REVIEW

in the competition for the academic position "professor" in the field of higher education 4. Natural sciences, professional field 4.3. Biological sciences, scientific specialty "Biochemistry", code 01.06.10, announced in the State Gazette, issue No.81/03.10.2025, for the needs of the "Pathology" section, IEMPAM - BAS

by Prof. DSc Ludmil Penuv Kirazov

The only candidate for the announced competition is Assoc. Prof. Ivan Angelov Iliev, PhD, from IEMPAM - BAS. As of the date of issuance of the certificate, Assoc. Prof. Iliev has 19 years and 7 months of work experience in the specialty at IEMPAM. He has submitted all the necessary documents in accordance with the legal requirements.

Brief biographical data about the candidate. Assoc. Prof. Iliev was born in 1976. He graduated with a bachelor's and master's degree in 2003 and 2005 at the Faculty of Biology of Sofia University "St. Kliment Ohridski" with a degree in "Molecular Biology". From 2003 to 2004 he worked at the Institute of Molecular Biology - BAS as a "Biologist - Molecular Biologist". He started working at IEMPAM in 2006. At the beginning of 2012 he defended his dissertation on the topic "Study of immunomodulatory properties of hemocyanins isolated from *Helix lucorum* and *Rapana venosa* in experimental immunotherapy of Graffi's myeloid tumor, Guerin's ascites tumor and trichinellosis". The same year he became a Chief Assistant Professor in the specialty "Immunology", and from 2018 to the present he is an Associate Professor in "Biochemistry".

Presented scientific production and scientometric data. Assoc. Prof. Iliev fulfills and significantly exceeds the minimum requirements for holding the academic position of "professor", which is briefly presented in the following table.

Indicator	A	B	Г	Д	E
Required points	50	100	220	120	150
Submitted points	50	215	481	514	190

The candidate declares that there is no data included that repeats the data submitted for the acquisition of the educational and scientific degree "doctor" and the academic position of "associate professor". He participated in the competition with 10 publications as the equivalent of a habilitation thesis and 23 publications in refereed and indexed publications in Web of Science and Scopus. The 33 presented publications have a total IF of 97.547. Of these, 15 are in Q1, 7 in Q2, 8 in Q3 and 3 in Q4, which emphasizes their high scientific quality. The citations that were not included in previous procedures are 257. The results have

been presented at 97 scientific forums with reports and posters. Assoc. Prof. Iliev has active project activities and has participated in 13 completed scientific projects, participated in 3 others for different periods of time and participates in 3 current projects. He has a registered utility model. He is the scientific supervisor of 1 successfully defended doctoral student and 7 successfully defended graduate students.

Analysis of scientific contributions. The main directions in the work of Assoc. Prof. Iliev and his contributions are in the following directions: 1. Research on the biological activity of different types of peptide analogues and the combination between peptides; 2. Research on the biological activity of newly synthesized substances; 3. Research on the biological activity of natural products; 4. Biological studies by differential scanning calorimetry; 5. Testing of new biocompatible coatings.

Contributions in direction 1. Research on the biological activity of different types of peptide analogues and the combination between peptides

The first studied group are analogues of the BIM-23052 peptide, known as an agonist of the somatostatin receptor sst5. By modulating its activity, the proliferation of tumor cells expressing this receptor can be affected. In a study, analogs of BIM-23052 containing halogenated Phe residues were tested. Their cytotoxic effects were tested in vitro against two human tumor cell lines - a breast cancer cell line and a hepatocellular cancer cell line, and on a human non-tumorigenic epithelial cell line. The tested peptides have high antitumor activity against the HepG2 cell line compared to breast cells. The target compounds show high hydrolytic stability under conditions mimicking physiological ones (1B - publication 1 of group indicators B). Biological evaluation of three new bioconjugates of monofluorinated analogs of BIM-23052 was performed. An analog with the highest selectivity against luminal type A breast cancer was demonstrated, but this bioconjugate is unstable under physiological conditions (5B). Analogues of BIM-23052 were studied, in which Phe residues were replaced by Tyr. The resulting analogues did not have a higher antiproliferative activity against tumor cells, but an analogue was obtained that was more lipophilic and could more easily pass the cell membrane (6B).

