REVIEWER REPORT

From Dr. Petko Nedyalkov Denev, professor at the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), Bulgarian Academy of Sciences (BAS)

on the materials, presented in competition for acquisition of academic degree **"associate professor"** at the IOCCP-BAS

in area of higher education: 4. **"Natural sciences, mathematics and informatics"**
professional field: 4.2. **"Chemical sciences"**
scientific specialty: "**Organic chemistry"**

1. General presentation of the received materials

By order № РД-09-126/1/23.06.2025 of the Director of IOCCP-BAS, I was appointed as a member of the scientific jury in a competition for the academic position of "associate professor" at IOCCP-BAS in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional direction 4.2. "Chemical Sciences", scientific specialty "Organic chemistry" for the needs of the laboratory "Structural Organic Analysis" (SOA) at IOCCP-BAS. Only chief assist. prof. Neda Anastassova from the SOA lab at IOCCP-BAS submitted documents for participation in the competition, announced in the State Gazette, issue 40 from 16.05.2025. At its first meeting, the scientific jury unanimously decided that the candidacy of chief assist. prof. Anastassova meets the criteria of the IOCCP-BAS for holding the academic position of "associate professor", and I was selected to prepare a review report on the materials submitted for the competition.

The set of materials on paper and electronic media submitted by assoc. prof. Anastassova is in accordance with the Regulations for the Development of the Academic Staff of the IOCCP-BAS for holding the academic position of "associate professor" and includes: Application to the Director of IOCCP-BAS for admission to the competition; Scientific CV; Reference (in the prescribed format) for meeting the minimum requirements in accordance with the Regulations and procedures for acquiring scientific degrees and holding academic positions at IOCCP-BAS; Diplomas for obtaining the educational and scientific degree "Doctor" and for being awarded the academic position of "Chief assistant professor"; Dissertation abstract for the award of the educational and scientific degree "Doctor"; Extended habilitation reference for scientific contributions in Bulgarian and English; Full list of scientific publications; List and copies of the publications submitted in the competition as an equivalent number of articles for the habilitation work under indicator group B; List and copies of the publications submitted in the competition under indicator group Г; List of conference participations, accompanied by supporting evidence; List of citations; List of participation in research projects; List and supporting evidence of received certificates and awards.

Chief assist. prof. Neda Anastassova has attached a list of twenty-six scientific publications, prepared in co-authorship throughout her professional career. In the current competition, she participates with seventeen publications, of which five are submitted as equivalent to a habilitation work and twelve are submitted under indicator group Г (according to Annex 1 of the Regulations for the Development of the Academic Staff at IOCCP). All seventeen scientific works are within the scope of the competition, cover the period 2018 - 2025, and have not been presented in other competitions and procedures in which the candidate has participated. Therefore, all materials are accepted for review and are taken into account in the final evaluation.

