R E V I E W

Considering the competition for the academic position "Professor" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Application of the principles and methods of cybernetics in various fields of science (*in silico* research of bioactive compounds ", announced in the State gazette issue 18 dated February 28, 2020.

For the needs of the department "QSAR and Molecular Modelling", Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences

by Prof. DSc Stefka Germanova Taneva, Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences

The only candidate in the competition is **Assoc. Prof. Ivanka Milosheva Tsakovska, PhD.**

General presentation of the materials received for review

The materials presented by the only candidate in the competition Assoc. Prof. Dr. Ivanka Milosheva Tsakovska from the department "QSAR and molecular modelling", IBPhBMI - BAS, are carefully prepared in accordance with the Rules for scientific development of the academic staff of IBPhBMI-BAS and the criteria for employment to the academic position of "professor". Lists for the scientific publications for participation in the competition for the academic position "Professor"; as well as those for the academic position "Associate Professor" and for the PhD degree; the citations of the scientific works of the candidate; copies of the publications and all required documents for participation in the competition are presented.

The total number of points on the scientometric indicators is **1880** (indicator A - 50, indicator B - 225, indicator D - 252, indicator D - 922 and indicator E - 431), which exceeds many times 600 points of the minimum national requirements and the regulations of ZRAS - IBPhBMI-BAS for holding the academic position of "professor".

Education, career development and specializations

Assoc. Prof. Dr. Ivanka Tsakovska graduated from UCTM, Sofia, in 1995 and holds a Master's degree in Chemical Engineering. In 2003 she defended her doctoral dissertation on the topic: "Quantitative dependences structure - activity of selected classes of biologically active compounds" in Central Laboratory of Biomedical Engineering, BAS.

During the period from 1996 to 2009 she was a specialist, full-time doctoral student, assistant and senior assistant at the Central Laboratory of Biomedical Engineering

“Prof. Ivan Daskalov”, BAS. In 2010 Dr. Tsakovska was elected as an "Associate Professor" at the Institute of Biophysics and Biomedical Engineering, BAS.

As a recipient of the Alexander von Humboldt Foundation research grant (2002-2003), Assoc. Prof. Tsakovska worked on establishing relationships structure-activity and pharmacological testing of modulators of multiple drug resistance in tumors. As a postdoctoral fellow (2005-2006) and a Contract agent (2006-2007) at the Joint Research Center of the European Commission, Ispra, Italy, she conducted research in the field of computational toxicology and expert activity in evaluation the risk of chemicals.

Assoc. Prof. Tsakovska has scientific cooperation with prestigious research institutes and universities: Joint Research Center, EC, Ispra and S-IN Soluzioni Informatiche SRL, Vicenza, Italy; John Moores University, Liverpool, Great Britain; Molecular Networks GmbH, Nuremberg, Germany; Altamira, LLC, Ohio, USA, as well as with the Faculty of Pharmacy, Medical University, Sofia.

Scientific indicators

The scientific output of Assoc. Prof. Dr. Tsakovska is presented by a total of 46 scientific papers (according to the presented lists of publications), cited 610 times, h-index 12 (Scopus).

Dr. Tsakovska participated in the competition with 24 publications (total IF of the publications - 65.65), which are subject to review. All articles are on the research topic of the competition and published after her habilitation as an Assoc. Professor.

The habilitation work (Indicator B) includes 9 publications, all with rank Q1(with IF from 1.833 to 11).

I would note two of the publications in very high impact factored journal (11):

- *Dinić J, Efferth T, Garcia-Sosa AT, Grahovac J, Padron JM, Pajeva I, Rizzolio F, Saponara S, Spengler G, Tsakovska I (2020) Repurposing old drugs to fight multidrug resistant cancers, Drug Resist. Updates, 52, 100713;*
- *Dallavalle S, Dobričić V, Lazzarato L, Gazzano E, Machuqueiro M, Pajeva I, Tsakovska I, Zidar N; Roberta Fruttero (2020) Improvement of conventional anti-cancer drugs as new tools against multidrug resistant tumors, Drug Resist. Updates, 50, 100682.*

The publications outside the habilitation work are 15: 12 are included in indicator Г7, 8 of them with IF (5 with rank Q1, 2 with Q2 and 1 with Q4); 3 with SJR (1 of them accepted for publication), 1 in a conference proceeding (Series G. Medicine, Pharmacy and Dental medicine, Union of Scientists in Bulgaria); and 3 book chapters (one of which is accepted for printing) are included in indicator Г8.

