R E V I E W

In the competition for obtaining the scientific title „**Professor**“ in professional direction 4.2 „Chemical sciences“, scientific specialty „Bioorganic chemistry, chemistry of natural and physiologically active substances“ for the needs of the Institute of Molecular Biology „Acad. Rumen Tsanev“ at BAS-Sofia

By Prof. D.Sc. Lyubomir Vezenkov, UCTM-Sofia

The competition announced in State Gazette, Extraordinary Issue 114/24.12.2025, advertisement № 87, only one candidate Associate Professor Nikolay Tzvetkov Tzvetkov appeared. N. Tzvetkov was born on October 18, 1972. After completing his secondary education, he studied at the University of Chemical Technology and Metallurgy, Sofia, majoring in Chemical Technologies, specializing in Organic Synthesis Technology. He graduated in 1998 after doing a thesis in Germany. Since 2000 year Nikolay Tzvetkov is a doctoral student at the Higher School of Chemistry and Biochemistry, University of Bielefeld, Germany, and in 2005 successfully defended a dissertation on „Photoreaktionen tricyclischer Cyclopropylketone: Aufbau von Polyquinanen und analoger Ringsysteme“ and received the scientific degree of doctor. From 2005 to 2006, he was a postdoctoral fellow at the same university, where he carried out scientific research on Stereo- and regioselective synthesis, isolation and characterization of „quasi“-sesquiterpenes and optically active compounds. From 2006 to 2008, he was a research assistant at the Pharma Centre Bonn, Germany – Schwarz Pharma AG, Monheim, Germany. From 2008 to 2013, Tzvetkov was a research assoc. lecturer at the BioPharma NeuroAlliance Consortium, Pharma Centre Bonn, Germany – UCB Pharma S.A., Belgium. From 2014 to 2015, he was a part-time head of research and development projects at Beraterm AD, Prateln (Basel), Switzerland. From 2014 to 2017, he was a Senior Research Assoc. Lecturer, Head of Research and Development Projects with Industrial Partners. From 2017 to 2018, he was an assistant professor, section „Molecular design and biochemical pharmacology Institute of Molecular Biology “Acad. Rumen Tsanev“, BAS , Sofia, Bulgaria. In 2018, he was elected Assoc. Professor in the same section, scientific specialty „Bioorganic chemistry, chemistry of natural and physiologically active substances“. Since 2019, he has been the head of this section. N. Tzvetkov is a member of the following scientific organizations: BPD, EPD, American Chemical Society, etc. He is the guest editor of a special edition „The 10th Bulgarian Peptide Symposium ” in the journal Current Research in Biotechnology. Since 2012, he has been an associate reviewer for a number of scientific publishers: J. Org. Chem., J. Med. Chem., ACS Med. Chem. etc.

Works with which the candidate is presented in the competition.

For participation in the announced competition, N. Tzvetkov has presented a total list of 101 publications and 7 patents. Among the candidate’s works, 2 publications are included in his doctoral dissertation (**1** and **2** of the general list of publications). Additionally, 19 publications were submitted for the academic position of "Assoc. Professor": 7 publications for equating to habilitation work (3–9 of the general list of publications) and 12 publications for the Assoc.

Professor competition (10–12, 15–21, 29, and 30 of the general list of publications). Publications not included in previous competitions amount to 60. I will focus solely on the scientific works related to the Professor competition, as the works submitted for the doctoral and Assoc. Professor competitions have already been evaluated.
The data provided by the candidate demonstrate that the national criteria for the academic position of "Professor" have been met.
Indicators from Group A (Ind. **1**, Dissertation for the academic degree of "Doctor") account for **50 points**.
Indicators from Group B (Ind. **4**, Habilitation thesis – Publications) include five publications: 4 in Q_1 journals (4 × 25 = **100 points**) and one in a Q_2 journal (1 × 20 = 20 points), totaling 120 points, with a minimum requirement of 100 points.
Indicators from Group D (Ind. **7**) include 15 publications: 10 in Q_1 journals (10 × 25 = **250 points**) and 5 in Q_2 journals (5 × 20 = **100 points**), totaling **350 points**, with a minimum requirement of 220 points. The total impact factor is 90.06.
Indicators from Group D (Ind. **11**, citations in scientific publications referenced in WoS and Scopus) include **312 citations**, corresponding to 624 points, with a minimum requirement of 100 points.
Indicators from Group E (Indicators $\mathbf{E_{15–18}}$) are as follows: Indicator $\mathbf{E_{15}}$ includes participation in 2 international scientific or educational projects (2 × 20 = **40 points**); Indicator $\mathbf{E_{16}}$ includes guidance of 2 national scientific or educational projects (2 × 20 = **40 points**); Indicator $\mathbf{E_{17}}$ includes guidance of a Bulgarian team in an international scientific or educational project (1 × 50 = **50 points**); Indicator $\mathbf{E_{18}}$ includes funds attracted from projects led by the candidate (**320,000 BGN**, equivalent to **64 points**). The total number of points is **194**, with a minimum requirement of 150 points.
The overall scientific research activity of Nikolay Tzvetkov is characterized by high scientific results, with excellent dissemination and presentation to the scientific community. According to Scopus and the Web of Science (WoS), his publications have received over 6,380 independent citations, with an h-index of 25, confirming the significant impact of his research. Of the scientific publications submitted for participation in the competition, the candidate is the first author of 4 publications and the last leading correspond author of 7 publications and the corresponding author of a total of 11 publications.
The analysis shows that for each group of indicators, the candidate meets and, in most cases, exceeds the required minimums. The total number of points for all groups of indicators is 1,338, with a minimum requirement of 640 points, which is almost twice as much.