The next group studied were $(KLAKLAK)_2$-NH_2 peptide analogues. This proapoptotic peptide is an alpha-helical amphipathic peptide, toxic to eukaryotic cells. It disrupts mitochondrial membranes and causes programmed cell death. It has antimicrobial and antitumor activity. The biological activity of 18 of its analogues, divided into three groups, was studied. The antibacterial properties of newly synthesized analogues were tested against Gram-negative microorganisms *Escherichia coli* K12. The results show that the introduction

of 1,8-naphthalimide-Gly- and Caf- increases the cytotoxicity and antiproliferative activity of the peptides, but not their selectivity (3B). In studies with a second group of analogues, it was shown that the length of the peptide chain plays an important role in their antiproliferative and antimicrobial activity. For the conjugates NphtG-$(KLAKLAK)_2$-NH_2 and Caf-$(KLAKLAK)_2$-NH_2, a good selectivity index against the tumor cell line MCF-7 was established, combined with cytotoxicity and antiproliferative properties. They showed good antifungal properties and complete hydrolytic stability for 72 hours (4B). In the third studied group of analogues of $(KLAKLAK)_2$ Leu was replaced with the unnatural amino acid nor-Leu and a second pharmacophore with proven anticancer properties was introduced into the peptide part. The analyses showed that the introduction of nor-Leu did not lead to an increase in antiproliferative activity, but the combination with the second pharmacophore (1,8-naphthalimide) in a hybrid structure led to a significant increase of antiproliferative properties. The peptide and the second pharmacophore have synergistic effect. Complete hydrolytic stability was observed for 72 hours in model systems. The compound is a good candidate for medical application in the treatment of breast adenocarcinoma type A (7B).

The cytotoxicity of FELL peptide analogues was studied. The FELL peptide has proven anti-inflammatory and opioid properties and is an interesting candidate for medical applications due to its easy synthesis. Determination of the analgesic activity of 7 FELL peptide analogues showed that D-Phe at the first position, combined with the two Leu residues, is the best combination. Lengthening the peptide chain with another hydrophobic residue has a positive effect on analgesia. The cytotoxicity of the final molecules is significantly lower than that of SLS from the positive control. They are also hydrolytically stable, which suggests their successful use in pharmacy (8B).

Aurein 1.2 is one of the most potent antimicrobial peptides with a short sequence (13AK) and shows moderate anticancer activity at concentrations that can kill bacterial and cancer cells without damaging healthy cells. 7 peptide analogues of Aurein 1.2 were synthesized and tested for antiproliferative effects and antibacterial activity. Due to their structure, these molecules may have increased photosensitivity, therefore a photosafety test was performed and a complete lack of phototoxic effect was shown (9B).

The biological activity of Temporin A peptide analogues was investigated. This is a highly hydrophobic antimicrobial peptide amide exhibiting activity especially against antibiotic-resistant Gram-positive cocci bacteria. Four peptide analogues, respectively modified at four different positions, were investigated and their antiproliferative activity was

established. It was shown that the most promising peptide for clinical application has low cyto- and phototoxicity and is hydrolytically stable in the tested model pH systems (10B).

The biological activity of a combination between aroyl-hydrazones and AVPI and RGD peptides was investigated. Aroyl-hydrazones have been intensively studied due to their anticancer, antibacterial and antimicrobial effects. They are known for their apoptotic potential and are interesting objects of pharmacological design. In the presented work, the antiproliferative effect of two new aroyl-hydrazones used in combination with AVPI and RGD peptides was investigated. Although aroyl-hydrazone derivatives used alone show high activity, the combination is not useful (2B).

Contributions in direction 2. Study of the biological activity of newly synthesized substances

17 aroyl-hydrazones derived from nicotinic acid hydrazide and isonicotinic acid hydrazide were studied. It was shown that the biological activity of the obtained compounds depends on the substituents in the salicylic part of the molecule. The results allow to assess the structure-activity relationship of the compounds and give a perspective for further development of this group as more potent and selective antineoplastic agents (1Г). New pyrrole-based carbohydrazides and hydrazones were characterized. The biological activity of the newly synthesized compounds was studied in vitro on tumor and non-tumor cell lines. They have low cytotoxicity and are not phototoxic. The cytotoxic effect of the compound with the highest antiproliferative activity correlates with its ability to induce apoptosis (18Г). In continuation of these studies, a series of compounds was created in which the halogen atom was replaced by a methyl group in the precursor pyrrole based carbohydrazide 2. The compounds have minimal cytotoxicity and do not have phototoxic effects (23Г). The antiproliferative activity of 15 derivatives of 3,5-diaryl-3,4-dihydro-2H-pyrrole-2-carboxylic acid was evaluated. The metabolic cycle of L-proline plays a crucial role in the survival and proliferation of cancer cells. In vitro screening of the synthesized compounds against human cancer cell lines shows that some of them exhibit good or high selectivity index and are a good basis for expanding the research (21Г).