The publications have been cited over 461 times (Scopus) which is proof of the relevance and importance of the research of Assoc. Prof. Tsakovska.

Major Scientific Contributions

The scientific works presented by Assoc. Prof. Tsakovska are in the field of modeling of toxic effects of biologically active compounds; elucidation of the relationship between the therapeutic/toxic effect and the chemical structure and properties of bioactive compounds; the mechanisms of interaction of bioactive compounds with target macromolecules; development of biological pathways leading to toxic effects as well as in the field of the regulatory computational toxicology. Modern *in silico* approaches combining ligand- and structure-based methods, pharmacophore and homology modeling, docking and virtual screening are applied.

In addition to the original scientific contributions listed below, several review papers are presented, discussing different approaches to combat multidrug resistance and the special importance of *in silico* structure-based approaches as well as their application alone or in combination; basic methods in computer-assisted drug design and computational toxicology; summarizing various aspects used in chemoinformatics to represent chemical structure and structural information in software applications for both store and retrieve structural data in large databases and using them for modeling purposes; overview of compounds from different chemotherapeutic classes with potential for new drug applications.

Habilitation work **(publications 1 - 9)** Dr. Tsakovska presented an introduction describing the importance of the research and the applied approaches included in the publications from the habilitation work, summarized the results related to modeling the interactions between biologically active compounds with proteins and nuclear receptors, the development of toxicity pathways of compounds with potential for hepatotoxic action and structure-based modeling of molecular events initiating those pathways.

P-glycoprotein (P-gp - ABC transport protein, which is a major factor in the mechanism of multiple drug resistance) and nuclear receptors (ERα (nuclear peroxisome proliferator-activated gamma receptor) and PPARγ (human estrogen receptor alpha) are target biomacromolecules in the treatment of a number of diseases.

Some of the most significant original scientific contributions are:

- In-depth analysis of compounds with potential as antitumor drugs demonstrates the essential role of *in silico* approaches, such as pharmacophore modeling, as well as pharmacophore- and docking-based virtual screening, for finding new applications of existing and detecting new chemotherapeutic agents, as well as the use of hybrid molecules to overcome multiple drug resistance.

- By molecular modeling of the structures of new inhibitors of the human chaperone (Hsp90), tested in resistant cancer (human non-small cell lung cancer and colorectal adenocarcinoma) cells with overexpression of P-gp, three of the eleven studied Hsp90 compounds were identified as dual targeting molecules - Hsp90 and P-gp inhibitors, which decrease the expression of P-gp in colorectal cancer cells. Their effect on

inhibition of the cell growth, P-gp activity and P-gp expression has been established. Based on docking simulations and interaction analysis, the potential site of binding of dual inhibitors to P-gp was identified.
This investigation has made a significant contribution to the establishment of a new class of dual HSP90 and P-gp inhibitors and have the potential to search for effective anticancer drugs.

- A pharmacophore model developed based on modeling of the structure of complexes of the human estrogen receptor alpha (ERα) with strong agonists and validated by molecular dynamic simulations in wild-type and mutant receptors, establishes additional structural element (hydrophobic/aromatic residue) to the already known pharmacophore features of ERα agonists. This pharmacophore model has the potential to distinguish compounds with potential for pro- and anti-estrogenic activity.

