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COMPUTATIONAL TOOLS FOR PREDICTION OF NUCLEAR RECEPTOR MEDIATED EFFECTS

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ABSTRACT— *Endocrine disrupting chemicals pose a significant threat to human health, society and the environment. Many of these chemicals elicit their toxicological effects through nuclear hormone receptors, like the estrogen receptor. Computational tools for predicting receptor mediated effects have been envisaged for their potential to be used for prioritization of chemicals for toxicological evaluation to reduce the amount of costly experimental testing and enable early alerts for newly designed compounds. In silico tools like knowledge-based expert systems and (quantitative) structure-activity relationship models have been created or upgraded on the yearly basis and also widely advertized to be used as primary screening technique in studies related to receptor mediated effects. The aim of this study is to provide an overview of the present most popular commercial and non-commercial in silico tools applicable for research studies in the field of receptor mediated effects.*

Keywords: *nuclear receptors, QSAR, computational toxicology, virtual screening*

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1. INTRODUCTION

Nuclear receptors (NRs) are a superfamily of ligand-dependent transcription factors that mediate the effects of hormones and other endogenous ligands to regulate the expression of specific genes. Members of the NR superfamily include receptors for various steroid hormones (estrogen, androgen, progesterone, and several corticosteroids), retinoic acid, thyroid hormones, vitamin D, and dietary lipids (the peroxisome proliferator activated receptor (PPAR)). A large number of 'orphan' NRs (e.g. pregnane X receptor, farnesoid X receptor and liver X receptor) have also been identified whose cognate ligands are still unknown (Giguere, 1999).

Many environmental chemicals can interfere with NRs, resulting in adverse effects on body systems. For example, in recent years, various agricultural, industrial, and household chemicals have been shown to directly or indirectly interfere with the endocrine system of wildlife species and humans (Lintelmann et al., 2003). Because these chemicals, called endocrine disruptors (EDCs), represent a potential threat to the male and female reproductive functions, there has been a rising scientific and regulatory interest in their identification (Gray et al., 2002). Numerous mechanisms of action have been associated with endocrine disruption, and a wide variety of *in vitro* tests have been developed to identify chemicals acting via these mechanisms (Jacobs et al. (2008).

Since June 2007, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation has been implicated in the European Union (REACH). The main goal of REACH is to protect human health and environment from hazardous chemicals. The REACH legislation drives industry to the responsible assessment and risk management of chemicals and to provide safety information to the users. In practice, this means proof of safety of chemicals sold or used by the industry. Testing the actions of all used chemicals – possible EDCs – against all the potential targets related to endocrine disruption is an important but also expensive and difficult, if not impossible, task, also due to the limited availability of suitable bioassays. Especially biological testing is time and cost intensive; therefore, more rational approaches to help to identify potentially harmful chemicals in a fast way are urgently needed. In this context, methods established in drug discovery and development, where the task is to identify bioactive compounds from millions of available substances, can be applied to EDCs research.

Computational methods are already a well established tool in drug discovery (Tanrikulu et al., 2013) and can also support EDCs studies, either in the identification of new EDCs or pointing into the right direction when finding the mechanism of action for already known EDCs. Additionally, quantitative structure–activity relationship (QSAR) models have proven their utility for identification of chemicals that might interact with nuclear receptors (Fang et al., 2003). The basis for any QSAR model is that the biological activity of a new or untested chemical can be inferred from the molecular structure, or properties, of similar compounds whose activities have already been assessed (Johnson et al., 1990).

The aim of this paper is to provide an overview of the most popular computational (in silico) tools for virtual identification of chemicals as potential ligands for nuclear receptors. Because the specified area is very broad the focus will be set on technical capabilities of single representative commercial and non-commercial systems for identification of chemicals as estrogen receptor binders. A highlight will be provided on recently developed models for identification of ligands for glucocorticoid and pregnane X receptors.
It is expected that the provided information will assist researchers to find the best possible solution by choosing most appropriate computational tool for investigations in the field of nuclear receptors toxicology research.

