

IN SILICO IDENTIFICATION OF HUMAN PREGNANE X RECEPTOR ACTIVATORS

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Abstract

The nuclear receptor member human pregnane X receptor (hPXR) regulates enzymes and transporters involved in xenobiotic detoxification as well as maintains homeostatic balance of bile acids, thyroid and steroid hormones. hPXR can be recognized and activated by a structurally diverse array of environmental chemicals and drug compounds to initiate adverse biological effects, such as perturbing normal physiological functions and causing dangerous drug–drug interactions and exhibiting a high promiscuity in its ligand spectrum. In the current study capabilities for structure-activity modeling incorporated in the platform QSAR Toolbox were employed for investigation the binding mode and structural basis of hPXR interactions with various activators and non-activators. A total of 348 molecules, representing a variety of chemical structures, constituted the training set of the model. Validation of the model showed a sensitivity of 70%, a specificity of 85%, and a concordance of 77%. The developed model provide knowledge about molecular descriptors that may influence the effect of molecules to hPXR.

Key words: human pregnane X receptor, QSAR, drug-drug interaction, metabolism, in silico tools

1. INTRODUCTION

The human pregnane X receptor (hPXR) is a transcriptional regulator of a large number of genes involved in xenobiotic metabolism and excretion (Kliewer et al., 2002), which is activated by a diverse array of endogenous and exogenous compounds including antibiotics, glucocorticoids, herbal extracts and environmental substances, and regulates the expression of cytochromes P450, multidrug resistance proteins and transporters involved in the metabolism and elimination of these potentially harmful chemicals from the body. hPXR can be activated by various environmental chemicals, including polychlorinated biphenyls, phthalates and xenoestrogens, causing genotoxicity, endocrine disruption and teratogenicity (Hurst and Waxman, 2004). hPXR has also been found to play an important role in dangerous drug–drug interactions by up-regulating the gene expression of various enzymes involved in drug metabolism pathways (Teotico et al., 2008).

Because of its vital role in drug metabolism, it has been found that hPXR is responsible for decreased drug efficacy and increased drug toxicity. The promiscuous nature of hPXR ligand binding is a contributing factor in drug–drug interactions due to its capability of binding a variety of structurally diverse molecules that induce CYP3A4. CYP3A4 is considered the main drug-metabolizing CYP in human liver. It accounts for up to 40% of total CYPs in this organ and is responsible for the metabolism of more than 60% of drugs on the market today (di Masi et al., 2009). Bioactivation through CYP3A4 is often suspected as an initiating event in chemical toxicity. The induction of metabolizing enzymes and transporters by hPXR has also been regarded as one of the major mechanisms of drug resistance (Chen and Nie, 2009). Activation of hPXR may accelerate the metabolism and elimination of chemotherapeutic agents, contributing to resistance to chemotherapy. Given the clinical liabilities associated with developing hPXR activating compounds into drugs, it has become one of the primary objectives for drug-discovery programs to attenuate hPXR activity (Gao et al., 2007).

Previously, the hPXR crystal structures in complex with its activators SR12813 (Watkins et al., 2001) and T1317 (Xue et al., 2007) were solved by X-ray crystallography, which provide atomic-level insights into the structural basis and molecular mechanism of hPXR–ligand recognition and binding.

With this knowledge, structure-based analysis and computational screening of hPXR ligands would be valuable for pharmaceutical and toxicological research. Beside those areas the extracted knowledge may help in identification of potential ligands in environment by screening of large inventories of practical concern. However, up to date there were only limited successes with structure-based modeling approaches to predict hPXR activity. This is because, on the one hand, unlike the steroid, retinoid and thyroid hormone receptors that are highly selective for their cognate hormones, hPXR has been evolved to detect structurally diverse compounds, resulting in promiscuous hPXR–ligand interaction; on the other hand, hPXR protein possesses significant flexibility and disorder, which further complicates the theoretical modeling and prediction of the interaction (Chrencik et al., 2005).

The activation of hPXR has the potential to initiate a broad spectrum of adverse effects, and in this respect identification of hPXR ligands would be important information for evaluating health risk of environmental chemicals and drugs. Experimental determination of toxicity requires resources both in terms of cost and time. Therefore reliable in silico alternatives such as quantitative structure–activity relationship (QSAR) models are becoming important tools for rapid and cost-effective prediction of biological activities. Such models may have a great potential for use in the early identification of large numbers of potential hPXR activators. To date, the lack of large sets of PXR data has restricted ligand-based computational models to a relatively small universe of molecules (Ekins et al., 2009 and Khandelwal et al., 2008).