Contributions in direction 3. Research on the biological activity of natural products

The cytotoxic effect of myosmine, which is a tobacco alkaloid found in food products and is a potential risk factor for the development of esophageal adenocarcinoma, was studied in vitro. The results can be used to assess potential risks and optimize diets for healthier nutrition (3Г). An in vitro study of the antitumor activity of extracts from the flowers of *Tanacetum vulgare* L. was conducted. 6 main compounds were obtained, which were tested on 9 cell

lines. Their selectivity and antitumor activity were established (4Г). In tests based on yeast and cell lines for potential antimutagenic, antirecombinogenic and antitumor effect, an extract of bitter apricot kernels was studied. It has been identified 1000 compounds, as well as 4 cyanogenic glycosides. Data on a strong effect of the extract were obtained (7Г). In a subsequent study, the antioxidant and antitumor potential of the polyphenolic fraction of grape marc obtained during the vinification of the Bulgarian grape variety Mavrud was reported. The studied extracts have a low cytotoxic effect, but inhibit cell proliferation in normal cells as well (13Г). Studies have been conducted on the anti-herpes simplex virus type 1 activity of essential oil and floral water from *Rosa damascena* Mill in retinal infection of rabbit cells in vitro and in silico. The two products have no significant effect on virus replication, but reduce the viral titer. When treating healthy cells with these products, they significantly protect them from subsequent infection with HSV-1 (22Г). The combined effect of European mistletoe extract (Iscador Q) and 11 standard chemotherapeutic agents on breast cancer cell lines was studied in vitro and promising therapeutic protocols were proposed compared to traditional monotherapies (16Г). An original nanocomposite hydroxypropyl-cellulose hydrogel containing polymer micelles loaded with essential oil of *Origanum vulgare ssp. hirtum* was studied, which has high therapeutic potential in the treatment of melanoma. A reduction in the cytotoxicity of the oil was achieved while maintaining its therapeutic potential (19Г). As an alternative to conventional anticancer drugs, mono- and diramnolipids and the combination with cisplatin were investigated. A possible mechanism for membrane remodeling through endosomal formation is proposed (8Г). The toxicity, antiproliferative activity and antitumor effect of hemocyanins from *Helix lucorum*, *Helix aspersa* and *Rapana venosa* were evaluated under in vitro and in vivo conditions. In vivo studies were conducted in hamsters transplanted with Graffi tumor (15Г). The results show that hemocyanins have antitumor activity against bladder cancer (6Г), colorectal carcinoma (5Г), breast carcinoma (10Г) and in vivo studies and show potential for the development of anticancer therapeutics.

Contributions in direction 4. Biological studies by differential scanning calorimetry

Differential scanning calorimetry was applied in albino Wistar rats to determine the specific calorimetric characteristics of the blood plasma proteome associated with stimulation of the immune response. The results contribute to a better understanding of the correlation between the calorimetric characteristics of blood plasma and the immunological status in experimental animals (2Г). The same method was used to analyze and compare the effects of two anticancer drugs, differing in their mode of action, miltefosine and cisplatin, on two breast cancer cell lines and one normal breast epithelial cell line. Different effects of

miltefosine and cisplatin on the thermodynamic behavior and viability of cancer and normal cells were demonstrated (9Г). Again, this method was used for the first time to study the denaturation profile of blood plasma from women suffering from early pregnancy loss, compared to healthy pregnant and non-pregnant women. The results show the potential of the method for the diagnosis of pathological changes (12Г).

Contributions in direction 5. Testing of new biocompatible coatings

In a conducted study, it was proven that the inclusion of one or more layers of graphene oxide in a multilayer coating of hyaluronic acid/chitosan is a way to regulate the degree of non-specific adhesion and growth of different cells. This may contribute to a new approach in adapting medical devices to cells and tissues (17Г, 14Г).

In addition, Assoc. Prof. Iliev participated in the creation of a specific and precise small-scale sample preparation method suitable for simultaneous HPLC-UV study of capecitabine and its 5'-dfcr metabolite in mouse blood plasma (11Г), as well as in the construction of a multidisciplinary educational experiment that can be applied in a laboratory practicum for students (20Г).

Conclusion: Assoc. Prof. Ivan Angelov Iliev is a scientist with high professional qualifications and a distinct research profile in the field of biochemistry. His work is valued in our country and abroad, as evidenced by the high citation rate of the submitted publications. The candidate's good relationship with other research groups is impressive. His scientometric indicators meet and in most criteria significantly exceed the requirements of the competition. All this gives me reason to give my positive assessment and to recommend to the esteemed Scientific Jury to submit a proposal to the Scientific Council of IEMPAM-BAS to vote for the election of Assoc. Prof. Iliev to the academic position of "Professor" in the scientific specialty "Biochemistry", code 01.06.10.

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