Center for Phytochemistry.

Written by: Assoc. Prof. Stela Toshkova Dragomanova, MScPharm, PhD, Department of Pharmacology, Toxicology, and Pharmacotherapy, Faculty of Pharmacy at Medical University “Prof. P. Stoyanov” – Varna; appointed member of the scientific jury by Order № РД-09-126/1/23.06.2025 of the Director of the Institute of Organic Chemistry with the Center for Phytochemistry, Bulgarian Academy of Sciences

1. CURRICULUM VITAE AND CAREER PROFILE OF THE CANDIDATE

Only one candidate has submitted the necessary documents to participate in the announced competition - Chief Assistant Professor Neda Orlinova Anastassova, PhD, from the Laboratory "Structural Organic Analysis" (SOA) at the Institute of Organic Chemistry with the Center for Phytochemistry (IOCCP) at the Bulgarian Academy of Sciences (BAS).

The collection of materials provided in paper form by Dr. Anastassova complies with the stipulations of the regulatory framework (Law on the Development of the Academic Staff of the Republic of Bulgaria, Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations for the Development of the Academic Staff of the IOCCP-BAS) and fulfills the criteria set by the IOCCP-BAS for the academic position of "associate professor".

The candidate, Dr. Anastassova, has submitted a total of 25 scientific papers. Among these, 17 scientific papers are considered for the final assessment and are outside the dissertation, while 13 research projects, of which she supervises 5, have been accepted for review. Additionally, 2 scientific papers related to the dissertation, 3 scientific papers concerning the acquisition of the title "chief assistant," and 3 scientific papers that fall outside the competition's scope have not been reviewed. The distribution of the scientific papers according to the relevant quartile (Q) is as follows: Q1 - 19 pieces, Q2 - 5 pieces, Q3 - 1 piece, and Q4 - 2 pieces. Furthermore, documents in the form of certificates and abstract books for participation in 41 scientific conferences, which include a total of 52 reports, have been provided. Participation in two industrial developments is also noted: a specialized electronic library aimed at studying artistic materials, as well as the identification and preservation of Bulgarian cultural heritage using infrared spectroscopy; and a small enterprise focused on organic cosmetics.

Brief biography of the candidate

Neda Anastassova completed her studies at the University of Chemical Technology and Metallurgy (UCTM) in Sofia, earning the educational and qualification degrees of "Bachelor of Engineering in Ecology and Environmental Protection" and "Bachelor of Organic Chemical Technologies." In 2012, she furthered her education by obtaining a "Master of Fine Organic Synthesis." That same year, she commenced her scientific career as a chemist in the Laboratory of Structural Organic Analysis at the Institute of Organic Chemistry with the Center for Phytochemistry, which is part of the Bulgarian Academy of Sciences. After a three-year hiatus, during which she served as a sales manager for laboratory equipment at "Biotehlab", she resumed her role as a chemist in the aforementioned laboratory in 2016. In October 2016, she was appointed as an assistant, and by May 2018, she advanced to the position of chief assistant at the Institute of Organic Chemistry with the Center for Phytochemistry at the Bulgarian Academy of Sciences. In 2017, she successfully defended her dissertation titled "Synthesis and study of hepatotoxicity and antioxidant activity of new N,N'-disubstituted benzimidazole-2-thiones" at the University of Chemical Technology and Metallurgy – Sofia.

The candidate has obtained additional qualifications in the following years: in 2019 at the Faculty of Chemistry, University of Barcelona, Spain, on the topic "Kinetic studies on the inhibition of beta-amyloid aggregation by benzimidazole derivatives"; in 2020 at the same institution on the topic "Consumption during the inhibited autoxidation of easily oxidizable substrates"; and in 2021 through the Erasmus+ program for training at the Faculty of Chemistry, Universidade Nova de Lisboa, Portugal, on the topic "Computational estimation of the structure and stability of reaction intermediates - radical and ionic species, characterization of transition states related to the formation of radical adducts, and evaluation of the free radical scavenging at the molecular level based on thermodynamic data for reactivity against various free radicals."

