REVIEW

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Institute of organic chemistry with Centre of Phytochemistry - BAS

*by competition: for holding the academic position "**Professor**" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, by scientific specialty: "**Principles and methods of cybernetics in different fields of science (in silico study of bioactive compounds)", to the section "QSAR and molecular modeling" at the Institute of Biophysics and Biomedical Engineering - BAS, announced in State Newspaper No. 18 / 28.02.2020.***

1. General presentation of the materials received for review

The terms and conditions of the procedure for acquiring the academic position "Professor" are observed and complied with the regulations. The only candidate is Associate Professor Dr. Ivanka Tsakovska in the section "QSAR and Molecular Modeling", IBPBME-BAS.

*From the submitted documents and references it is evident that in the announced competition associate professor Ivanka Tsakovska participates with a scientific asset **that fully meets the requirements and meets the criteria for holding the academic position "professor" according to LDASRB and the Rules of IBPBME-BAS.***

Indicator	*Minimal national requirements*	*Minimal requirements of BAS*	*Points declared by the candidate*
A	*50*	*50*	*50*
C	*100*	*100*	*225*
G	*200*	*220*	*252*
D	*100*	*120*	*922*
E	*150*	*150*	*431*
Additional requirements of IBPBME-BAS		*15 publications in the Journals with IF*	*17 publications in the Journals with IF; h index=12 (Scopus)*

2. Biographical and professional data about the candidate

Associate Professor Ivanka Tsakovska was born on July 6, 1972. in the town of Radomir, Bulgaria. She graduated from NNMG "Acad. Chakalov" profile "Chemistry" in 1990, after which she graduated as a master "chemical engineer" at the University of Chemical Technology and Metallurgy (UCTM) - Sofia in 1995, specialty "Chemical technologies and materials for microelectronics and electronic elements". In 2004 received the educational and scientific degree "PhD" after successfully defending a dissertation on "Quantitative dependences structure-activity of selected classes of biologically active compounds" under the guidance of then Associate Professor Ilza Pazheva. Since 1996 she has been working at IBPBME-BAS successively as a specialist, full-time doctoral student, assistant, chief assistant and associate professor.

Assoc. Prof. Ivanka Tsakovska is the winner of the awards: "Evrika" for a young scientist, 2004 and the award for young scientists "Prof. Marin Drinov", 2003, awarded by BAS, as well as for the best completed youth project at the National Science Fund, which prove the high appreciation of her scientific work from the very beginning as a young scientist. The research interests of Assoc. Prof. Ivanka Tsakovska are mainly in the field of quantitative structure-activity relationships of biologically active compounds, drug design, molecular modeling of ligand-protein interactions, computational toxicology. Assoc. Prof. Ivanka Tsakovsaka has been continuously improving her qualification throughout the period of her academic development. In 2002-2003. is a fellow of the Alexander von Humboldt Foundation, Germany, where she

conducts research in the field of structure-activity relationships of pharmacological testing of multiple drug resistance modulators in tumors. In 2005-2007 she participated in the work of the Research Center of the European Commission in Italy, first as a post-doctoral student (2005-2006), and then (2006-2007) Assoc. Prof. Tsakovska is a supervisor of a graduate and a lecturer in the master's program. Since 2008 was elected associate professor at the same section of IBPBME-BAS.

Staff training. Supervisor of 2 graduates and co-supervisor of 2 doctoral students, lecturer in a master's course at Sofia University (2018-2019).

Management and participation in research projects. Assoc. Prof. Ivanka Tsakovska has been a participant and coordinator in a number of research projects (total 11), incl. under COST action, 7th EU Framework Program (Health Program).