2. Outline of *in silico* techniques for modeling of NRs

Ligand specificity of NRs is crucial in terms of both cellular transcription and therapeutic applications. To understand the essential interactions that determine specificity, it is important to identify the key ligand structural features and crucial receptor residues that are involved. Depending of what kind information is available for investigated NRs the analyzed effect could be modeled either by the principle of *similarity* — similar compounds are assumed to produce similar effects — or by the principle of *complementarity* — the receptor of a biologically active compound is complementary to the compound itself (i.e. a lock-and-key model) (Kubinyi, 2002).

The choice of the appropriate method depends mainly on prior knowledge of the molecular structure of the active molecule and its receptor. For example, the ligand based method allows analysis of similar to the natural ligand molecules resulting in definition of specific rules related to receptor-ligand interaction. By contrast, knowledge of the 3D structure of the receptor or at least of its active site(s) allows screening for complementary molecules that fit the active site (i.e. the use of receptor-based methods). For more details one could refer to comprehensive reviews

(Halperin et al. (2002), Lyne, (2002), Stahl, (2000)) which supply excellent descriptions of these methods and their use in identification of potential ligands as targets for different receptors.

3. Computer programs suitable for research on nuclear receptors

Nowadays, a large number of computer programs allow identification of ligands for variety NRs. This is not surprising due to the fact that the easiest and most convenient way of applying QSAR models is via user-friendly software interface. An overview of the most popular software tools is provided in Table 1, alongside with a short description in general terms. For more details readers are encouraged to visit the official web site of the respective product.

Table 1. Software tools for predictions related to the nuclear receptors.

ChemBench http://chembench.mml.unc.edu/	Quantitative prediction of binding affinity to ERα and ERβ.
Endocrine Disruptor Knowledge Base (EDKB) http://www.fda.gov/	Quantitative models to predict the binding affinity of compounds to the estrogen and androgen nuclear receptor proteins.
OECD (Q)SAR Toolbox http://www.qsartoolbox.org/	Binary prediction of ER binders/non-binders.
ACD/Tox Suite (ToxBoxes) http://www.acdlabs.com/products/pc_admet/tox/tox/modules.php	Binary prediction of ER binders/non-binders.
ADMET Predictor http://www.simulations-plus.com/	Qualitative and quantitative prediction of binding affinity to ER.
Derek Nexus http://www.lhasalimited.org/	Classification models (different levels of likelihood) based on 23 alerts for developmental toxicity; 4 alerts for estrogenicity.
MolCode Toolbox http://molcode.com/	Quantitative prediction of binding affinity to ER and aryl hydrocarbon receptor (AhR).
TIssue MEtabolism Simulator http://oasis-lmc.org/	Binary prediction of ER, AR and AhR binders/ non-binders.
Toxboxes http://pharma-algorithms.com/tox_boxes.htm	Classification model for the prediction of ER binding.
VirtualToxLab http://www.biograf.ch	Prediction of endocrine disruption potential based on simulations of compound interactions with AR, AhR, ER, thyroid, glucocorticoid, liver X.

In general, all commercial products can be used for ultimate predictions without any possibility for manual improvement of the incorporated models. On the other hand a number of non commercial platforms became attractive for researchers allowing development of models and their application for endpoint prediction (QSAR Toolbox, Toxtree, Ambit). Moreover, in many cases improvement of existing models can be achieved by addition of private in-house experimental data. An example for development of QSAR models for identification of ligands for glucocorticoid and PXR receptors by using QSAR Toolbox will be provided in sections 5.1. and 5.2.

4. Applicability of commercial and non-commercial *in silico* tools for identification of endocrine disrupting chemicals

4.1. Prediction of estrogen binding potential of structural diverse chemicals by OASIS TIssue MEtabolism Simulator (TIMES)

OASIS TIMES (Serafimova et al., 2007) is a heuristic algorithm for generation of metabolic maps from a library of biotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. The TIMES platform is also used to predict different endpoints including receptor mediated endpoints for estrogen, androgen and aryl hydrocarbon binding affinity. They are based on the Common Reactivity Pattern (COREPA) methodology (Mekenyan et al., 2004). The COREPA approach is a probabilistic classification method which assesses the impact of molecular flexibility on stereo electronic properties of chemicals. Similarity between chemicals is analyzed by comparing their conformational distributions, and the system automatically identifies the parameter that best discriminate chemicals in groups. A Bayesian decision tree is then developed for classifying untested chemicals.