Dr. Anastassova has received numerous accolades over the years: four distinguished scientific reports for the period from 2012 to 2020, an award from the National Competition "Acad. Yuhnovski" for "Outstanding Young Scientist in the Field of Organic Chemistry" in 2019, as well as an award for "Best Popular Science Article" from the magazine "BG Science" in 2022.

2. OVERVIEW OF THE CANDIDATE'S ACTIVITIES

Evaluation of the candidate's scientific and applied scientific activities

For the competition, Dr. Anastassova has submitted a list comprising 5 scientific publications in Q1[a] journals, which are peer-reviewed and indexed in globally recognized scientific information databases, rather than the primary habilitation work - a monograph (indicator B). Additionally, there are 16 more publications (not related to the dissertation work or the competition for senior assistant professor), of which 12 are published in scientific journals that are peer-reviewed and indexed in esteemed databases, 3 are articles in non-refereed journals with scientific review, and one interview is included, which is a popular publication in the magazine BG Nauka from 2019. Ten of the articles have appeared in journals with an impact factor, such as the *Journal of Cellular Biochemistry* (IF = 3.448), *Spectrochimica Acta Part A-Molecular and Biomolecular Spectroscopy* (IF = 4.098), *Pharmacological Reports* (IF = 3.027), *Journal of Molecular Structure* (IF = 3.841), *Crystals* (IF = 2.4), *Pharmacia* (IF = 1.1), *Journal of Physical Chemistry B* (IF = 2.8), *Journal of Colloid and Interface Science* (IF = 9.7), among

others. In twelve of the publications submitted for the competition, the candidate is listed as the first author. The author's individual *h*-index stands at 9 according to Web of Science. The candidate has contributed to 52 reports and posters presented at 41 national and international scientific forums. From the provided references, 19 publications were identified, accumulating a total of 210 citations in scientific works indexed in WoS or Scopus for the period from 2019 to 2025, which corresponds to 420 credits under indicator D, significantly surpassing the minimum required threshold of 70 points by more than six times.

Evidence of the strong professional and academic credentials of Senior Assistant Professor Anastassova includes her leadership of five national research projects, one of which received funding through the Bulgaria-China bilateral cooperation program. Additionally, she has been involved in research teams for another eight projects with national funding.

Evaluation of teaching and learning activities

The educational endeavors of Senior Assistant Professor Neda Anastassova primarily focus on the training in organic and pharmaceutical chemistry and synthesis, aligning with the scientific field relevant to the announced competition. The candidate is actively involved in the education of students and graduates, including serving as a mentor in the organic synthesis of compounds with potential pharmacological activity for master's students in pharmacy, as part of the "Student Practices" initiative by the Ministry of Education and Science. Additionally, she supervises and co-supervises bachelor's and master's theses for students at the University of Chemical Technology and Metallurgy and the Faculty of Pharmacy at the Medical University-Sofia. Furthermore, she conducts laboratory exercises in the Technology of Organic Synthesis at the Department of "Organic Synthesis and Fuels" at the University of Chemical Technology and Metallurgy.

Characterization and evaluation of the contributions of scientific works

The research activities of the candidate Neda Anastassova encompass investigations in organic and pharmaceutical chemistry, specifically aimed at the creation of novel multitarget agents for addressing neurodegenerative diseases. Her research is concentrated on various contemporary areas, where significant scientific and applied contributions are also achieved. The key contributions can be categorized as follows:

• Design, synthesis, and structural as well as spectral analyses of hybrids composed of benzimidazole and indole;

• Examination of the pharmacological effects of these compounds across multiple in vitro models;

• Assessment of the radical-scavenging capabilities of the compounds in diverse in vitro settings

• Execution of an in vivo study utilizing a rat model for dementia;

• Quantum chemical computations aimed at clarifying the mechanism of action.