In this study a QSAR model was built for identification of hPXR ligands based on data obtained by human hPXR LBD binding assay. This approach can be used to elucidate the role of hPXR in toxicity and to increase the mechanistic understanding of toxic effects.

2. MATERIALS AND METHODS

2.1. Data sets

The training set consisted 348 chemicals with experimental data for hPXR binding taken from a single source (Matter et al., 2008). The classification scheme of hPXR activators and non-activators is based on half maximal effective concentration (EC₅₀) which refers to the concentration of a drug, antibody or toxicant able to induce a halfway response between the baseline and maximum after some specified exposure time. pEC₅₀ values were computed from $\log(\text{EC}_{50})$, with EC₅₀ in [M] (Matter et al., 2008). Compounds with a pEC₅₀ value > 4 were classified as hPXR activators and pEC₅₀ <4 as non-activators. The dataset was split randomly into a training set of 266 chemicals (used for model development) and a test set of 82 chemicals for validation of the ultimate model.

2.2. Modeling methodology

An important goal in QSAR modeling is to find a correlation between the structural features of ligands and their biological activity, that is, their ability to bind to specific target proteins. In general, a model can be derived by forming a relationship based on structural variation within the group of molecules under investigation and other parameters that describe their biological activities.

There are a number of software packages that allows such model development and subsequent screening of chemical datasets [Cronin et al., 2003]. It should be noted that the larger part of them are commercial and expensive which in general restrict their use to narrow range of customers. On the other hand a number of non commercial platforms became attractive for researchers allowing development of models and their application for endpoint prediction [Toxtree, Ambit]. In the current study the investigation was performed by using non-commercial software tool described in next section.

2.3. OECD QSAR Toolbox.

The development of the system was coordinated by the Organisation for Economic Co-operation and Development (OECD) and it was designed, encoded and currently maintained by the Laboratory of Mathematical Chemistry at “prof. Dr. Assen Zlatarov” University, Bourgas, Bulgaria [OASIS]. More specifically, the purpose of the system is to provide category formation and read-across to fill gaps in data needed for safety/hazard assessment of chemicals. In brief, analogues of a target chemical can be identified; data for these can be retrieved from included databases and read-across or trend analysis can be used to fill the data gap.

During the analysis investigated chemical(s) undergoes initial categorization by mechanism or mode of action by using profilers available within the software. Each profile consist a set of rules related to specific or general structural requirements. As an example a general profile encoding rules for organic functional groups can be applied for identification of specific functional groups in the chemicals under study. More specific structural moieties can be found by application of endpoint dependant profiles as those for DNA or protein binding.

Since the Toolbox allows manual incorporation of new profiles investigators are encouraged to developed and use their own profiling schemes for biological/toxic endpoints.

In the current study new profiling scheme for identification of potential hPXR binders is developed. The whole modeling process was performed in the Toolbox environment which includes analysis of the training set chemicals, construction of profiling scheme and validation of the model.

3. RESULTS AND DISCUSSION

The training set chemicals were imported into the Toolbox and classified in categories by using the profiler for organic functional groups. The encoded structural features represent functional groups which can be treated as pharmacophores with impact to the hPXR binding effect.

As a result of precise investigation of a set of 11 from all identified functional groups are considered as important toxicophores related to hPXR binding effect. They are summarized in Table 1.

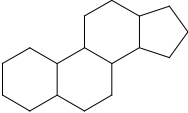
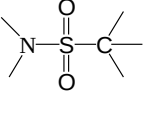
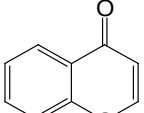
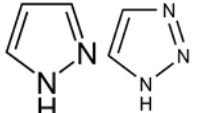
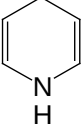
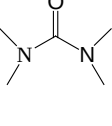
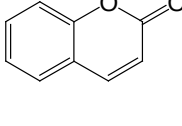
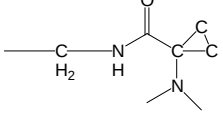
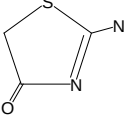
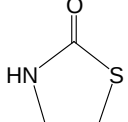
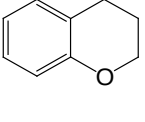
			
1. Steroids	2. Sulfonamides	3. Flavonoids	4. Pyrazoles and triazoles
			
5. Unsaturated heterocyclic amines	6. Urea derivatives	7. Coumarins	8. Carboxamides
			
9. Iminothiazoldione	10. Oxothiazolidine	11. Dihydrobenzopyran	

Table 1. Identified toxicophores supposed to modulate hPXR activation

A set of predictive models was developed based on a local groups formed from respective chemicals with same structural feature. In some cases additional molecular descriptor is required in order to distinguish hPXR activators from non-activators in a same category. Details on model development for each group are given in next sub-sections.