General characteristics of the applicant's scientific and applied activity

Nikolay Tzvetkov’s research and applied scientific work establish him as a specialist in the synthesis of biologically active compounds, particularly for biomedical applications. His expertise spans multiple domains, including: Planning, leading, and executing the design and synthesis of low-molecular-weight substances and short-chain peptides. Conducting in vitro and in vivo studies of natural extracts enriched in specific bioactive compounds, as well as low-

molecular-weight organic compounds and short-chain peptides as model systems. These studies focus on potential therapeutic applications in cancer, neurodegenerative diseases, and autoimmune disorders. Additionally, he possesses extensive experience in applying in silico molecular modeling and quantum chemical computational methods—both independently and in conjunction with experimental studies—to address critical questions. His work includes the prediction and visualization of protein-ligand interactions, contributing to a deeper understanding of molecular mechanisms in drug design and development.

Scientific contributions: The thematic focus in the research activity of Nikolay Tzvetkov is in the field of pharmaceutical chemistry. The candidate's scientific contributions highlight the interdisciplinary nature of his research. The results supporting these contributions can generally be classified into the following interrelated directions:

1. **Scientific contributions from Group B indicators, Metric 4**

A series of 8 (pyrrolo-pyridin-5-yl) benzamide derivatives were synthesized and subjected to comprehensive analysis. In vitro biological screening was conducted on all newly synthesized compounds to evaluate their ability to inhibit rat and human monoamine oxidase-B (MAO-B) enzymes. Through structure-activity relationship (SAR) analysis and structural optimization, potent, competitive, and reversible MAO-B inhibitors were identified, exhibiting improved physicochemical and drug-like properties. Among these, compounds 14 and 15 demonstrated an excellent pharmacological profile, making them promising model structures for further investigation in in vivo models of Alzheimer's and Parkinson's diseases.

A comparative analysis was performed on two classes of compounds: indazole-5-carboxamide derivatives (compounds 11–16) and (indazole-5-yl) methanimine derivatives (compounds 17–22). This involved in vitro screening against human MAO-A and MAO-B enzymes, as well as acetylcholinesterase and butylcholinesterase. Additional studies included a preliminary hepatotoxicity assessment, molecular modeling, binding affinity evaluation, photochemical stability testing, and drug-likeness assessment. The SAR analysis revealed that these compounds act as selective and potent inhibitors of human MAO-B, with IC_{50} values ranging from the low nanomolar to picomolar range.

A systematic search was conducted in the Web of Science Core Collection database, and the retrieved scientific articles were analyzed using VOS viewer software. This research identified and analyzed the 100 most cited articles in the field of neurotensins, highlighting emerging trends and significant pharmacological topics. A separate study focused on publication trends related to monoamine oxidases, providing insights into current research directions in the field.
Another notable study involved the design of peptide mimetics with modifications at positions 8 and/or 9 in the structure of the parent peptide neurotensin (NT) (8–13), while preserving residues 10–13. Using standard solid-phase peptide synthesis with the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group, the peptide NT (8–13), its analogs (10–14), and their precursor (9) were successfully synthesized and characterized. Radioligand binding assays experimentally confirmed the importance of residues at positions 8 and/or 9 in NT (8–13) and its analogs (10–14) for binding affinity to hNTS1 and hNTS2 receptors. Substitution of Arg_8 and/or Arg_9 with Lys and/or Cav residues yielded analogs with equivalent

affinity for both receptors. Subsequent in vitro ADME-T studies and in vivo behavioral assessments demonstrated that peptide mimetic 10 exhibits enhanced stability, improved blood-brain barrier permeability, and significant improvements in motor and cognitive functions in a mouse model of MPTP-induced parkinsonism, compared to the parent peptide NT (8–13).