The model for estrogen receptor (ER) binding is based on the assumption that distances between electrophilic sites in the receptor determine the requirements for binding mechanism (Mekenyan et al., 2012). A training set including 645 structures (tested *in vitro* by using purified full-length human ER) is used for deriving the model. Experimental results are expressed as relative binding affinity (RBA, %), with estradiol having an affinity of 100%.

Analysis of common reactivity patterns of the most active ER binders (RBA>10%), based on the distance between nucleophilic sites, resulted in identification of distinct interaction types, summarized as steroid-like A–B type, modulated by steric and electronic interactions (analyzed by COREPA); an A–C type, where the local hydrophobic interactions were found to be significant; and a mixed A–B–C type, modulated by stereoelectronic parameters. A fourth mechanism called A-type has been specified for the 0.1<RBA<10% and 0.001<RBA<0.1% bins which accounts for phenolic chemicals. The mechanisms A–B, A–C, and A–B–C were not identified at the lowest activity range (0.0001<RBA<0.001%). The chemicals eliciting activity in this range are organized into chemical classes, such as halogen-containing chemicals, phthalates, flavones, esters, and ketones, or chemicals interacting by A-type mechanism. For each class, additional parametric requirements were specified for ER binding.

The ultimate model is organized as a battery of all models related to the respective potency bins. When a chemical is submitted for prediction the requirements for high ER binding are first applied. If the chemical does not respond to all of them, then the requirements for the lower activity bins are applied sequentially. If the chemical passes through the activity bins without meeting a binding requirement, then the ultimate prediction is not ER binder.

The model performance is assessed on the basis of prediction made for the training set chemicals. In total the predictive performance shows 82% sensitivity (correct predicted binders) and specificity of 85% for non-ER-binders.

The performance of the model is further evaluated by external validation study. It is based on predictions for 232 not used during model development. Without discrimination in respect to the potency bins (i.e. ER binders or non binders) 78% of the chemicals are correctly predicted.

In conclusion, the use of the presented model is recommended as highly reliable tool for identification of chemical as binders toward the estrogen receptor. However it should be noted that the OASIS TIMES is commercial software which can be used under rules of license agreement with the developer.

4.2. Application of the OECD QSAR Toolbox for prediction of estrogen binders

The OECD QSAR Toolbox is a standalone software application for filling gaps in (eco)toxicity data. It is developed by ECHA and the OECD (http://www.qsartoolbox.org/).

The Toolbox is open source software intended to be used for grouping approaches such as read across and category definition. Several grouping tools are provided. It is possible to group according to (1) predefined groups such as categories derived from the US EPA New chemical or the OECD HPV program; (2) mechanistic aspects e.g. DNA binding or biodegradation; (3) endpoint specific aspects e.g. based on a certain reactivity observed in in vitro/in vivo assays; and (4) empiric methods e.g. chemical elements or organic functional groups.

The Toolbox also includes a range of profilers to quickly evaluate chemicals for common mechanisms or modes of action. In order to support read-across and trend analysis, the Toolbox contains numerous databases with results from experimental studies, a profiler for ER binding and a database of underlying ER binding data.

The ER binding profiler requires only chemical structure information describing the two-dimensional (2D) structure of molecules (for instance coded in SMILES format or directly drawn by the user) as an input. According to the classification scheme, cyclic chemical structures weighting less than 500 Daltons (Da) and bearing an OH and/or an NH_2 group are considered as binders. On the other hand, a chemical is considered as a non-binder if it does not satisfy these rules or if its OH or NH_2 groups are impaired by ortho di-substitutions. This set of criteria was derived on the basis of the findings reported in the scientific literature (Schultz et al., 2002). Details for each category are given in Table 2.

Table 2. Structural criteria for the characterization of the binding potency of chemicals according to the ER binding profiler incorporated in the (Q)SAR Toolbox.

