Abstracts

of selected publications of Assoc. Prof. Ivanka Tsakovska

for participation in the competition for occupying the academic position of Professor

1. 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 Resistance Updates, 50, 100682.

Multidrug resistance (MDR) is the dominant cause of the failure of cancer chemotherapy. The design of antitumor drugs that are able to evade MDR is rapidly evolving, showing that this area of biomedical research attracts great interest in the scientific community. The current review explores promising recent approaches that have been developed with the aim of circumventing or overcoming MDR. Encouraging results have been obtained in the investigation of the MDR-modulating properties of various classes of natural compounds and their analogues. Inhibition of P-gp or downregulation of its expression have proven to be the main mechanisms by which MDR can be surmounted. The use of hybrid molecules that are able to simultaneously interact with two or more cancer cell targets is currently being explored as a means to circumvent drug resistance. This strategy is based on the design of hybrid compounds that are obtained either by merging the structural features of separate drugs, or by conjugating two drugs or pharmacophores via cleavable/non-cleavable linkers. The approach is highly promising due to the pharmacokinetic and pharmacodynamic advantages that can be achieved over the independent administration of the two individual components. However, it should be stressed that the task of obtaining successful multivalent drugs is a very challenging one. The conjugation of anticancer agents with nitric oxide (NO) donors has recently been developed, creating a particular class of hybrid that can combat tumor drug resistance. Appropriate NO donors have been shown to reverse drug resistance via nitration of ABC transporters and by interfering with a number of metabolic enzymes and signaling pathways. In fact, hybrid compounds that are produced by covalently attaching NO-donors and antitumor drugs have been shown to elicit a synergistic cytotoxic effect in a variety of drug resistant cancer cell lines. Another strategy to circumvent MDR is based on nanocarrier-mediated transport and the controlled release of chemotherapeutic drugs and P-gp inhibitors. Their pharmacokinetics are governed by the nanoparticle or polymer carrier and make use of the enhanced permeation and retention (EPR) effect, which can increase selective delivery to cancer cells. These systems are usually internalized by cancer cells via endocytosis and accumulate in endosomes and lysosomes, thus preventing rapid efflux. Other modalities to combat MDR are described in this review, including the pharmaco-modulation of acridine, which is a well-known scaffold in the development of bioactive compounds, the use of natural compounds as means to reverse MDR, and the conjugation of anticancer drugs with carriers that target specific tumor-cell components. Finally, the outstanding potential of *in silico* structure-based methods as a means to evaluate the ability of antitumor drugs to interact with drug transporters is also highlighted in this review. Structure-based design methods, which utilize 3D structural data of proteins and their complexes with ligands, are the most effective of the *in silico* methods available, as they provide a prediction regarding the interaction between transport proteins and their substrates and inhibitors. The recently resolved X-ray structure of human P-gp can help predict the interaction sites of designed compounds, providing insight into their binding mode and directing possible rational modifications to prevent them from becoming P-gp drug substrates. In summary, although major efforts were invested in the search for new tools to combat drug resistant tumors, they all require further implementation and methodological development. Further investigation and progress in the abovementioned strategies will provide significant advances in the rational combat against cancer MDR.

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2. Tsakovska I, Alov P, Ikonomov N, Atanassova V, Vassilev P, Roeva O, Jereva D, Atanassov K, Pajeva I, Pencheva T InterCriteria Analysis Implementation for Exploration of the Performance of Various Docking Scoring Functions. Studies in Computational Intelligence, приета за печат.

The present study describes an implementation of InterCriteria Analysis (ICrA) in the field of the computer-aided drug design and computational toxicology. ICrA strives to go beyond the nature of the criteria involved in a process of evaluation of multiple objects against multiple criteria, and, thus to discover some dependencies between the criteria themselves. The approach is based on the apparatus of the index matrices and the intuitionistic fuzzy sets. In this study new software capabilities, implemented in order to apply ICrA to *in silico* drug design, are presented. As a case study, ICrA is implemented to explore the performance of various scoring functions in docking. Docking, which is the most commonly used structure-based drug design method, has been applied to predict the binding mode and to provide a measure for the ligand binding affinity to the protein. In particular, ligands of the peroxisome proliferator-activated nuclear receptor gamma (PPARg), involved in the control of a number of physiologically significant processes, have been investigated towards prediction of their binding to the protein. A dataset of 160 tyrosine-based PPARg agonists with experimentally determined binding affinities has been used in this investigation. Docking combined with the in-house developed pharmacophore model as a filter has been applied. Various scoring functions and docking protocols have been tested in the molecular modelling platform MOE (v. 2019.01). ICrA has been applied to assess dependencies among the scoring functions. The analysis has demonstrated high positive consonance for two of the scoring functions – London dG and Alpha HB. None of the functions could be distinguished as a good predictor of the experimental binding affinity.

