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Modeling Organic Anion-Transporting Polypeptide 1B1 Inhibition to Elucidate Interaction Risks in Early Drug Design

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ABSTRACT

The importance of transporter proteins for the disposition of drugs has become increasingly apparent during the past decade. A noted drug-drug interaction risk is the inhibition of organic anion-transporting polypeptides (OATPs), key transporters for the liver uptake of the widely used statins. We show here the development of a ligand-based *in silico* model for interaction with OATP1B1, an important representative of the OATP family. The model is based on a structural overlay of 6 known OATP1B1 inhibitors. A data set of about 150 compounds with published OATP1B1 inhibition data was compared to the resulting "transportophor," and a similarity threshold was defined to distinguish between active and inactive molecules. In addition, using a statistical model based on physicochemical properties of the compounds as prefilter was found to enhance the overall predictivity of the model (final accuracy 0.73, specificity 074, and sensitivity 0.71, based on 126 compounds). The combined model was validated using an in-house data set (accuracy, specificity, and sensitivity were 0.63, 0.59, and 0.78, respectively; 62 compounds). The model gives also a structural overlay to the most similar template enabling visualization of where a change in a given structure might reduce the interaction with the transporter.

Introduction

During the past decade, the importance of transporter proteins for the uptake of drugs into various tissues, and the resulting potential for drug-drug interactions, has become more visible.[1-6] The continuous effort in drug design to find metabolically stable compounds with high affinity to the target under consideration is likely driving this development. A goal during drug design is to aim for compounds with high lipophilic ligand efficiency,[7] that is, compounds that have a high-specific affinity to the target but as low lipophilicity as possible to reduce unspecific binding to other targets including metabolizing enzymes. Metabolically stable compounds cannot depend on metabolic clearance for their elimination from the body but require other mechanisms including active transport.[8] Moreover, such compounds may also rely on uptake via special transporter proteins for being distributed to the tissue of interest because more hydrophilic compounds are less likely to cross lipophilic membranes through a purely passive mechanism.

Organic anion-transporting polypeptides (OATPs) are transporters at the basolateral side of hepatocytes. They have been shown to be of importance for liver uptake and thereby for clearance of endogenous acidic compounds, such as bile acids or bilirubin.[9-11] This pathway is also used by certain amphiphilic drugs. For example, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, also known as statins, make use of OATPs to reach their target within the hepatocyte. In addition, transport via OATPs ensures clearance from the blood and thereby reduces the risk of toxic side effects.[12] Inhibition of OATPs can therefore lead to interaction with a drug's disposition, its safety risks, and action. New pharmaceutics likely to be comedicated with statins need to avoid any interaction with OATPs.[13] Successful drug design in the cardiovascular area, where statins are considered standard of care,[14] therefore depends on a good understanding of the structure-transporter activity relationship early on the drug discovery process.

OATP1B1 inhibition potential can be measured *in vitro* through determining how much the transport of a known OATP1B1

Abbreviations used: 2D, two dimensional; 3D, three dimensional; DDI, drug-drug interaction; HEK cells, human embryonic kidney cells; IC50, inhibition concentration to give 50% inhibition (i.e., half maximal inhibitory concentration); OATP, organic anion-transporting polypeptide; PCA, principal component analysis; PLS, projection to latent structures (also known as partial least squares analysis); r^2, coefficient of determination; r^2(CV), cross-validated coefficient of determination; TS score, transportophor similarity score.

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substrate is hindered by addition of the query compound using OATP1B1-transfected cell lines.[15,16] Fluorescent marker substrates have been suggested for utilization in high throughput screening.[17-20] Knowledge of the substrate used is important to understand which data are comparable because inhibition constants vary depending on the probe substrate used.[9,16,21] Specific drugs known to be OATP1B1 substrates or estradiol-17β-glucuronide have been suggested as most relevant probe substrates for drug-drug interaction risk assessment.[4,21]

In early drug discovery, and to more actively guide compound design, *in silico* models can be used. Various approaches to model transporter interactions have been reported,[22-25] including an increasing number of models for OATP1B1 interactions: In 2005, Chang et al.[26] defined a pharmacophore for OATP1B1 substrates consisting of 2 hydrogen bond acceptors, 1 hydrogen bond donor and a central lipophilic region, based on literature data for human and the rat. Inhibitor models for OATP1B1 most often use quantitative structure activity–relationship approaches.[15,16,27-31] Lipophilicity and hydrogen bonding were found to be important molecular properties that influence OATP1B1 inhibition in most of these studies.[28,30] Using comparative molecular field analysis, Gui et al.[32] analyzed 18 diverse inhibitors and proposed an interaction mode with a central core and peripheral polar regions, one of which is acidic, as structural features that promote interactions with the transporter. It was discussed that pharmacophore models might be difficult to obtain for diverse sets of compounds[30] since the varying inhibition constants depending on the substrate used,[9] as well as biphasic binding kinetics for example in the case of estrone-3-sulfate,[33,34] indicate the possibility of different binding sites.