2. Short biography of the applicant

Neda Anastassova obtained a Bachelor's degrees in "Organic Chemical Technologies" and in "Environmental Engineering and Environmental Protection" from the University of Chemical Technology and Metallurgy (UCTM), Sofia, in 2011, and a Master's degree in "Fine Organic Synthesis" from the same university in 2012. In the periods January 2012 – February 2013 and March 2016 – September 2016, she worked as a "chemist" in the SOA laboratory at IOCCP-BAS, and in 2017 she obtained a PhD degree in "Pharmaceutical Chemistry" from UCTM, Sofia, after defending a dissertation entitled "Synthesis and study of hepatotoxicity and antioxidant activity of new N,N′-disubstituted benzimidazole-2-thiones." This doctoral dissertation largely shaped Neda Anastassova's future scientific interests, focused mainly on the synthesis and characterization of the biological activity of benzimidazole derivatives. In the period October 2016 – May 2018, she held the position of "assistant professor" at IOCCP-BAS, after which she was elected "chief assistant professor" in the SOA lLaboratory at the Institute, a position she still holds today. Between 2019 and 2021, she completed three specializations abroad (NOVA University Lisbon, Portugal; University of Bologna, Italy; and University of Barcelona, Spain), which demonstrates Dr. Anastassova's aspiration to acquire new knowledge and further develop her expertise. She has participated in the development of a specialized electronic library for the study of artistic materials, identification, and preservation of Bulgarian cultural heritage using infrared spectroscopy. Throughout her professional career, Dr. Anastassova has shown very active involvement in scientific and research projects, including as a project leader. Notably, she has led four projects funded under competitions for fundamental scientific research for young scientists and postdoctoral researchers, as well as one bilateral cooperation project between Bulgaria and China funded by the Bulgarian National Science Fund. This highlights Dr. Anastassova as an active scientist with a strong inclination toward building scientific collaborations. In 2019, her scientific work was awarded with the prestigious award for young scientists "Outstanding Young Scientist in the Field of Organic Chemistry" from the National Competition named after Acad. Ivan Yukhnovsky. The teaching and educational activities of chief assist. prof. Anastassova include supervision and co-supervision of three graduate

students, mentorship of two students under the Ministry of Education and Science project “Student Practices,” as well as conducting practical laboratory courses in “Technology of Organic Synthesis” at UCTM, Sofia.

3. General characteristics of the applicant's activities

Assessment of scientometric indicators

In **indicators group A** from of the Regulations for the Implementation of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), Dr. Anastassova has presented an abstract of a dissertation for the acquisition PhD degree in "Pharmaceutical Chemistry" entitled "Synthesis and study of hepatotoxicity and antioxidant activity of new N,N'-disubstituted benzimidazole-2-thiones". This brings her 50 points on this indicator.

Indicators group B: Five of the publications in the competition are presented by the candidate as equivalent to a habilitation thesis. Their topic is mainly on the synthesis and biological activity of new arylhydrazone derivatives. All of them are in Q1 editions, and the total IF of these publications is over 27.5, which is a very good certificate of their quality. In four of them, Dr. Anastassova is the first, and in one, the second author, which is indicative of her significant role in their development and shaping. These publications bring Dr. Anastassova 125 points, which exceeds the required minimum of 100 points of the IOCCP-BAS for holding the position of "associate professor".

Indicators group Г: Twelve publications are presented under this group of indicators, all of which have an impact factor and/or impact rank. Here, a small reduction in the candidate's points should be made, since in the year of publication of G12, the journal *Bulgarian Chemical Communications* no longer has an impact factor, but only an impact rank (SJR). According to the RIADASRB and the regulations of IOCCP-BAS, this publication carries 10 points, instead of 12 points. Thus, Dr. Anastassova collects 237 points with a required minimum of 220 points.

Indicators groups Д and Ж: A Scopus reference at the time of writing the review indicates a total of 240 citations for Dr. Anastassova’s entire scientific career. In the current competition, she applied with a list of 210 citations, in addition to those submitted for awarding the academic position “chief assistant professor” and the PhD degree. This brings her 420 points on this indicator, which is six times higher than the required minimum of 70 points required in IOCCP-BAS for holding the academic position of “associate professor”. These citations are mainly from foreign authors in foreign journals of large academic publishers and were received mainly in the last six years. The overall H-index of Dr. Anastassova's publications (with excluded self-citations) in Scopus is 8. Thus, by this indicator, she not only meets, but also exceeds one of the specific

requirements of the IOCCP-BAS for holding the academic position of “associate professor” for an H-index of 5. It is very important to note here that Dr. Anastassova's H-index is formed by 8 scientific publications out of a total of 21 referenced in Scopus. The high comparative share of these publications from the total number (over 38%) is indicative of the high quality of Dr. Anastassova's scientific production.