3.1. Steroids

A general structural rule related to steroidal chemical structures was constructed. It allows identification of diverse steroidal structures with aromatic or non aromatic A ring . It was found that a total number of 42 chemicals from the training set correspond to this rule (Table 1, #1). In order to explain the difference between activators (30 chemicals) and non activators (12 chemicals) additional investigation was performed to distinguish both groups. As a result a specific range of the descriptor van der Waals partial positive surface area (VWPPSA) was identified ($19 \div 32.4 \text{ \AA}^2(\text{a.u})$) which discriminate 23 from all 30 steroids PXR activators (sensitivity 77%) at the cost of 3 false positives, i.e., non activators with values in the “active” parameter range (specificity 75%). From mechanistic point of view the discriminating parameter is associated with electrostatic interactions between ligands and the receptor. In respect to the range of this parameter the hPXR activators exhibit lower values in comparison with non activators. It can be concluded that structural variations which lead to increasing of VWPPSA may result in inactivation of the steroidal chemicals toward hPXR.

3.2. Sulfonamides

A subset of 17 chemicals containing sulfonamide group was identified by application of a general structural rule for this chemical class (Table 1 #2). In the next step an attempt for separation between activators (13 chemicals) and non activators (4 chemicals) was done based on their hydrophobicity. The octanol/water partition coefficient $\log K_{ow}$ is associated with molecular hydrophobicity/hydrophilicity, which is thought to play a crucial role in promoting the binding of lipophilic ligands into the hydrophobic hPXR pocket. The identified $\log K_{ow}$ for activators is in the range from 3.15 to 5.50 in which 12 out of 14 chemicals fall in (86% sensitivity). All non-activators have values below 3.15 and it is evident that they are more hydrophilic as compared to activators. This is expected if considering that the hPXR pocket is highly hydrophobic that prefers hydrophobic activators rather than hydrophilic non activators.

3.3. Flavonoids

Flavonoids are a diverse group of phytochemicals sharing a common structural diphenylpyran skeleton, with two benzene rings linked through a heterocyclic pyran or pyrone ring. They generally have high biological activity and are considered to be beneficial due to their antioxidant properties and their ability to modulate several enzyme and receptor pathways.

The structural rule (Table 1, #3) constructed for this class identifies 7 structures. Six of them are hPXR activators by experimental data. In this case additional analysis for other molecular requirements cannot be performed due to lack of non activators. However, it can be assumed that structural features in flavonoids necessary for activation of the receptor are (1) the presence of hydroxyl groups, especially two of them generating the 3,4-catechol moiety; and (2) a planar molecule due to the presence of a C2–C3 double bond (Figure 1).

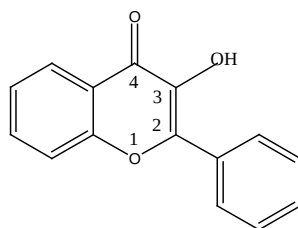


Fig. 1. General representation of Flavonoids and respective important features related to hPXR binding

3.4. Pyrazoles and triazoles

In many chemical structures pyrazole and triazoles moieties are considered to be important for pronouncement of diverse biological responses. As representative in this group clotrimazole was found to be hPXR activator. The binding affect can be related to formation of hydrogen bond between imidazole moiety of clotrimazole with specific part from the receptor. On the other hand it is assumed that van der Waals hydrophobic interactions contribute favorable for interaction between clotrimazole and the receptor.

Analysis of the training set shows that 27 chemicals are classified as pyrazoles and triazoles based on the respective structural rule (Table 1 #4). Eighteen chemicals have activating affect whereas nine chemicals are non activators. Next, both groups were separated by making use the parameter van der Waals surface (VWSurf. [$\text{\AA}^2$]). It was assumed that this parameter estimates the interacting areas of ligands and the receptor. The identified range $307\div 367 \text{ \AA}^2$ was found to be discriminative for 15 out of all 18 activators (sensitivity 83%). All non activators are out of this range which leads to maximal value for specificity (100%).