- Toxicity pathways induced by ligand-dependent dysregulation of PPARγ by complete agonists leading to non-alcoholic steatosis have been developed. The Mode of Action/Adverse outcome pathway approach was applied to assess biological toxicity pathways and the sequence of key events.
The performed assay proves: (i) tissue-specific hepatotoxic activity of compounds mediated by their interaction with PPARγ; (ii) two tissue-specific pathways of hepatotoxic action - in the liver and in adipose tissue (activation of the receptor by full agonists in hepatocytes and inhibition of the receptor by antagonists in adipocytes); (iii) potential events at different levels of biological organization and the molecular initiating event to an organ-level response.
- A virtual library (with public access) with structural and biological data of the largest set of PPARγ agonists has been built; the structure of the available PPARγ complexes in Protein Data Bank was analyzed and a pharmacophore model of complete PPARγ agonists was developed. The model describes the main features of the binding of full agonists and defines the critical set of pharmacophore points for full agonists, and can be used for future studies of binding of partial agonists.

- 3D QSAR model based on molecular similarity indices has been developed, which predicts the transactivation activity of complete PPARγ agonists. A virtual screening protocol is proposed, combining the developed pharmacophore and 3D QSAR models, which allows assessment and prioritization of potential prosthetogenic ligands, whose action is carried out by overactivation of PPARγ in hepatocytes. The statistical parameters obtained during validation of the model confirm it as reliable for prognostic purposes.

- Molecular-dynamic simulations to establish the structure and dynamics of the PPARγ receptor in antagonistic form and the binding of PPARγ antagonists reveal that the receptor does not have a defined site of antagonist binding, but different ways of binding with different probabilities.

Scientific publications outside the habilitation work (publications 10 - 24)

The publications outside the habilitation work present research data related to the application of *in silico* approaches to search for new target biomacromolecules, establishing of their mechanisms of action and therapeutic applications; predicting the

therapeutic/toxic effects of natural compound, their pharmacokinetic properties, possible metabolic transformations and interactions with target proteins; application of a new mathematical (intercriteria) analysis of docking results for groups of bioactive compounds.

- The mechanisms of action and potential pharmacological applications of flavonolignans found in the medicinal plant milk thistle (Silybum marianum) have been predicted.

- The most probable metabolic transformations (glucuronidation and oxidative demethylation) and potential toxicity of flavonolignans have been envisaged; three main potential toxic effects in mammals (chromosomal aberrations *in vitro*, ERα modulation and skin sensitization) have been identified.

- Two new potential target proteins for flavonolignans, BRAF kinase and SMO, were proposed based on the established similarity between flavonolignans and structurally similar drugs selected by DrugBank (Vemurafenib and Vismodegib) and subsequent docking simulations, which was confirmed by *in vitro* studies. The possibility of using these natural compounds as potential structures in the design of new drugs with antitumor activity has been proposed.

- The potential estrogenic activity of silibin (silibins and 2,3-dehydro derivatives of silibins) chemical structures, having hepatoprotective, cytoprotective, antioxidant and chemopreventive properties, has been identified. It has been proven that stereoisomeric forms of silibin can interact in a stereospecific manner with ERα.

- A network of pharmacological action pathways initiated by modulation of nuclear receptors and protein kinases is presented and their potential molecular targets are identified. Based on *in silico* evaluation of the pharmacokinetic properties and toxicity of 26 natural compounds with experimentally proven potential to alleviate non-alcoholic steatosis of the liver, 12 compounds modulating the largest number of experimentally supported key events were identified.

- Pharmacophore models of partial PPARγ (a target protein in many pathologies associated with the metabolic syndrome, including type 2 diabetes) agonists and a protocol for virtual screening of triterpenoid compounds of natural origin to predict their partial agonism against PPARγ have been developed. The protocol can be applied for virtual screening of leading structures in the design of antidiabetic drugs.

- Existing QSAR models were analyzed and new QSAR models were developed for predicting the antiradical activity of polyphenolic compounds which have a high predictive ability and the possibility of mechanistic interpretation.

- *In silico* studies to assess gastrointestinal absorption of bioactive substances predict values that correlate with *in vivo* data. The model is included in a platform with open access for data processing and analysis.

- Intercriteria analysis of experimental data on the binding affinity of agonists (complete and tyrosine derivatives as agonists) to the PPARγ receptor and of structurally similar compounds (3-amidinophenylalanine derivatives) to proteins (thrombin, trypsin and factor X) has revealed: (1) positive consonance of the evaluation functions with respect

to tyrosine derivatives as PPARγ agonists in the docking algorithms embedded in the commercial molecular modeling software MOE; (2) lack of clear coherence between the individual assessment functions with respect to 3-amidinophenylalanine derivatives. This suggests that the multicriteria analysis has applications in the field of computer-assisted drug design and computational toxicology.

