

## OPINION

by Assoc. Prof. Emilia Borislavova Petrova, PhD, Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences, member of Scientific jury appointed by Order № HO-05-05-11/21.10.2025 of the Director of IEMPAM

Regarding: Competition for the academic position Professor in scientific specialty Biochemistry, Professional field 4.3. Biological Sciences, Field of higher education 4. Natural sciences, mathematics and informatics, in the Department of Pathology, IEMPAM, announced in the State Gazette no. 81 of 03.10.2025

In the current competition for the academic position Professor documents have been submitted by one candidate – Ivan Angelov Iliev, PhD, Associate Professor at the Department of Pathology of the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM). The applied materials for participation in the competition are in accordance with the requirements of The Law on Academic Staff Development in the Republic of Bulgaria, the Regulations for its implementation and the internal regulations of IEMPAM. The required documentation has been presented in full.

Ivan Iliev graduated in 2005 from the Faculty of Biology of Sofia University “St. Kliment Ohridski”, where he obtained a Master’s degree and a professional qualification as Molecular biologist – Master in Biochemistry. He started his research activity at the Institute of Molecular Biology at the Bulgarian Academy of Sciences, where he worked from 2003 to 2004.

At the beginning of his academic career, he held the positions of Research Associate III and Research Associate II (2006-2010) at the Institute of Experimental Pathology and Parasitology – BAS. From 2008 to 2012, he was a part-time doctoral student at IEMPAM, where in 2012 he obtained a PhD in Immunology (*Indicator A*). In the period 2012-2018 he held the position of Assistant Professor at the Department of Pathology of IEMPAM, and in 2018 he habilitated as an Associate Professor in Biochemistry. Ivan Iliev has more than 20 years of professional experience.

The results of the overall research activity of Assoc. Prof. Ivan Iliev are presented in a total of 119 publications with a total impact factor (IF) of 186.541 and an individual IF of 29.651. The publications have been cited 415 times (*h*-index 13). The candidate’s publications reveal his excellent methodological training and skills to apply a wide range of modern techniques (cytological, biochemical, histological, microscopic, etc.).

In the current competition, Assoc. Prof. Iliev presented 33 scientific publications (10 under *Indicator B* and 23 under *Indicator I*) in Web of Science and Scopus refereed journals, published after his habilitation as an Associate Professor. Of these, 26 are with impact factor (total IF: 97.547) and 7 are with SJR (SJR: 1.650). Ranked by quartiles, the publications are distributed as follows: 15 articles in Q1, 7 in Q2, 8 in Q3, and 3 in Q4. One registered utility model is also attached. The list of citations (*Indicator D*) includes 257 citations (Web of Science and Scopus) of 38 publications, which shows the wide visibility of the candidate’s scientific results. The publications and citations in this competition do not repeat those submitted for the PhD degree and for the academic position of Associate Professor.

The candidate’s scientific interests and research cover relevant issues in the field of biochemistry, biophysics, and cell biology. They are focused on determining the biological activity

of newly synthesized substances and natural products, as well as the combined effects of already well-studied compounds in model biological systems (different types of tumor and normal cell lines and experimental animals). In the course of the work, a cell collection of 12 normal and 19 tumor cell lines was created, which were successfully used for *in vitro* experiments. Two new cell lines were also created and characterized.

For the first time in Bulgaria, the *in vitro* method for testing the safety of new chemical substances, BALB 3T3 Neutral Red Uptake test, has been introduced and adapted to the LED solar simulator HeliosiO. It allows for quick and high-quality testing of the phototoxicity of synthetic and natural substances with potential antitumor activity. Given the significant number of newly synthesized compounds worldwide, testing them using the phototoxicity determination method will contribute to protecting health and improving quality of life.

The scientific contributions of Assoc. Prof. Iliev, based on the works submitted for evaluation, cover several thematic areas that correspond to the subject of the announced competition: **1)** Biological activity of various peptide analogues; **2)** Biological activity of newly synthesized substances; **3)** Biological activity of natural products; **4)** Biological research through differential scanning calorimetry; **5)** Creation and testing of new biocompatible coatings.

The research contributions from the **first** thematic area reveal structural-functional relationships in several peptide analogues:

1. Seventeen peptide analogues of BIM-23052 (a synthetic analogue of somatostatin) with various chemical modifications (introduction of fluorinated Phe; replacement of Phe with Tyr) were synthesized, leading to higher stability of the molecule in a biological environment, reduced toxicity, and increased antiproliferative activity against MDA-MB-231 and HepG2 tumor cells.