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3. Diukendjieva A**,** Zaharieva M, Mori M, Alov P**, Tsakovska I,** Pencheva T**,** Najdenski H, Křen V, Felici C, Bufalieri F, Di Marcotullio L, Botta B, Botta M, Pajeva I (2020) Dual SMO/BRAF Inhibition by Flavonolignans from Silybum marianum, Antioxidants, 9(5), 384.

Silymarin is the standardized extract from the fruits of Silybum marianum (L.) Gaertn., a well-known hepatoprotectant and antioxidant. Recently, bioactive compounds of silymarin, i.e., silybins and their 2,3-dehydro derivatives, have been shown to exert anticancer activities, yet with unclear mechanisms. This study combines *in silico* and *in vitro* methods to reveal the potential interactions of optically pure silybins and dehydrosilybins with novel protein targets. The shape and chemical similarity with approved drugs were evaluated *in silico*, and the potential for interaction with the Hedgehog pathway receptor Smoothened (SMO) and BRAF kinase was confirmed by molecular docking. *In vitro* studies on SMO and BRAF V600E kinase activity and in BRAF V600E A-375 human melanoma cell lines were further performed to examine their effects on these proteins and cancer cell lines and to corroborate computational predictions. Our *in silico* results direct to new potential targets of silymarin constituents as dual inhibitors of BRAF and SMO, two major targets in anticancer therapy. The experimental studies confirm that BRAF kinase and SMO may be involved in mechanisms of anticancer activities, demonstrating dose-dependent profiles, with dehydrosilybins showing stronger effects than silybins. The results of this work outline the dual SMO/BRAF effect of flavonolignans from Silybum marianum with potential clinical significance. Our approach can be applied to other natural products to reveal their potential targets and mechanism of action.

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4. Kochev N, Jeliazkova N, **Tsakovska I (2020)** Cheminformatics representation of chemical structures – a milestone for successful big data modelling. In *Big Data in Predictive Toxicology*, pages 69-107, Royal Society of Chemistry, doi: 10.1039/9781782623656-00069.

Within the computational toxicology field, the representation of a chemical structure is considered as a key to predict/retrieve the toxicity information for a substance. Chemoinformatics provides efficient tools to computationally handle the chemical information. This is even more important in a big data era with an increasing amount of information on chemical compounds available, the endeavour to link activity information to chemicals, also across different databases, and the need of unambiguous identification of chemicals and

taking into account structural features for modelling. This chapter gives an overview of the different aspects of chemical structure representation used in chemoinformatics. Various techniques for chemical information formalisation are provided, together with the different levels of structure representation starting from 0D (0 dimension) and going to the more complex 3D and 4D as essential for interactions with biomacromolecules. Structural descriptors that represent the chemical structure in the bioactivity modelling are introduced. Furthermore, the challenges in unique structure representations, chemical substances representation, as well as specific issues such as handling aromaticity and tautomerism are discussed. The approaches show how to represent structural information within chemical software applications in the context of storing/searching structural data in big databases and its use for predictive modelling purposes.

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5. Diukendjieva A, Alov P, **Tsakovska I**, Pencheva T, Richarz A, Kren V, Cronin M, Pajeva I (2019), *In vitro* and *in silico* studies of the membrane permeability of natural flavonoids from Silybum marianum (L.) Gaertn. and their derivatives, Phytomedicine, 53, 79.

Background: In recent years the number of natural products used as pharmaceuticals, components of dietary supplements and cosmetics has increased tremendously requiring more extensive evaluation of their pharmacokinetic properties.

Purpose: This study aims at combining *in vitro* and *in silico* methods to evaluate the gastrointestinal absorption (GIA) of natural flavonolignans from milk thistle (Silybum marianum (L.) Gaertn.) and their derivatives.

Methods: A parallel artificial membrane permeability assay (PAMPA) was used to evaluate the transcellular permeability of the plant main components. A dataset of 269 compounds with measured PAMPA values and specialized software tools for calculating molecular descriptors were utilized to develop a quantitative structureactivity relationship (QSAR) model to predict PAMPA permeability.