The research activities of the candidate encompass several multidisciplinary advancements in collaboration with scientific teams across diverse fields, such as neurobiology, pharmacology, physics and biophysics, biochemistry, among others.

The publications included in this habilitation report focus on the design, synthesis, and biological assessment of novel compounds created through the molecular hybridization of pharmacophores with known activity. These compounds are designed to target critical

pathogenetic mechanisms, with the potential to provide neuroprotective and modifying effects in neurodegenerative conditions, particularly in Parkinson's disease and Alzheimer's disease.

The scholarly contributions of Senior Assistant Professor Anastassova are characterized by originality in the design, synthesis, and characterization (including quantum chemical, toxicological, and pharmacological aspects) of novel compounds that influence various mechanisms involved in the pathogenesis of neurodegeneration, a significant health and social issue. A notable scientific and practical achievement of the candidate is the creation of new indole and benzimidazole derivatives, which were developed through the molecular hybridization of pharmacophores with established activity. Beyond their structural characterization, the pharmacological assessment yielded both in vitro and in vivo experimental data regarding their cytotoxicity, biocompatibility, neuroprotective and antioxidant properties, as well as their capacity to inhibit MAOB. It is important to highlight the candidate's contributions to the development of potential agents with antioxidant neuroprotective effects, as the findings indicate a distinct correlation between the structure of the synthesized molecules and their biological activity. In a rat model of 6-OHDA-induced injury, several of the compounds exhibited effects that were either superior to or comparable with the reference compounds melatonin and rasagiline, enhancing the viability of brain neurons and increasing GSH levels. A crucial aspect of scientific research involves investigations aimed at validating the established antioxidant properties of newly synthesized compounds through various oxidative stress models. These include a model of oxidative stress induced by H2O2 in SH-SY5Y cells; oxidative stress triggered by t-BuOOH in isolated rat brain mitochondria; and lipid peroxidation facilitated by a combination of iron and ascorbic acid, which generates the Fenton reaction and produces hydroxyl radicals (•OH). The radical-scavenging capabilities of benzimidazole and indole hybrids were also examined using several in vitro models, such as Fe (II)-induced damage to lecithin and deoxyribose, the assessment of reducing ability towards Fe (III), and the capacity to scavenge superoxide radicals and hypochlorite anions via the enzyme system xanthine/xanthine oxidase. The findings indicate a distinct influence of molecular structure on antioxidant activity. The data derived from studies on radical-scavenging activity enhance the understanding of the structure-activity relationship regarding the antioxidant effects of substituents in hydrazone side chains. The significant role of the catechol group in exhibiting a robust antioxidant effect has been confirmed, both in systems involving biologically relevant molecules such as lecithin and deoxyribose, and against various free radicals. Furthermore, quantum chemical calculations aimed at elucidating the most likely mechanisms of free radical scavenging—namely hydrogen atom transfer (fHAT), electron transfer (SET), and radical adduct formation (RAF)—in both polar (water) and non-polar (benzene) environments, reveal that the fHAT mechanism is the primary pathway for the neutralization of •OH, as well as $•OCH_3$ and •OOH radicals.

Another prominent scientific area of focus for Anastassova is the investigation of the inhibitory effects of newly synthesized derivatives on MAOB, which is a crucial target for addressing Parkinson's disease. All selected monosubstituted and disubstituted benzimidazoles, chosen based on their favorable safety profile and neuroprotective properties, exhibit statistically significant inhibitory effects against hMAOB. The selectivity for MAOB is essential in the creation of inhibitors aimed at treating neurodegenerative disorders. The absence of inhibitory effects on MAOA mitigates the risk of adverse reactions linked to elevated levels of serotonin and noradrenaline. In this regard, the discovery of three selective MAOB inhibitors

(5a, 5d, and 5e) marks a noteworthy advancement, showcasing the potential of hydrazone derivatives of indole-3-propionic acid in the development of novel neuroprotective agents.