3.5. Unsaturated heterocyclic amines

Based on the structural criteria for this class (Table 1, #5) a set of seven chemical is defined. All they were hPXR activators. As representative chemical Felodipine shows strong activating effect. It is supposed that felodipine interacts with PXR through mostly hydrophobic interactions. In general the interactions for this class are considered to be also partially electrostatic however formation of hydrogen bonds is also supposed to influence the ultimate binding effect.

The proposed rule contains definition for structural requirement only. It can be expected that additional molecular descriptors may be relevant for definition of more specific rule, however the limited number of chemicals in this group are insufficient for such kind analysis.

3.6 Urea derivatives

A lot of evidences have been published that variety of urea derivatives may exert receptor mediated effects. For example one could mention the effect of phenylurea herbicides diuron, fenuron, monuron, linuron in activation of several receptors [Sharma et al., 2011]. In the current study based on structural rule (Table 1, #6) eighteen urea derivatives chemicals have been found. In respect to their hPXR effect 15 are activators and 3 non activators. In such a case with minimal number of chemicals in one from both groups (activators/non activators) it is difficult to introduce additional requirement for their better separation. However, in order to specify the rule more precisely an analysis was performed aiming to distinguish both groups by making use a relevant descriptor. The best discriminating parameter was found to be the minimum distance between two parallel surfaces where the molecule could be inscribed (*Diammin* [$\text{\AA}$]). Fourteen out of total fifteen activators were found to fall in specific range $6.4\div 7.9 \text{ \AA}$ with sensitivity of 93%. It was found also that all non activators have values below this range. One may assume that this parameter can be related to the planarity of the “active” spatial

orientation of the ligand. Following this hypothesis it is expected that there is a “planarity” threshold (assessed by *Diammin*) that can be used as measure for activating hPXR effect of urea derivatives ligands.

3.7. Coumarins

The coumarins are of great interest due to their biological properties. In particular, their physiological, bacteriostatic and anti-tumour activity makes these compounds attractive for further backbone derivatisation and screening as novel therapeutic agents. Both coumarin and coumarin derivatives have shown promise as potential inhibitors of cellular proliferation in various carcinoma cell lines [Egan et al., 1997].

A total number of five coumarins correspond to the structural rule (Table 1, #7) used in this study. As representative chemical the *7-hydroxycoumarin* was found to be hPXR activator. Another activator found in this class is *6-methoxy-7-hydroxycoumarin*. The limited number of coumarins (3 activators and 2 non activators) does not allow identification of further intrinsic chemical features that can be used for more precise prediction of new chemicals. However a structural comparison between activators and non activators results in conclusion that substituents in 3rd position in the general coumarin structure may eliminate the hPXR activity (Figure 2).

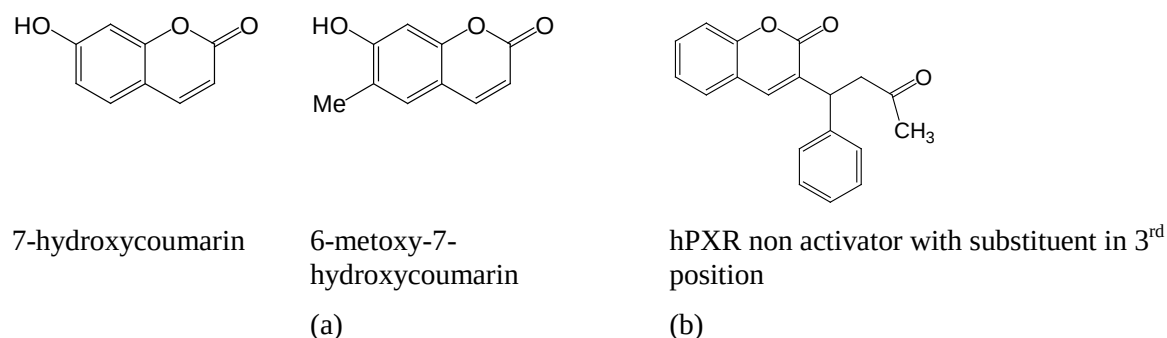


Fig 2. Representative coumarins as hPXR activators (a) and non activator due to presence of substituent in 3rd position (b)