Thus, the candidate's summarized points for the individual groups of indicators are as follows:

Indicator	Minimal requirements of IOCCP-BAS	Points of the candidate
А	50	50
Б	-	-
В	100	125
Г	220	237
Д	70	420
Е	-	-
Ж (Н-индекс)	≥ 5	8

As is evident from this summarized information, Dr. Anastassova's scientific output not only covers, but also significantly exceeds, the minimum scientometric requirements set out in the ADASRB and the Regulations of the IOCCP-BAS and the Bulgarian Academy of Sciences for the academic position of "associate professor".

Evaluation of contributions

All scientific works presented by chief assist. prof. Dr. Anastassova correspond to the subject matter of the competition. The main scientific contributions of these works are related to: 1) Design, synthesis, spectral and structural studies of benzimidazole and indole hybrids; 2) Investigation of the pharmacological activity of the compounds in various *in vitro* models; 3) Investigation of the radical-scavenging properties of the compounds in various *in vitro* models; 4) Conducting *in vivo* studies in a rat model of dementia; and 5) Quantum-chemical calculations to clarify the mechanism of action.

The submitted habilitation reference encompasses a wide cycle of studies dedicated to the development of new multitarget compounds for the therapy of neurodegenerative diseases, primarily Parkinson’s disease and Alzheimer’s disease, and impresses with its complexity, methodological consistency, and depth of analysis. The author approaches the problem with a clear understanding of the multifactorial pathogenesis of these diseases and the limited effectiveness of existing therapies, proposing an interesting concept for multitarget molecules aimed simultaneously at inhibiting monoamine oxidase B (MAO-

B), counteracting oxidative stress, modulating mitochondrial functions and redox homeostasis, as well as counteracting neuroinflammatory processes.

At the core of the research is the synthesis of 40 new arylhydrazone derivatives, divided into five series including benzimidazole and indole hybrids with various substituents, aiming to optimize the structure–activity relationship [B1–B5]. By applying diverse analytical approaches – IR, ^{1}H- and ^{13}C-NMR spectroscopy, high-resolution mass spectrometry, X-ray structural analysis [Г5], as well as quantum-chemical calculations – the structural characteristics, conformational behavior, and possible reactive centers in the molecules were clarified. The presence of conformers, demonstrated by theoretical and experimental data, provides additional insight into the stereoelectronic features of the studied compounds and their potential significance for biological activity. Of particular interest is the systematic toxicological assessment carried out on cell lines (SH-SY5Y), isolated synaptosomes, mitochondria, and erythrocytes, which made it possible to outline compounds with the most favorable safety profile, such as 2e, 3e, and 5a, and to eliminate potentially toxic structures at early stages of the research. Such an approach is valuable in drug development, as it minimizes the risk of adverse effects in later phases. The data on neuroprotective activity are extensive and convincing. In models of H_2O_2- and 6-OHDA-induced oxidative stress, as well as in systems with isolated mitochondria and microsomes, several compounds (1h, 2e, 4a, 5a, etc.) demonstrated significant protection, often exceeding reference agents such as melatonin and rasagiline. Special attention deserves the 2-hydroxy-4-methoxy derivative 2e, which in some models showed higher effectiveness even than catechol analogues, traditionally considered leading due to their strong antioxidant potential [B5]. Quantum-chemical calculations shed light on this result, showing that in 2e the delocalization of spin density after hydrogen atom transfer is more pronounced, leading to more effective scavenging of free radicals via the HAT mechanism, while the SET pathway was ruled out as unlikely [Г2]. This achieves an impressive synergy between experiment and theory, enhancing the reliability of the conclusions.