Furthermore, another avenue of experimental research involves the examination of a specific indole derivative using a model of scopolamine-induced dementia of Alzheimer's disease type, in comparison to the standard treatment rivastigmine. Behavioral assessments reveal beneficial effects of the compound on memory; biochemical analyses indicate elevated levels of ACh and reduced AChE activity in the hippocampus, returning them to baseline levels; a decrease in oxidative stress caused by the toxic agent is also observed, along with a restoration of BDNF levels. Consequently, the investigations suggest that the compound is a promising subject for further detailed studies as a multitarget therapeutic agent for neurodegenerative diseases.

I evaluate the contributions made towards the development of novel substances exhibiting neuroprotective properties as a commendable attribute of an active researcher. A crucial aspect of the candidate's scientific research activities is the investigation of potential targets for new pharmacological agents that may influence not only Parkinson's disease but also other neurodegenerative disorders.

The future research prospects outlined in the provided Habilitation Report indicate a need for ongoing efforts in the design and synthesis of new compounds, as well as a more thorough and comprehensive examination of substances that already demonstrate neuroprotective potential. A contemporary focus is the anticipated development of nanoparticles aimed at exploring synergistic effects, including those related to antitumor activity.

The assessment of Dr. Neda Anastassova's scientific research activities reveals her status as an established scientist with a notable publication record that has garnered significant attention in specialized medical literature. Evidence of this is her receipt of the 2019 Award from the National Competition “Acad. Yukhnovsky” for “Outstanding Young Scientist in the Field of Organic Chemistry,” along with her role as a guest editor for the *Antioxidants* (MDPI) Special Issue: Targeting Oxidative Stress in Parkinson's Disease with Multi-Target Compounds during 2023-2024.

The candidate's scientific research activities affirm her established position within the scientific community. The original and corroborative results documented in the references for her scientific contributions represent a substantial portion of her scientific and evidentiary portfolio.

3. RECOMMENDATIONS AND REMARKS

I hold no criticisms regarding the candidate. I suggest that she continue her strong engagement in publications and projects, along with his commitment to collaborating with students.

CONCLUSION

The documents and materials presented by Senior Assist. Prof. Neda Anastassova meet all the requirements of Law on the Development of the Academic Staff of the Republic of Bulgaria, Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations for the Development of the Academic Staff of the IOCCP-BAS).

The candidate has submitted a considerable number of scientific publications that were released subsequent to the materials utilized in the defense of her doctoral thesis and and for obtaining the position of "chief assistant". The works of Dr. Anastassova encompass original scientific and applied contributions that have garnered international acclaim, as a portion of these have been featured in journals and scientific compilations published by global academic publishing entities, and the theoretical advancements possess practical relevance. The scientific qualifications of the candidate are indisputable. I assert that the scientific works presented by chief assistant Neda Anastassova exhibit contemporary scientific and applied value and represent an original contribution within the scope of the announced competition for the associate professor position in the discipline of "Organic Chemistry".

The accomplishments attained by Neda Anastassova in her scientific and research activities align entirely with the specific stipulations outlined in the Regulations of the Institute of Organic Chemistry with the Center for Phytochemistry-BAS regarding the implementation of the Act on the Development of the Academic Staff of the Republic of Bulgaria.

Upon reviewing the materials and scientific articles submitted for the competition, and evaluating their significance along with the scientific, scientific-applied, and applied contributions they encompass, I confidently provide my favorable assessment and recommend that the Scientific Jury prepare a report-proposal to the Scientific Council of the Institute of Organic Chemistry with the Center for Phytochemistry-BAS for the appointment of Senior Assistant Professor Neda Anastassova to the academic position of "Associate Professor" at the Institute of Organic Chemistry with the Center for Phytochemistry-BAS in the scientific specialty "Organic Chemistry", within the professional field 4.2. Chemical Sciences.

11.09.2025 **Who prepared the opinion**: .....

Varna (**Assoc. Prof. Stela Toshkova Dragomanova, MScPharm, PhD**)