2. Scientific contributions from Group D indicators, Metric 7

The more significant contributions are as follows:

Of research on the basis of natural products:

An in vivo comparative study was conducted to evaluate the neuroprotective effects of three natural bio-antioxidants—ellagic acid, alpha-lipoic acid, and myrtenal on an experimental model of Parkinson's disease (PD) using a 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in rats. The study included a biochemical evaluation of key oxidative stress parameters, as well as dopamine (DA) levels in brain homogenates. The findings revealed that all three compounds improved learning and memory performance, as well as neuromuscular coordination. Biochemical analyses demonstrated that these compounds significantly reduced lipid peroxidation levels, restored catalase activity, and normalized DA levels. Among the tested compounds, myrtenal exhibited the most pronounced antiparkinsonian effects.

In folk medicine, the leaves of Haberlea rhodopensis Friv. have been traditionally used to treat wounds and infectious diseases in livestock, while the herb itself is still employed for detoxifying the stomach, liver, kidneys, and blood vessels. Due to its myconoside content, extracts of this plant are also valued as cosmetic ingredients. A study was conducted to assess the cytotoxic and antiproliferative activity of two herbal extracts of Haberlea rhodopensis on various cancer cell lines and one normal cell line, using doxorubicin as a reference compound. The results indicated that both extracts exhibited reduced inhibitory activity compared to doxorubicin across all tested cell lines. However, the myconoside-enriched extract (Extract 2) demonstrated enhanced cytotoxicity and antiproliferative effects compared to Extract 1. Specifically, Extract 2 showed a significant increase in cytotoxicity after 24 hours and antiproliferative activity after 48 and 72 hours at the highest tested concentration of 100 μg/mL.

Another study focused on bitter hop compounds, including α-acids, β-acids, and xanthohumol, and their effects on anorexigenic hormone secretion. The findings revealed that these compounds stimulate the release of glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK), while selectively increasing the expression of mouse bitter taste receptors (Tas2rs). Specifically, xanthohumol and α-acids enhanced the expression of Tas2r138 and Tas2r130–Tas2r138, respectively, whereas β-acids upregulated multiple bitter receptors, including Tas2r119, Tas2r105, Tas2r138, Tas2r120, and Tas2r130. A notable contribution of this research was the first-ever molecular modeling of the mouse Tas2r138 receptor and its human ortholog T2R38, which served to validate the experimental data.

In a separate in vivo study, the protective mechanism of a standardized polyphenol-enriched extract of Geranium sanguineum L. (PPhC) was investigated on inhibited monooxygenase activities in the context of influenza virus infection (IVI) in animals. The study established that PPhC may be beneficial for preventing and treating IVI by mitigating oxidative damage in the liver and restoring inhibited drug metabolism.

Through bibliometric analysis of data from the Web of Science database, the candidate identified that curcumin, flavanones, resveratrol, carotenoids, polyphenols, flavonols, flavones, and berberine are the most frequently cited natural products studied for diabetes treatment. In another study analyzing 22,885 publications, the role of reactive oxygen species (ROS) in neurodegeneration was examined, providing key insights and future research directions.

The candidate's research also contributes to understanding essential pathophysiological processes, including the proliferation of vascular smooth muscle cells in cardiovascular diseases. This work highlights the importance of signaling cascades in regulating vascular cell proliferation, offering valuable perspectives for disease mechanism studies and therapeutic development.

Application of In Silico Molecular Modeling Methods in Combination with Experimentally Obtained Data

Nikolay Tzvetkov's research demonstrates a pioneering application of in silico molecular modeling methods, integrated with experimental data, to address complex scientific challenges. His work leverages modern computational platforms and customized procedural frameworks to solve specific scientific problems, leading to innovative solutions in drug discovery and molecular design. This interdisciplinary approach has enabled the successful execution of several high-impact projects: One key achievement involved the identification of multi-target lead ligands through the virtual screening of 650,000 compounds. The objective was to discover molecules with simultaneous predicted activity against three critical proteins linked to neurodegenerative diseases: acetylcholinesterase, histone deacetylase 2, and monoamine oxidase B. This research highlights the potential for developing multi-target therapeutics capable of addressing multiple pathological mechanisms in neurodegenerative disorders.

Another significant contribution was the development of a phytochemical approach to identify 4-(α-L-rhamnosyloxy)-benzyl isothiocyanates, which exhibit promising activity against multidrug-resistant Staphylococcus aureus. This work underscores the potential of natural product-based strategies in combating antibiotic-resistant bacterial strains.