Expert activity. Her professional experience and competence in this field have become the basis for her inclusion in a number of expert commissions and councils. She has been a member of the Expert Council at the Ministry of Environment and Water since 2015 and a member of the General Assembly of BAS since 2016, a member and Chairman of the Supervisory Board of IPFBME-BAS since 2019. Expert participation in the work of TC NES (European Technical Committee for New and Existing Substances) 2016. Reviewer in over 20 scientific journals and research projects at the EU

***3. Assessment of the candidate's research activity**. The main research interests of Assoc. Prof. Ivanka Tsakovska are focused on the application of in silico (computer-assisted) approaches for the purposes of targeted drug design and risk assessment of biologically active compounds on human health and the environment (computational toxicology). For that reason, I think that the research activity of Assoc. Prof. Ivanka Tsakovska was carried out purposefully on scientific topics in an extremely important and topical field.*

Her work at the JRC, Italy, was focused on implementing in silico approaches in the field of so-called regulatory computational toxicology, the main purpose of which is to support the risk assessment of chemicals under European legislative directives such as REACH (EC 1907/2006, Registration, Evaluation, Authorization and Restriction of Chemicals) and the Regulation of the European Parliament and of the Council on cosmetic products (Regulation (EC) No 1223/2009). This allowed Assoc. Prof. Tsakovska to initiate the expansion of the section's activities in this field.

The main achievements in this direction include modeling the toxic effects of compounds that are important for the European regulatory authorities related to the control of chemicals, as well as the development of biological pathways leading to toxic effects. The main activities in this field are within the COSMOS project (http://www.cosmostox.eu) as part of the Research Initiative under the 7th Framework Program of the European Commission "SEURAT-1" (Safety Evaluation Ultimately Replacing Animal Testing), in which IBPBME-BAS is a partner, and her participation is in her capacity as a team leader from IBPBME-BAS. This research initiative is a major step in the European Research Area and regulators.

For the realization of her research work Assoc. Prof. Ivanka Tsakovska has accumulated a lot of theoretical knowledge, skills and competencies in the field of chemistry, computer-assisted approaches for the purposes of targeted drug design and computational toxicology.

Assessment of compliance with the minimum national scientometric indicators for the academic position "Professor".

*The scientific production of Assoc. Prof. Dr. Ivanka Tsakovska is presented according to the requirements of IBPBME-BAS. Apply in the competition for "Professor" with a total of **24 scientific works** of which: with impact factor: **17** (Total impact factor - **65.65**), distributed by quarters according to SJR as follows: **Q1 – 14 pcs., Q2 – 2 pcs., Q4 – 1 issue**; Without impact factor - 4 ; Chapters from collective monographs - 3 pcs. First author and / or author for correspondence - in 5 publications.*

According to indicators group A *Assoc. Prof. Ivanka Tsakovska presents a dissertation for awarding educational and scientific degree "**PhD**" on the topic: "**Quantitative dependences structure-activity of selected classes of biologically active compounds",** successfully defended on 23.10.2003**.***

By indicators group C. Monographic work or equivalent publications:

*Assoc. Prof. Ivanka Tsakovska participated in the competition with an equivalent number of articles for habilitation work **9 pcs.** (presented in a separate list) in editions that are referenced and indexed in Web of Science and Scopus, all with impact factor and **all are from the highest quertel Q1**, which bring it **225 points**, exceeding the required 100points. **This gives me reason to accept that the presented articles completely replace habilitation work.***

*Publications, chapters from collective monographs and utility models Assoc. Prof. Dr. Ivanka Tsakovska presents by **group of indicators G: 12 scientific publications** that are referenced and indexed in Web of Science and Scopus, with impact factor: **Q1 - 5 pcs., Q2 - 2 pcs. and Q4 - 1 issue**, **4** in Journals without impact factor and **3** chapters in collective monographs, which bring her **252 points.***

Citation in national and foreign literature. *Assoc. Prof. Ivanka Tsakovska provides evidence for a total of 461 citations (excluding self-citations) of papers in scientific journals, reflected in the Web of Science and Scopus databases for the period 2004-2019, which bring her 922 points for group of **indicators D.***

Participation in national scientific or educational projects. *Assoc. Prof. Ivanka Tsakovska has participated in a total of **8** projects funded by national and international sources, and by EU Operational Programs, which bring her **100** points. She is the head of **3** contracts, which brings her **90** points; For attracted funds for projects managed by the applicant receives another **216** points, together with a co-supervisor of PhD student (**25** points), or a total of **431** points for group of **indicators E.***

From the presented reference Sample for the criteria for the academic position "Professor" and the presented evidence it is evident that Assoc. Prof. Ivanka Tsakovska in number of points significantly exceeds those studied by IBPBME-BAS.