Categories of ER binders	**Predictive rule**
Very strong binders	200<MW≤500 Daltons (Da) in combination with two non-impaired OH groups attached to two different five or six carbon-atom rings.
Strong binders	Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and 200<MW≤500 Daltons (Da)
Moderate binders	Chemicals with a single five or six-member carbon ring structure with an unhindered hydroxyl-group (-OH) or amino-group (-NH2) (one in the para- or meta-position on the ring)
Weak binders	Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxy or amino group and MW < 170 Da
Non binders	• impaired OH or NH_2 group • no presence of OH or NH_2 group • non-cyclic structure • exceeded upper limit related to molecular weight (MW > 500)

In a recent study performed by Todorov (2015, a) the predictive ability of the ER profiler has been assessed by predictions for 1235 chemicals which are part from larger database included in Toolbox. The performance in terms of sensitivity (correct predicted ER binders) shows that the ER profiler is able to identify 88% of all ER binders in the set. In respect to specificity (correct predicted non ER binders) the performance is 63% only. The rate of false positives was found to be relatively high which prompts the obvious need for further precise reevaluation of the rules expecting decreased number of wrong predicted non binders.

In order to assess the predictive ability of the profiler with account to predefined activity bins the analyzed chemicals has been splitted in groups in a same manner as this one used in the TIMES ER model (see section 4.1.).

The obtained prediction results with detailed information for the contribution of each single ER binding category from the ER profiler for all five binding potency bins are presented in Table 3.

Table 3. Prediction results for all external set chemicals segmented by ER binding categories

ER binding category	Number of chemicals	Predictions				
		Very strong RBA≥10	Strong 0.1≤RBA<10	Moderate 0.001≤RBA<0.1	Weak 10^{-4}≤RBA<10^{-3}	Non binders
Very strong	257	153	95	-	-	9
Strong	347	186	99	-	-	62
Moderate	235	87	85	11	8	44
Weak	42	6	7	7	12	10
Non binders	353	11	47	26	47	222

*RBA – Relative binding affinity (%)

As far as general predictive aspects of the ER profiler are concerned, the prediction of moderate and weak binding affinities is less reliable than the prediction of strong and very strong ER binders. This result suggests that the profiler should be improved especially for lower ER binders by addition of specific rules for this activity bin. Concerning predictive rules for strong and very strong binders it is necessary to be further modified in order to be more distinctive among both groups. A way to increase the predictive performance is to account 3D characteristics of the ligands. For example this can be achieved by using distances between specific active sites (OH groups) as pre-filtering criteria. While the current version of the QSAR Toolbox (3.3) does not allow technical use of distances based on 3D chemical geometry such kind of analysis could be performed by external *in silico* applications and may be applied preliminary before application of the ER-profiler.

5. Recently developed QSAR models for Pregnane X and glucocorticoid receptors by making use of QSAR Toolbox

5.1. QSAR model for Pregnane X receptor

The activation of pregnane X receptor (PXR) regulates the expression of metabolizing enzymes such as cytochrome P450 enzymes (CYP3A4, CYP2B6 and CYP2C8/9) and glutathione-S-transferases, as well as important drug transporters (P-glycoprotein, multidrug resistance protein as well as others) (Kliewer et al., 2002). Because the CYP enzymes metabolize the majority of clinically important drugs, inadvertent upregulation by PXR agonists may increase the metabolism and excretion of other co-administered therapeutic agents and cause undesirable drug–drug interactions or the generation of toxic levels of a drug metabolite. Hence, the activation of PXR has the potential to initiate a broad spectrum of adverse effects, and in this respect identification of PXR ligands is important information for evaluating health risk of drugs and environmental chemicals.

As a result of analysis of 348 training set chemicals by making use of the incorporated profiler for organic functional groups in the QSAR Toolbox a set of 11 chemical categories related to PXR activators have been proposed (Todorov, 2015, b). Each category represents functional groups which can be treated as pharmacophores with impact to the PXR binding effect. As next step a set of predictive models was developed based on additional analysis of the chemicals in

each category. In some cases additional molecular descriptor is required in order to distinguish PXR activators from non-activators in a same category. Details for all categories are presented in Table 4.