Additionally, a combined single-crystal X-ray diffraction (SCXRD) and molecular modeling approach was employed to design novel type II topoisomerase inhibitors targeting the human topoisomerase II (hTOP2) isoform. This led to the synthesis and characterization of two new 4-substituted 2-(5,5-dimethyl-3-styrylcyclohex-2-enylidene) malononitrile derivatives (compounds **1** and **2**). Molecular docking and virtual screening, utilizing the crystallographic structures of compounds 1 and 2, were performed to predict their binding affinity (Ki HYDE values) to the hTOP2β isoform. The antiproliferative activity of these compounds was evaluated in human hepatocarcinoma HepG2 cells and compared to the reference inhibitor etoposide. The results revealed that compounds **1** and **2** exhibited higher binding affinity to hTOP2β than etoposide, although their antiproliferative activity was lower than that of the reference compound.

In another theoretical study, a comparative analysis was conducted between two clinically relevant drugs: the antiviral favipiravir (FAV) and the anti-thalassemia drug deferiprone (DFP). Both compounds were explored as potential structural scaffolds for designing novel antivirals, particularly against COVID-19. The study utilized a combinatorial virus-host target approach,

employing molecular modeling to assess interactions with two critical biological targets: SARS-CoV-2-ACE**2** and SARS-CoV-2-Mpro. Additionally, the tautomeric forms of FAV and DFP were investigated using quantum chemical calculations and UV-Vis spectroscopy in various solvents and in the presence of bivalent ions. A key finding was the greater stability of enol forms in organic solvents, providing valuable insights for drug optimization and design.

Further theoretical and experimental advancements were achieved in a study focused on the design, synthesis, and characterization of new peptide mimetics and conjugates of the tetrapeptide AVPI. This research combined molecular modeling with in vitro biological evaluations of AVPI, its mimetics, and AVPI-conjugates. By integrating HYDE assays and molecular docking, critical insights were obtained regarding protein-ligand interactions within the binding sites of two key biological targets: IAP1-BIR3 and XIAP-BIR3. Notably, AVPI and its analog AVHypI demonstrated similar binding interactions in both proteins. The in vitro evaluations included cytotoxicity assays (MTT) and parallel artificial membrane permeability assays (PAMPA). The study revealed that modifying AVPI—by replacing Pro_3 with Hyp_3 and extending the C-terminus with RGD analogs—significantly enhanced the antiproliferative effects of AVPI-conjugates across all tested cancer cell lines (MDA-MB-231, MCF-7, HepG2, and HT-29) compared to the parent AVPI peptide. The most active conjugate, AVHyp-AgbGD, exhibited IC_{50} values of 348 μM (MDA-MB-231), 457 μM (MCF-7), 399 μM (HepG2), and 578 μM (HT-29). Additionally, PAMPA results indicated that the substitution of Pro with Hyp improved blood-brain barrier permeability in the AVHypI peptide, positioning it as a promising candidate for further therapeutic development.

Applicant's participation in doctoral guidance and work with students:

Nikolay Tzvetkov is the supervisor for three full-time doctoral students, demonstrating his commitment to mentoring the next generation of researchers.

At the Center for Pharmacy, Rhenish Friedrich-Wilhelm University of Bonn, Germany, he contributed significantly to the training of pharmacy students pursuing their Master's degree. Between 2008 and 2013, he conducted practical classes and examinations in the following disciplines: General and Analytical Chemistry of Inorganic Medicinal, Auxiliary, and Harmful Substances, Instrumental Analysis. These courses were delivered to first- and fourth-semester students in the full-time pharmacy program. Additionally, from 2014 to 2017, he continued his involvement in the training of pharmacy students at the same university, further supporting their progression toward the Master's degree.

Critical remarks

I have no critical remarks to make to the material presented. I personally know Assoc. Professor Tzvetkov and in my opinion he has shown that he possesses both professional and personal qualities corresponding to the scientific degree „Professor". I am convinced that he will continue to develop and deepen its research activity at a high level as he has done before.

Conclusion

The documents and materials submitted by Nikolay Tzvetkov fully comply with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria

(ZRASRB), as well as the Regulations for the Implementation of ZRASRB at the Institute of Molecular Biology „Acad. Rumen Tsanev“BAS. All scientific works presented in this competition were published following his appointment to the academic position of Assoc. Professor, ensuring their relevance to his current candidacy. Nikolay Tzvetkov’s scientific contributions include original and applied research that has garnered international recognition, underscoring his expertise and impact in the field. Beyond his scientific achievements, he possesses valuable administrative and organizational experience, currently serving as the Head of the „Molecular Design and Biochemical Pharmacology“ Section at the Institute of Molecular Biology „Acad. Rumen Tsanev“BAS. Additionally, he has successfully led multiple scientific projects, further demonstrating his leadership capabilities. Based on the comprehensive evaluation of his qualifications, scientific contributions, and administrative experience, I am fully confident in providing a positive assessment of Nikolay Tzvetkov’s candidacy. I strongly recommend that the respected members of the Scientific Jury cast a favorable vote in support of his appointment to the academic position of Professor in Professional Direction 4.2 „Chemical Sciences“, scientific specialty „Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances“.

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