The results of the inhibitory activity against MAO-B are also of particular importance. Selective inhibitors were identified among compounds 2e, 5a, 5d, and 5e, for which molecular docking revealed a favorable orientation in the enzyme's active site and the formation of stable hydrogen bonds with key amino acids [B3]. The selectivity towards MAO-B over MAO-A, established for some of these derivatives, is critically important to avoid side effects associated with nonspecific inhibition and increased serotonin and noradrenaline levels. A significant contribution is also represented by studies on radical-scavenging properties, where different reactive oxygen species were systematically examined and the key role of the catechol group in antioxidant activity was demonstrated. Some compounds, such as 5a, 3a, and 4a, showed efficacy comparable to or even higher than that of reference antioxidants such as Trolox and quercetin, positioning them as particularly promising for a multitarget approach to neurodegenerative diseases [B1–B3]. In the presented *in vivo* studies in rats with a scopolamine-induced dementia model, the 3,4-dihydroxyl derivative 4b was found to restore cognitive functions, influence oxidative

stress and neuroinflammatory markers, and exhibit an effect comparable to or even better than rivastigmine [B2]. Thus, a successful transition was made from cellular and subcellular models to the whole organism, which is a crucial step in the preclinical evaluation of new drug candidates.

Overall, the presented work impresses with its consistency and interdisciplinary approach. Dr. Anastassova not only synthesizes and characterizes new structures but also participates in the systematic assessment of their toxicity, antioxidant and neuroprotective properties, mechanisms of action, and pharmacological potential, supporting the experimental data with theoretical calculations. The identification of lead structures such as 2e, 4b, 5a, and 5e with a favorable safety profile, strong neuroprotective activity, antioxidant properties, and selective inhibition of MAO-B represents a significant contribution to the development of multitarget therapeutic strategies for neurodegenerative diseases and creates a solid foundation for future pharmacological and clinical studies.

4. Evaluation of the candidate's personal contribution

The scientific contributions presented in the competition are the result of interdisciplinary research at the border between organic chemistry, pharmaceutical chemistry, bioorganic chemistry and molecular biology, and all scientific publications in the competition are co-authored. The fact that Dr. Anastassova is the first and/or corresponding author in over 50% of them leaves no doubt about her essential role and personal contribution to their development and publication.

5. Critical comments and recommendations

I have no significant comments on the submitted materials and documents, nor on the candidacy of Dr. Anastassova. It should be noted that the documents for the submitted competition are well-structured and arranged, which facilitates their analysis and evaluation. The habilitation reference is meaningful and presents well the scientific contributions of Dr. Anastassova.

6. Personal impressions

I know Dr. Anastassova vaguely, mainly as a colleague from the IOCCP-BAS, as well as from some presentations of her scientific developments at scientific forums and within the framework of various initiatives at the Institute. My impression is that she is a very active and thorough young researcher.

CONCLUSION

The documents and materials submitted by the sole candidate in the competition, chief assist. prof. Neda Anastassova, meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, the Regulations for the Implementation of the ADASRB at the Bulgarian Academy of Sciences and the Regulations of the IOCCP-BAS. She has presented a sufficient number of scientific papers beyond those used in the procedures for the defense of the PhD degree and acquisition of the academic position "chief assistant professor". All scientific publications are of a high scientific level and contain original scientific contributions on the design, synthesis, structural characterization and biological activity of new benzimidazole and indole compounds. From the documents presented, it is clear that chief assist. prof. Anastassova is an active and promising researcher with a desire to continuously upgrade and acquire new knowledge. In her scientific research activities, she has achieved significant and interesting results that significantly exceed the specific requirements of the Regulations of the IOCCP-BAS for holding the academic position of "associate professor".

Because of all this, after a thorough acquaintance with the materials and scientific works presented in the competition, an analysis of their significance and the scientific and applied contributions contained in them, **with full conviction I give my positive assessment** and recommend to the Scientific Jury to prepare a report-proposal to the Scientific Council of the IOCCP-BAS for the election of chief assist. prof. Dr. Neda Anastassova to the academic position of "associate professor" at the IOCCP-BAS in professional field 4.2. "Chemical Sciences", scientific specialty "Organic chemistry" for the needs of the SOA laboratory at the IOCCP-BAS.

16.09.2025 Reviewer: .....

Prof. Petko Denev, PhD