4. Scientific contributions related to habilitation work (publications in indicator C4)

The habilitation work includes 9 publications thematically combining studies on modeling the interactions between small biologically active compounds and proteins, including transport proteins (β-glycoprotein, P-gp) and nuclear receptors (estrogen receptor alpha, ERα and peroxisome proliferator). activated gamma receptor, PPARγ). ***They all have a high impact factor and Q1.*** *The results are applicable in the field of targeted drug design (in particular - overcoming tumors, as well as in the assessment of toxic effects. Two publications, together with partners from the COST Action, (STRATAGEM COST Action, https: / /stratagem-cost.eu), in which Assoc. Prof. Tsakovska summarize new modern approaches to overcoming multiple drug resistance (Dallavalle et al., 2020; Dinić et al., 2020). The Bulgarian participants are the only ones among the author's team engaged in analyzing the potential of in silico approaches as a means of assessing the ability of antitumor drugs to interact with transport proteins and in particular with P-gp (Dallavalle et al., 2020). The publication focuses on approaches that use 3D structural information about proteins and their ligand complexes and are among the most effective in silico approaches in terms of the reliability of predicted interactions of transport proteins with their substrates / inhibitors. It is these approaches that are the basis of the research of Assoc. Prof. Tsakovska, presented further in the habilitation thesis.*

High appreciation should also be given to the work of Dinic et al, 2020, which presents a number of practical examples of applications of in silico approaches such as pharmacophore modeling and pharmacophore- and docking-based virtual screening, aimed at quickly and efficiently finding new applications. of existing drugs to overcome multiple drug resistance. In silico approaches have also proven their effectiveness as a tool in assessing the risk of endocrine-active substances. Assoc. Prof. Tsakovska presents a critical analysis of the molecular modeling approaches used in the study of these interactions in

relation to the manifested biological effects (Tsakovska et al, 2011).

The availability of information on potential steatogenic effects of complete PPARγ agonists provoked a number of studies by the candidate, which laid the foundation for solving two main tasks in this direction: 1. Prediction of toxicological pathways initiated by the interaction of full agonists - PPARγ and leading to hepatic steatosis; 2. Development of in silico structure-based models for prediction of the molecular initiating event. The obtained results and the used approaches are summarized in the work of Al Sharif et al., 2019. Within the COSMOS project and in fulfillment of these research goals a dissertation on the topic "Study of the ligand-dependent dysregulation of PPARγ: adverse outcome" was successfully defended. pathways development and molecular modeling "by PhD student Marilyn Al Sharif under the supervision of Assoc. Prof. Tsakovska and the supervision of Corresponding Member Ilza Pazheva.

Major scientific contributions

Molecular modeling of ligands and their interactions with P-gp. *This is the first study of ligand interactions with the newly obtained human P-gp structure, published in the specialized literature (Alam et al, 2019). The authors conclude that their site partially overlaps with the binding site of the P-gp substrate Rhodamine 123, which identifies the compounds as competitive inhibitors of this P-gp substrate. The result will support the further targeted design of a new class of dual HSP90 and P-gp inhibitors.*

Molecular modeling of Erα ligands. *High appreciation should be given to the developed extended pharmacophore model of ERα agonists, which has been validated using molecular dynamic simulations in normal and mutated form of the receptor. The identified new pharmacophore trait indicates that it may be useful in the selection of potentially strong ERα agonists, as well as in the precise discrimination of substances that do not bind to ERα. The analysis shows that combining the pharmacophore model with structural descriptors for shape and polarity is an effective means of distinguishing compounds with potential pro- and antiestrogenic activity..*

Development of toxicological pathways of chemicals with potential for hepatotoxic action and structure-based modeling of molecular initiating events in these pathways. Two tissue-specific pathways of hepatotoxic action (in the liver and in adipose tissue) have been described, with a molecularly initiating event ligand-dependent dysregulation of PPARγ by binding to full agonists and a final toxic effect - nonalcoholic steatotic liver disease.