Table 4. Identified structural alerts associated with PXR activating effect

#	Chemical category	Structural Rule	Molecular descriptor
1	Steroids	+	3D
2	Sulfonamides	+	logKow
3	Flavonoids	+	-
4	Pyrazoles and triazoles	+	3D
5	Unsaturated heterocyclic amines	+	-
6	Urea derivatives	+	3D
7	Coumarins	+	-
8	Carboxamides	+	-
9	Iminothiazoldiones	+	-
10	Oxothiazolidines	+	-
11	Dihydrobenzopyranes	+	-

Six of the alerts were identified as ones that cause effects without the need of modulating factors (additional molecular descriptors). In two cases, global physicochemical (2D) parameters such as logKow and molecular weight were imposed as modulating factors. In three cases only, quantum-chemical requirements (based on 3D calculations) were used to assess the degree to which the alerts are affected by the rest of the molecules.

The performance of the model was assessed by application over the training set chemicals. Concerning activators - 72% sensitivity is reached. In respect to non-activators the specificity is 86% resulting from correct predictions for non-activators.

An external validation was performed by standard random selection of chemicals before analysis of the training set. The result shows sensitivity and specificity - 70% and 85% respectively which confirms the stability of the predictions similar to these obtained for the training set.

Beside the standard validation procedure the model has been further evaluated by its application on a set of 37 chemicals (drugs and pesticides) experimentally tested for PXR activating effect. It was found that significant part of the investigated chemicals (70%) can not be predicted successfully as a result of lack of any previously identified structural fragments. In order to improve the model concerning pesticides a nine new structural alerts have been encoded as rules and implemented in the model. Regarding the group of drugs three new chemical categories were defined. It is expected that this upgrade of the model will be valuable in further screening of chemical datasets.

5.2. QSAR model for glucocorticoid receptor

Because of their ability to exert intense biological effects in almost any organ, corticosteroids are one of the most widely used drug classes (Avery et al., 2003). These steroids exert their main

effect by binding to glucocorticoid receptors (GRs), a member of the steroid–thyroid–retinoid receptor super-family (Kumar et al. 1999, Wardell et al. 2006). GRs are predominantly localized to the cytoplasm of target cells and move into the nuclear compartment only on binding of the glucocorticoid. Unfortunately, because of the intrinsic multiple activities of steroids and structural similar xenobiotics and because of the ubiquitous distribution of the corticosteroid receptors, unwanted side-effects such as osteoporosis, hypertension, insulin resistance, weight gain, fat redistribution, growth inhibition, and others (Buchman, 2001), can be initiated. Thus it became evident that early identification of potential binders toward GR could be classified as one of the issues of high toxicology concern.

In a recent study, a new QSAR model for prediction the binding potential of steroidal structures toward GR has been described (Todorov 2014). The investigated chemicals forming the training set are initially categorized according to their binding potency (expressed as relative binding affinity, %) as strong, moderate and weak binders. The binding effect is further analyzed in the QSAR Toolbox by contrasting the groups of Strong-Moderate as well as Moderate-Weak binders regarding large number of molecular descriptors. It has been found that measurement of ligands surface could be used successfully in discrimination of the binders in both groups. The parameter van der Waals surface area is used as discriminating parameter between strong and moderate binders with overall statistical performance of 73% correct predicted binders. More specific descriptor van der Waals partial negative surface area has been found to discriminate moderate from weak binders with performance of 88% correct results.

The results suggest that identified descriptors and their specific ranges are reliable and can be used as preliminary in silico evaluation in identification of steroidal structures as potential glucocorticoid binders.

6. CONCLUSIONS

It is evident that significant progress has been made in developing and validating QSAR models for the prediction of binding affinity for nuclear receptors. Almost all of the present commercial computational tools have been upgraded with modules for predictions of receptor mediated effects which make them very valuable for prioritizing potential EDCs for biological evaluation, thereby helping to find a majority of EDCs already at the beginning of systematic in vitro screening campaigns. On the other hand it should be emphasized the growing use of free computational tools such as OECD QSAR Toolbox which also allows application of scientific robust models for different biological endpoints including receptor mediated effects. In addition, a special attention should be set on the possibility of using these platforms for development and easy dissemination of newly derived models. However, the main problem which arises is the absence of standardization in terms of officially accepted guidelines pointing out good and acceptable practices for model development studies. Thus, harmonization, systematization, and standardization of criteria and methods involved in the *in silico* toxicology discovery should be the critical issue at the international level for the nearest future.

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