Results: The PAMPA permeabilities of 7 compounds constituting the main components of the milk thistle were measured and their GIA was evaluated. A freely-available and easy to use QSAR model predicting PAMPA permeability from calculated physico-chemical molecular descriptors was derived and validated on an external dataset of 783 compounds with known GIA. The predicted permeability values correlated well with obtained *in vitro* results. The QSAR model was further applied to predict the GIA of 31 experimentally untested flavonolignans.

Conclusions: According to both *in vitro* and *in silico* results most flavonolignans are highly permeable in the gastrointestinal tract, which is a prerequisite for sufficient bioavailability and use as lead structures in drug development. The combined *in vitro*/*in silico* approach can be used for the preliminary evaluation of GIA and to guide further laboratory experiments on pharmacokinetic characterization of bioactive compounds, including natural products.

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6. Dinić J, Podolski-Renić A, Jovanović M, Musso L, **Tsakovska I**, Pajeva I, Dallavalle S, Pešić M. (2019) Novel Heat Shock Protein 90 Inhibitors Suppress P-Glycoprotein Activity and Overcome Multidrug Resistance in Cancer Cells, Int J Mol Sci, 20, 4575.

Heat Shock Protein 90 (Hsp90) chaperone interacts with a broad range of client proteins involved in cancerogenesis and cancer progression. However, Hsp90 inhibitors were unsuccessful as anticancer agents due to their high toxicity, lack of selectivity against cancer cells and extrusion by membrane transporters responsible for multidrug resistance (MDR) such as P-glycoprotein (P-gp). Recognizing the potential of new compounds to inhibit P-gp function and/or expression is essential in the search for effective anticancer drugs. Eleven Hsp90 inhibitors containing an isoxazolonaphtoquinone core were synthesized and evaluated in two MDR models comprised of sensitive and corresponding resistant cancer cells with P-gp overexpression (human non-small cell lung carcinoma and colorectal adenocarcinoma). We investigated the effect of Hsp90 inhibitors on cell growth inhibition, P-gp activity and P-gp expression. Structure–activity relationship analysis was performed in respect to cell growth and P-gp inhibition. Compounds 5, 7, and 9 directly interacted with P-

gp and inhibited its ATPase activity. Their potential P-gp binding site was identified by molecular docking studies. In addition, these compounds downregulated P-gp expression in MDR colorectal carcinoma cells, showed good relative selectivity towards cancer cells, while compound 5 reversed resistance to doxorubicin and paclitaxel in concentration-dependent manner. Therefore, compounds 5, 7 and 9 could be promising candidates for treating cancers with P-gp overexpression.

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7. Al Sharif M, **Tsakovska I**, Pajeva I, Alov P, Fioravanzo E, Bassan A, Kovarich S, Yang C, Mostrag-Szlichtyng A, Vitcheva V, Worth AP, Richarz AN, Cronin MTD (2017) The application of molecular modelling in the safety assessment of chemicals: A case study on ligand-dependent PPARγ dysregulation. Toxicology, 392, 140.

The aim of this paper was to provide a proof of concept demonstrating that molecular modelling methodologies can be employed as a part of an integrated strategy to support toxicity prediction consistent with the mode of action/adverse outcome pathway (MoA/AOP) framework. To illustrate the role of molecular modelling in predictive toxicology, a case study was undertaken in which molecular modelling methodologies were employed to predict the activation of the peroxisome proliferator- activated nuclear receptor g (PPARg) as a potential molecular initiating event (MIE) for liver steatosis. A stepwise procedure combining different *in silico* approaches (virtual screening based on docking and pharmacophore filtering, and molecular field analysis) was developed to screen for PPARg full agonists and to predict their transactivation activity (EC50). The performance metrics of the classification model to predict PPARg full agonists were balanced accuracy = 81%, sensitivity = 85% and specificity = 76%. The 3D QSAR model developed to predict EC50 of PPARg full agonists had the following statistical parameters: q2cv = 0.610, Nopt = 7, SEPcv = 0.505, r2pr = 0.552. To support the linkage of PPARg agonism predictions to prosteatotic potential, molecular modelling was combined with independently performed mechanistic mining of available *in vivo* toxicity data followed by ToxPrint chemotypes analysis. The approaches investigated demonstrated a potential to predict the MIE, to facilitate the process of MoA/AOP elaboration, to increase the scientific confidence in AOP, and to become a basis for 3D chemotype development.