2. The biological activity of 18 peptide analogues of (KLAKLAK)<sub>2</sub> – a synthetic peptide with antimicrobial and antitumor activity – was investigated. It has been proven that the introduction of 1,8-naphthalimide-Gly- and Caf- increases the cytotoxicity and antiproliferative activity of the peptides, but not their selectivity. It has been established that the amino acid  $\beta$ -Ala in the structure of these analogues is responsible for their high biological activity and selectivity. Data have been obtained on the crucial role of peptide chain length in antiproliferative and antimicrobial activity. A significant increase of the antiproliferative properties has been demonstrated upon introduction of the unusual amino acid nor-Leu at the place of Leu in the primary structure of the analogues in combination with a second pharmacophore (1,8-naphthalimide) in the hybrid structure, which is due to a synergistic effect between the two parts of the hybrid molecule. This makes the compound a very good candidate for potential application in medicine in the treatment of type A adenocarcinoma of the mammary gland.

3. Analgesic activity has been established for 7 FELL peptide analogues when D-Phe occupies the first position in the molecule, in combination with the two Leu residues at the third and fourth positions. The analgesic effect increases with the lengthening of the peptide chain by adding one more hydrophobic residue. The complete hydrolytic stability of FELL analogues allows their safe and effective use in pharmacy.

4. The replacement of lysine in positions 7 and 8 in 7 peptide analogues of Aurein 1.2 (a peptide with antimicrobial and anticancer properties) leads to improved antitumor activity. Some of the analogues have been found to have reduced toxicity and a complete lack of phototoxic effect.

5. It has been established that among the four tested peptide analogues of Temporin A (antimicrobial peptide), the most promising analogue is the one in which the Ser residue at position 10 is replaced with a more hydrophobic OH-containing Tyr residue. The compound has very good

antiproliferative activity against luminal type A breast cancer, low cyto- and phototoxicity, and is hydrolytically stable.

6. The antiproliferative activity of two new aroyl hydrazones used in combination with AVPI- and RGD-peptides in triple-negative, basal-type breast cancer (MDA-MB-231) has been established.

The **second** thematic area highlights contributions related to the synthesis and characterization of new pyrrole-based carbohydrazides (1) and hydrazones (1A–D). The compounds show low cytotoxicity, no phototoxic effect, high selectivity index, and antiproliferative activity in an in vitro melanoma model.

Among 15 newly synthesized derivatives of 3,5-Diaryl-3,4-dihydro-2H-pyrrole-2-carboxylic Acid, the highest selectivity was found for the compound *rel*-(2R,3S)-5(4-methylphenyl)-3-phenyl-3,4-dihydro-2H-pyrrole-2-carbonitrile in lung alveolar adenocarcinoma cells.

The **third** thematic area focuses on the biological activity of a wide range of natural products:

1. It has been established that miosmine (a tobacco alkaloid) causes a significant dose-dependent decrease in the viability and proliferative activity of tumor cell lines (erythroleukemia and hepatocellular carcinoma). Vitamin C, combined with miosmine, exhibits modulating activity on the cytotoxic effect. The results contribute to the assessment of potential health risks associated with dietary intake of miosmine.

2. The anti-tumor effect of extracts from the flowers of *Tanacetum vulgare L.* has been established in a breast cancer cell line.

3. High selectivity of bitter apricot kernel extract in hepatocellular carcinoma (HepG2) and high antiproliferative effect have been established.

4. The antioxidant and antitumor potential of the polyphenolic fraction from grape pomace obtained from the vinification of the local Bulgarian grape variety Mavrud has been established. The MCF-7 cell line is significantly more sensitive to the studied extracts compared to MDA-MB-231 cells. A correlation has been established between antioxidant activity and the total polyphenol content in the extract.

5. The *in vitro* antiviral activity of essential oil and floral water from *Rosa damascena Mill* against herpes simplex type 1 on rabbit retinal cells has been established.

6. The combined effect of European mistletoe extract (*IsCADOR Qu*) and 11 standard chemotherapeutic agents on two breast cancer cell lines has been established, which is promising in the development of therapeutic protocols for breast cancer compared to traditional monotherapies.