In silico modeling of the molecular initiating event in hepatocytes

Development of a pharmacophore model. *A study was performed to develop an in silico model to predict the binding of complete PPARγ agonists whose interaction with the receptor may initiate hepatotoxicity (particularly nonalcoholic steatosis of the liver). The reliability of the modeling depends largely on the quality of the available experimental data. High appreciation should also be given to the purpose-built virtual library with structural and biological data on PPARγ agonists. This is the most complete and extensive set of PPARγ agonists with public access (http://biomed.bas.bg/qsarmm/). The library has been downloaded 239 times in the last year. Based on this and after analysis of all available PPARγ complexes in Protein Data Bank (PDB, https://www.rcsb.org), a pharmacophore model of complete PPARγ agonists was built, which was used to develop a virtual screening protocol. of compounds with full agonistic activity (Tsakovska et al., 2014).*

Development of 3D QSAR models for prediction of transactivation activity of PPARγ complete agonists. *A pharmacophore model of complete PPARγ agonists has been developed and based on it a protocol for virtual screening of compounds - complete PPARγ agonists has been developed and successfully applied. A 3D QSAR model based on molecular similarity indices was derived that predicted the transactivation activity of complete PPARγ agonists. The model is based on the largest and structurally diverse group of compounds, which is part of the developed and publicly available database with structural and biological data for PPARγ agonists (available at*

http://biomed.bas.bg/qsarmm/).

In silico modeling of the molecular initiating event in adipocytes. *The experimental methods used so far have not established the structure of PPARγ in antagonistic form, nor the way of binding the antagonists. In this regard, numerous molecular-dynamic simulations have been performed that give an idea of the structure and dynamics of the receptor, both when it is not bound to a ligand and when it is bound to an antagonist (Fratev et al., 2015). As a result of performed molecular-dynamic simulations, the dynamics of PPARγ in its antagonistic form was studied. It has been found that antagonists do not have a defined place in the receptor, but show different ways of binding with different probability of realization.*

5. Scientific contributions outside habilitation work (publications in indicators G7 and 8)

In silico studies of flavonolignans contained in the medicinal plant milk thistle (Silybum marianum).

Their potential pharmacological effects, ADMET properties (absorption, distribution, metabolism, excretion and toxicity), mechanisms of action and potential new pharmacological applications are predicted. These studies are the basis of a dissertation under development on "In vitro and in silico studies of ADME / Tox properties and molecular interactions of flavonolignans from Silybum Marianum l. (Milk Thistle)" by PhD student Antonia Dyukendzhieva, under the supervision of Corresponding Member Ilza Pazheva and Assoc. Prof. Ivanka Tsakovska.

In silico prediction of metabolism and toxicity. *In the work of Diukendjieva et al. 2017 predicted the possibility of three major toxic effects in mammals (chromosomal aberrations in vitro, modulation of ERa and skin sensitization) and two most likely metabolic transformations (glucuronidation and oxidative demethylation). Based on the identified potential estrogenic activity of the studied silibin, docking simulations were performed and it was confirmed that silibin A and B can occupy the active site in the ligand-binding domain of ERa by showing different stereospecific positions and, respectively, interactions with the receptor. For the studied flavonolignans, three main potential toxic effects in mammals (chromosomal aberrations in vitro, ERa modulation and skin sensitization) and two most probable metabolic transformations (glucuronidation and oxidative demethylation) were identified. It has been found that the stereoisomeric forms of silibin (A and B forms) can interact with ERa as the interaction at the binding site is realized in a stereospecific manner.*

Finding new potential target proteins and new pharmacological applications*. Two new potential target proteins for the studied flavonolignans - BRAF kinase and SMO - have been identified and the possibility of using these natural compounds as potential leading structures in the design of drugs with antitumor activity has been shown.*

Investigation of natural compounds using the MoA / in silico approach*. A network of pharmacological pathways potentially initiated by modulation of nuclear receptors and protein kinases and their target biomolecules crucial for the regulation of lipid metabolism, inflammatory processes and hepatocyte autophagy is described. An in silico evaluation of the pharmacokinetic properties and toxicity of 12 natural compounds was made with promising experimental evidence for the relief of non-alcoholic steatosis of the liver (Al Sharif et al., 2017b).*