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8. Jereva D, Fratev F, **Tsakovska I**, Alov P, Pencheva T, Pajeva I (2017) Molecular Dynamics Simulation of the Human Estrogen Receptor Alpha: Contribution to the Pharmacophore of the Agonists. Math Comput Simulat, 133, 124.

Human estrogen receptor alpha (ERα) is one of the most studied targets for *in silico* screening of bioactive compounds. The estrogenic activity of a vast number of chemicals has been studied for their potentially adverse effects on the hormone regulation of the endocrine system. The commonly accepted presentation of the ERα agonist pharmacophore includes terminal phenolic groups and a hydrophobic rigid backbone. In this study we report on molecular dynamics (MD) simulations of ERα to get a deeper structural insight into the agonist–receptor interactions and the pharmacophore pattern of compounds with agonistic activity. We rely on a crystallographic structure of a complex of ERα (PDB ID 2P15) with an agonist of picomolar affinity. As the X-ray structure has a mutation next to a key structural element for ERα agonistic activity (helix H12, Y537S), a series of MD simulations have been performed on the mutated and on the wild type receptor to prove the stability of the agonist–receptor interactions. No significant difference in the ligand–protein interactions has been detected between the studied proteins implying that the Y537S mutant structure can be used for refinement of the pharmacophore model of the ERα agonists. The results suggest that the pharmacophore of compounds with ERα agonistic activity can be extended by a feature that occupies a free hydrophobic region of the binding pocket. The extended pharmacophore model has been evaluated by a pharmacophore-based virtual screening of databases of ERα binders and decoys. The results also imply that MD simulations are a powerful *in silico* tool for both protein dynamics and structure investigation, especially when mutations are available that can potentially disturb the protein structure and functions.

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9. Tsakovska I, Al Sharif M, Alov P, Diukendjieva A, Fioravanzo E, Cronin MTD, Pajeva I (2014) Molecular modelling study of the PPARγ receptor in relation to the Mode of Action/Adverse Outcome Pathway framework for liver steatosis. Int J Mol Sci, 15(5), 7651.

The comprehensive understanding of the precise mode of action and/or adverse outcome pathway (MoA/AOP) of chemicals has become a key step toward the development of a new generation of predictive toxicology tools. One of the challenges of this process is to test the feasibility of the molecular modelling approaches to explore key molecular initiating events (MIE) within the integrated strategy of MoA/AOP characterisation. The description of MoAs leading to toxicity and liver damage has been the focus of much interest. Growing evidence underlines liver PPARγ ligand-dependent activation as a key MIE in the elicitation of liver steatosis. Synthetic PPARγ full agonists are of special concern, since they may trigger a number of adverse effects not observed with partial agonists. In this study, molecular modelling was performed based on the PPARγ complexes with full agonists extracted from the Protein Data Bank. The receptor binding pocket was analysed, and the specific ligand-receptor interactions were identified for the most active ligands. A pharmacophore model was derived, and the most important pharmacophore features were outlined and characterised in relation to their specific role for PPARγ activation. The results are useful for the characterisation of the chemical space of PPARγ full agonists and could facilitate the development of preliminary filtering rules for the effective virtual ligand screening of compounds with PPARγ full agonistic activity.

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10. Tsakovska I, Pajeva I, Alov P, Worth A (2011) Recent advances in the molecular modelling of estrogen receptor-mediated toxicity. Adv Protein Chem Struct Biol,85, 217.

In the past two decades, there has been increasing concern about the potentially adverse effects of exogenous endocrine active substances (EAS) that alter the function of the endocrine system by interfering with hormone regulation. The mechanistic pathways by which EAS may elicit adverse effects, such as developmental and reproductive toxicity, often involve direct binding to nuclear hormone receptors. Certainly, the best studied nuclear receptor is the estrogen receptor (ER). Large-scale *in vitro* and *in vivo* methods have been developed to assess the estrogenic toxicity of chemicals. However, there are financial and animal welfare concerns related to their application. Quantitative structure–activity relationship (QSAR) approaches have proven their utility as a priority setting tool in the risk assessment of EAS. In addition, the models help to clarify the binding mode of the interacting substances. As estrogen-mediated effects are usually related to ligand–receptor interactions, and as there have been comprehensive structural studies on the ER, molecular modeling together with other *in silico* approaches provide a suitable means of studying these estrogenic effects. This chapter provides an overview of the molecular modeling approaches applied to ligand–ER interactions. The progress in the field is outlined, and some critical issues are analyzed based on recently published models where these approaches are applied.