7. Data have been obtained showing that encapsulation of essential oil from *Origanum vulgare ssp. hirtum* in mycelium and gel reduces the oil's cytotoxicity but preserves its therapeutic potential in normal and tumor cells of the skin.

8. The antiproliferative activity of mono- and di-ramnolipids (RL-1 and RL-2) has been established in breast cancer (MCF-7 and MDA-MB-231). When combined with cisplatin, a synergistic effect is observed only in the triple-negative cell line MDA-MB-231.

9. An antiproliferative and antitumor effect of 9 hemocyanins has been established under in vivo conditions (hamsters transplanted with Graffi tumors) and tumor cell lines (breast cancer, bladder cancer, colorectal carcinoma). It has been proven that the antitumor activity of the studied hemocyanins is due to both immune stimulation and direct effects on tumor cells.

The studies in the **fourth** thematic area are based on the differential scanning calorimetry (DSC) method. The specific calorimetric characteristics of the proteome in blood plasma during

immune response stimulation in an experimental *in vivo* model of treatment with highly immunogenic egg albumin were studied. A common feature of the thermograms of treated male and female animals is the expansion of the transitions above 75 °C and the appearance of an exothermic transition above 90 °C.

Based on the plasma thermograms of patients suffering from early pregnancy loss, convincing data were obtained that the DSC approach has the potential to distinguish pathological changes.

It has been established by differential scanning calorimetry that highly metastatic MDA-MB-231 cells are much more susceptible to the anticancer drugs cisplatin and miltefosine than low-metastatic MCF-7 cells.

A contribution in the **fifth** thematic area is the creation of new biocompatible coatings for regulating cell adhesion with the aim of successful implantation and proper functioning of temporary and permanent medical devices.

In addition to his research activity, Assoc. Prof. Ivan Iliev is also actively involved in educational activities aimed at training young specialists in various fields of biochemistry. He is the supervisor of one successfully graduated PhD student in Biochemistry (*Indicator E*). In the period 2014-2022, he is the scientific supervisor of five and consultant to two graduates from Sofia University “St. Kliment Ohridski” and the University of Chemical Technology and Metallurgy - Sofia. All of them have successfully defended their theses.

Assoc. Prof. Iliev is a lecturer in the specialized course “Animal Cell Culture” at the Training Center of the Bulgarian Academy of Sciences with a workload of 78 academic hours. He has conducted exercises with students, PhD students, and young scientists on a project of IEMPAM under The Science and Education for Smart Growth Operational Programme. He has presented eight lectures in English to students and PhD students at Pamukkale University, Department of Medical Biology, Turkey.

The candidate’s project activity is a significant element of his research work and is focused on current issues in the field of biomedicine, with priority given to the prevention and treatment of oncological diseases. Assoc. Prof. Iliev has participated in the implementation of 19 projects, 3 of which are ongoing. The projects are funded by the National Scientific Program BioActiveMed, the National Recovery and Sustainability Plan, The Bulgarian National Science Fund, including the Bilateral Cooperation Program, as well as European programs (COST, Operational Programme Human Resources Development, and The Science and Education for Smart Growth Operational Programme). In the current competition under *Indicator E*, the candidate has included his participation in 13 of the above-mentioned projects (12 national and 1 international).

Assoc. Prof. Iliev actively participates in scientific events in Bulgaria and abroad – a total of 53 forums since his habilitation as an Associate Professor, at which he has presented 87 reports and posters.

The materials submitted for the competition undoubtedly demonstrate the candidate’s high scientometric performance, exceeding the required minimum in all indicators. His total score is 1450 out of the required 640.

## Conclusion

Based on the materials submitted for the competition, I consider that Assoc. Prof. Ivan Iliev fully meets the requirements of The Law on Academic Staff Development in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of IEMPAM for holding the academic position of Professor. He fulfils and significantly exceeds the required scientific metrics according to the relevant regulatory documents.

Assoc. Prof. Iliev is an established scientist with proven high research qualities and significant publications corresponding to the profile of the announced competition. The results of his scientific research have substantial fundamental and applied scientific value in the field of human medicine.

Considering the above, I give my **positive assessment** with full conviction and recommend the honorable Scientific Jury to propose **Assoc. Prof. Ivan Angelov Iliev** for election to the academic position Professor in the scientific specialty Biochemistry, Professional field 4.3. Biological Sciences, Field of higher education 4. Natural Sciences, Mathematics and Informatics.



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