Development of pharmacophore models of partial PPARγ agonists. *Pharmacophore models of partial PPARγ agonists (target biomacromolecule in the treatment of type 2 diabetes) have been developed, which can be used to screen leading structures in the design of new drugs in antidiabetic therapy. A potential mechanism of action - partial agonism of PPARγ - of triterpenoid compounds of natural origin with antidiabetic activity has been established.*

Analysis of existing QSAR models for predicting antiradical activity of polyphenolic compounds and development of new QSAR models. *QSAR models have been developed to predict the antiradical (radical scavenging) activity of polyphenolic compounds measured in stoichiometric tests. The models are characterized by high predictive power and the possibility of mechanistic interpretation.*

In silico studies to assess gastrointestinal absorption and skin permeability of biologically active substances. *A new*

QSAR model for predicting membrane permeability has been developed, which is in good agreement with in vivo gastrointestinal absorption data and can be used for in silico assessment of gastrointestinal absorption. The model is implemented in the data processing and analysis platform of the COSMOS project (http://knimewebportal.cosmostox.eu/) and is freely available.

Evaluation of the applicability of the Intercriteria analysis in the selection of evaluation functions in the docking algorithms, set in the software program MOE. *The applicability of the Intercriteria Analysis for the selection of evaluation functions in the software program MOE in docking of ligands in different proteins was evaluated: 1. With respect to tyrosine derivatives as agonists of PPARγ it was found that the evaluation functions London dG and Alpha HB consonance; Alpha HB, predicts binding position well; 2. With regard to 3-amidinophenylalanine derivatives - ligands of thrombin, trypsin and factor X - no clear agreement was registered between the individual evaluation functions in MOE, ie. each of them individually carries significant information about the connection.*

6. Evaluation of the personal contribution of the candidate. *It is evident from the presented materials that to a large extent the obtained results and the formulated contributions are a personal merit of Assoc. Prof. Iveanka Tsakovska. She is the first or corresponding author of 5 publications out of 24. The results obtained are from work on a total of 11 projects, 3 of which under her leadership, funded by National Programs and EU Operational Programs.*

I declare that I have not found a form of plagiarism in the materials submitted by Assoc. Prof. Ivanka Tsakovska for participation in the competition.

7. Critical remarks and recommendations: *I have no critical remarks. In the future, I recommend that he be actively involved in the training of PhD students as a research supervisor and with lectures at the Training Center at the Bulgarian Academy of Sciences in order to pass on his experience to younger colleagues.*

8. Personal impressions. *I know Assoc. Prof. Ivanka Tsakovska from our joint work in an expert panel at the National Science Foundation (VNEK in Chemical Sciences). I have excellent impressions of her as a colleague and scientist.*

Conclusion

From the submitted documents and references it is evident that in the announced competition associate professor Ivanka Tsakovska participates with a scientific asset ***that fully meets the requirements and meets the criteria for holding the academic position "professor" according to LDASRB and the Rules of IBPBME-BAS.****From the presented reference Sample for the criteria for the academic position "Professor" and the presented evidence* ***it is evident that Assoc. Prof. Ivanka Tsakovska in number of points significantly exceeds those studied by IBPBME-BAS.****Having in mind everything said so far, my overall impression of the documents submitted at the competition, as well as my personal excellent impression of the candidate, I am convinced that Associate Professor Ivanka Milosheva Tsakovska fully meets the requirements for* ***LD****ASRB and the specific requirements of IB****P****BM****E****-BAS for holding the acad****emic position of*** *"Professor". It is a built scientist with a sufficient volume of scientific and applied science. In the works of the candidate there are original scientific and applied contributions that are internationally recognized. Based on all this, I strongly recommend the esteemed members of the scientific jury to vote positively for the award of the academic position "Professor" to Associate Professor Ivanka Tsakovska in the scientific specialty "Principles and methods of cybernetics in various fields of science (in silico study of bioactive compounds)", to the department of "QSAR and molecular modeling" at the Institute of Biophysics and Biomedical Engineering - BAS.*

01.09.2020 *Reviewer*

(Prof. Vessela Kancheva, PhD)