The aim of the present work was the development of a fast ligand-based *in silico* model for inhibition of OATP1B1 that can be used to guide drug design in early drug discovery. We used a rapid pharmacophore type approach, alignment of molecular shape,[35,36] as primary method, based on a small set of established OATP1B1 inhibitors. Note that these compounds also can be transported by the protein and most likely act as competitive inhibitors. Comparing query structures to this alignment, designated "transportophor," resulted in both a similarity score and the best structural fit. The similarity score was able to indicate whether a compound had low or high risk for OATP1B1 inhibition, and a similarity score threshold was defined. The best structural fit showed which parts of a query structure were aligned with the features of a known inhibitor, thereby indicating which part of the molecule may be important for the interaction with the transporter. However, we found that a prefilter based on physicochemical descriptors, using the projection to latent structure (PLS) method, clearly enhanced the prediction quality, as shown in the external validation using in-house compounds.

Materials and Methods

Compound Sets

Three different compound data sets were used in this study. Data set 1 consists of 6 known OATP1B1 inhibitors (estrone-3-sulfate, estradiol-17β-glucuronide, taurocholic acid, rosuvastatin, pitavastatin, and pravastatin)[15,26] and was used to define the alignment rule for the transportophor generation (see Fig. 1). Three compounds belong to the class of endogenous steroids and the remaining are statins, that is, compounds of particular interest in the cardiovascular research area. The activity for the selected compounds ranges from 60% inhibition for pravastatin and rosuvastatin to >90% for pitavastatin. All 6 compounds have a negatively charged moiety, a lipophilic core and at least 1 hydrogen bond acceptor. The negatively charged moiety is not always a carboxylic acid but can be any acid isostere.

Data set 2 comprises 146 compounds reported by Karlgren et al.[15] and was used for determination of the similarity threshold to discriminate between active and inactive compounds through comparison to the transportophor. The data set was also used as training set for the PLS model based on physicochemical parameters. Inhibition of OATP1B1-mediated transport had been measured as described in human embryonic kidney cells transfected with OATP1B1, using estradiol-17β-glucuronide (0.5 μM) as substrate. The test compound concentration was 20 μM.[15]

Data set 3 is a set of 63 AstraZeneca proprietary compounds that was used for validation. OATP1B1 inhibition measurements for these compounds were performed in human embryonic kidney cells transfected with OATP1B1 as described by Soars et al.,[16] using estradiol-17β-glucuronide as substrate. The compounds were

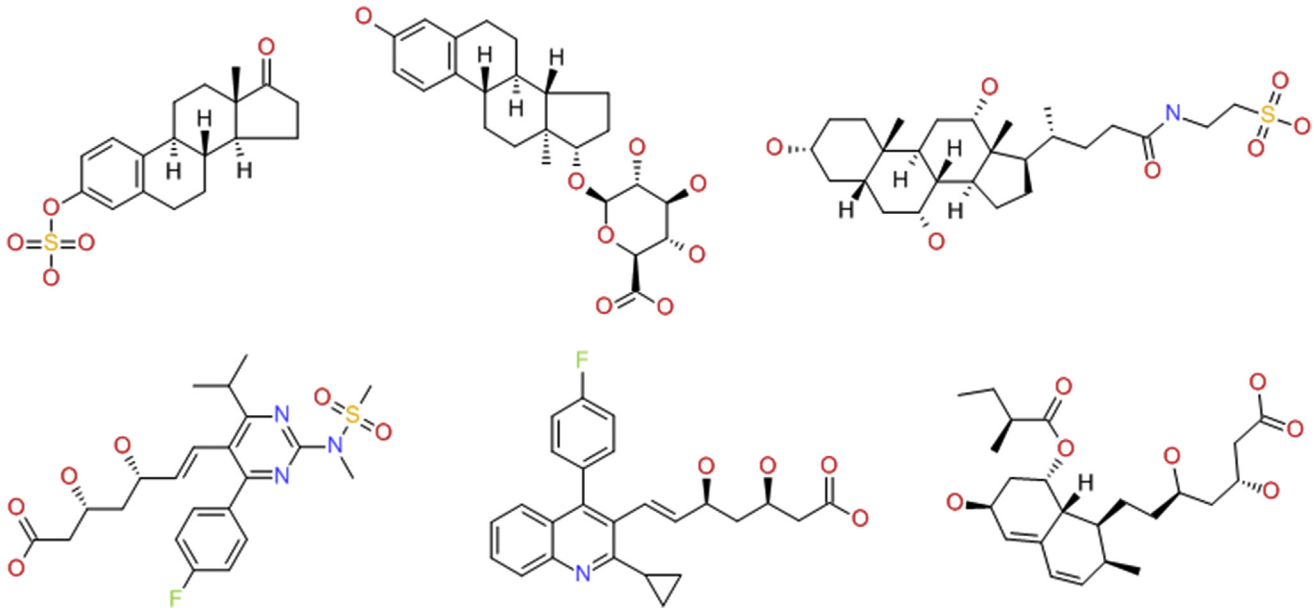

Figure 1. Six known OATP1B1 inhibitors: upper row: estrone-3-sulfate, estradiol-17β-glucuronide, taurocholic acid; lower row: rosuvastatin, pitavastatin, pravastatin.