3.8. Carboxamides

Carboxamides have received considerable attention owing to their wide range of biological and pharmacological activities [Konopa et al.,]. Thirteen chemicals have been found in the training set to belong to this chemical class. The larger part represents hPXR activators (13 chemicals) whereas two chemicals are non activators only. It was found that all activators are also polyfunctional compounds. In this respect along with the main structural rule (Table 1, #8) additional requirement for presence of aromatic ring is also added. It was also found that there are two chlorine atoms placed in meta position in the aromatic ring. Therefore an additional requirement specifying this finding was added in the ultimate construction of the rule for this chemical class.

3.9 Iminothiazolidiones

Iminothiazolidiones (Table 1, #9) are found to be represent as a set of eleven chemicals in the training set. The larger part are hPXR activators (9 chemicals). The insufficiency of non activators does not allow more specific definition in the group in terms of addition of other molecular descriptors. Nevertheless a general requirement for hydrophobicity by making use of octanol/water partition coefficient ($\log K_{ow}$) can be attributed. This is the range of $1.45 \div 3.70$ in which all activators fall in. In order to confirm the role of this parameter it is obvious that further investigations are needed by using

more expanded set of representative chemicals in this group. However the reliability of this chemical class in respect to hPXR can be supported by evidences that compounds bearing similar - thiazoline moiety have been reported to exhibit a wide spectrum of biological effects [Gaumont et al., 2009].

3.10 Oxothiazolidines

In the current analysis three hPXR activators containing 2-oxo-thiazolidine fragment (Table 1, #10) which are derivatives of thiazolidinone with a carbonyl group at the 2nd position was found. A literature search revealed that oxo-thiazolidine derivatives may exhibit variety of biological responses [Andres et al., 2000] which supports their activating effect of hPXR. This finding was used to introduce this structural rule as important for hPXR activation however further clarification based on additional chemicals in this group is required.

3.11. Dihydrobenzopyranes

Dihydrobenzopyran (Chromane) is one of the known pharmacophore which appears as an important structural component in natural compounds and generated great attention because of their interesting biological activity. It is a heterocyclic ring system consisting of a benzene ring fused to a pyran ring. It is known that certain natural and synthetic derivatives possess important biological activities including interactions with estrogen receptor [Nareshkumar et al., 2009]. The structural rule for this chemical class (Table 1, #11) identifies a total number of six chemicals in the training set. Four of them exhibits hPXR activating effect. It should be noted that all structures have substituents hydroxyl or methyl group in at least three position in the aromatic ring.

3.12. Performance and validation of the model

The ultimate model organized as a combination of all structural alerts (Table 1) and respective additional requirements was assessed by its application over the training set chemicals. The sensitivity is 72% in terms of correct predicted activators versus all activators in the set (114/158). In respect to non-activators the specificity is 86% resulting from correct predictions for 93 from all 108 hPXR non-activators. Given the fact that experimental error should be taken into account and the type of the model is a QSAR expert system a concordance of 77% is promising for reliable use in further predictions of untested chemicals.

An external validation was performed by standard random selection of chemicals before analysis of the training set. Based on application of the model over 82 chemicals the sensitivity was found to be 70% as a result of correct predictions for 30 from all 43 activators. Specificity was found to be 85% and the concordance is 77% which confirms the stability of the predictions obtained for the training set. However it should be noted that beside the good performance the model should be validated by using other available experimental data. Such a study will be a subject of further development of the model.

4. CONCLUSIONS

There are nowadays increasing interest in the QSAR studies, which consist of an important methodology that has been frequently used in medicinal chemistry and environmental science. Frequently, the experimental determination of biological properties of substances is very complex, time consuming and costly. On the other hand, the use of QSAR methods allows one to estimate through calculations and structural analysis which substances will be active or toxic, saving time money and effort. In the presented work, a QSAR model for identification of hPXR activators have been developed. It is based on a set of structural alerts expertly specified by making use of non commercial in silico tool. Eleven toxicophores related to different chemical classes was constructed as profiling scheme ready to be implemented and used in the OECD QSAR Toolbox. The applicability of

the model was assessed by internal (over the training set) and external (preliminary separated chemicals) validation procedure. The obtained results suggest that the model is reliable and can be used for screening purposes of structural heterogeneous chemical inventories.

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