Personal contribution of the candidate

Assoc. Prof. Tsakovska is the lead author of 5 and corresponding author of 6 of the peer-reviewed publications. Despite the fact that the publications are in author teams with scientists from Bulgarian scientific institutions and foreign research centers, it is clear that she has a significant contribution to all published works.

Prospects for future research

Dr. Tsakovska outlined the prospects for future research on the bases of the data obtained so far: (i) extending the research on modeling of interactions with transport proteins and nuclear receptors in respect to their role as targets in a number of therapies; (ii) model studies on natural products for the purpose of targeted drug design; (iii) developing sustainable models for predicting the toxic effects of substances, e.g. those that are part of cosmetics and food supplements.

Participation in research projects

Assoc. Prof. Tsakovska participated in 8 projects (1 funded by the European Commission and 7 national, granted by the National Scientific Fund and the Ministry of Education and Science) and participated in two European networks: COST action to the European Commission.

The project under the 7th Framework Program of the European Commission (COSMETICS Europe), of which she was a coordinator of the Bulgarian team, deserves special attention.

Teaching activity

Dr. Tsakovska was a lecturer in "QSAR and molecular modeling" in a master's course at the Faculty of Chemistry and Pharmacy - Sofia University, 2008-2019.

She has supervised diploma works of 2 students; and co-supervised two doctoral students, one successfully defended PhD Thesis in 2016 and another one with upcoming defense.

Expert activities

Assoc. Prof. Tsakovska has considerable expert and reviewer activities:

- member of the General Assembly of BAS since 2016; member of the Expert Council for Evaluation of Priority Substances at the Ministry of Environment and Water since 2015; member of the Scientific Council and Deputy Chairman of the Scientific Council of the Institute of biophysics and biomedical engineering - BAS.

- expert in the work of the European Technical Committee for New and Existing Substances (2006) and in the work of the EU (Q)SAR Working Group, led by the European Chemical Bureau, Joint Research Center, Italy (2006 - 2007).
- reviewer of international scientific journals and research projects at the European Commission and the Bulgarian National Science Fund; member of the editorial boards of Computational Molecular Bioscience and Austin Journal of Computational Biology and Bioinformatics
- member of scientific juries for awarding PhD degree;
- member of the Union of Scientists in Bulgaria and The Cheminformatics and QSAR Society.

Awards

Dr. Tsakovska received Eureka Award of the Eureka Foundation and the Higher Attestation Commission for achievements in Science, 2004.

She was also awarded for her research activity as a young scientist:

- National Award "Marin Drinov" (competition 2003) of BAS for young scientists for scientific achievements in the field of quantitative modeling structure-activity dependencies of biologically active compounds;
- Award of the National Fund for Science Research, MES, for the best youth project in the competition "Young Scientists", 2002.

CONCLUSION

The research investigations of Dr. Tsakovska are extremely important for the assessment of toxic/therapeutic effects of biologically active and natural compounds, and provide perspectives for future applications of modern *in silico* approaches. In addition to original publications in journals with a high impact factor and their international recognition, the expert and reviewer activities of Dr. Tsakovska are an additional certificate for her expertise.

I would like to emphasize the fact that the scientific indicators of Assoc. Prof. Ivanka Tsakovska significantly exceed the recommended requirements for the academic position of "professor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Application of ADASRB in BAS and the specific requirements of IBPhBMI-BAS.

This gives me a reason to express my positive opinion on the application of Assoc. Prof. Tsakovska for the academic position "Professor" and to strongly recommend the members of the scientific jury to vote positively and the Scientific Council of IBPhBMI-BAS to elect Assoc. Prof. Dr. Ivanka Milosheva Tsakovska for the academic position "Professor" in a professional field 4.3. Biological sciences, scientific specialty "Application of the principles and methods of cybernetics in various fields of science (in silico study of bioactive compounds)".

Sofia
4 September 2020 /prof. Stefka Germanova Taneva/