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Prediction of binding affinity and efficacy of thyroid hormone receptor ligands using QSAR and structure-based modeling methods

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ABSTRACT

The thyroid hormone receptor (THR) is an important member of the nuclear receptor family that can be activated by endocrine disrupting chemicals (EDC). Quantitative Structure–Activity Relationship (QSAR) models have been developed to facilitate the prioritization of THR-mediated EDC for the experimental validation. The largest database of binding affinities available at the time of the study for ligand binding domain (LBD) of THRβ was assembled to generate both continuous and classification QSAR models with an external accuracy of $R^2 = 0.55$ and CCR = 0.76, respectively. In addition, for the first time a QSAR model was developed to predict binding affinities of antagonists inhibiting the interaction of coactivators with the AF-2 domain of THRβ ($R^2 = 0.70$). Furthermore, molecular docking studies were performed for a set of THRβ ligands (57 agonists and 15 antagonists of LBD, 210 antagonists of the AF-2 domain, supplemented by putative decoys/non-binders) using several THRβ structures retrieved from the Protein Data Bank. We found that two agonist-bound THRβ conformations could effectively discriminate their corresponding ligands from presumed non-binders. Moreover, one of the agonist conformations could discriminate agonists from antagonists. Finally, we have conducted virtual screening of a chemical library compiled by the EPA as part of the Tox21 program to identify potential THRβ-mediated EDCs using both QSAR models and docking. We concluded that the library is unlikely to have any EDC that would bind to the THRβ. Models developed in this study can be employed either to identify environmental chemicals interacting with the THR or, conversely, to eliminate the THR-mediated mechanism of action for chemicals of concern.

Introduction

Endocrine disrupting chemicals (EDCs) are natural or synthetic compounds that have the potential to interfere with the endocrine system, often through imitating or blocking endogenous hormones (Rogers et al., 2013). Fetal and early life exposures appear to have more severe effects than exposure in adulthood on developmental, reproductive, cardiovascular, metabolic, and immune systems (Birnbaum and Fenton, 2003; Rubin and Soto, 2009).

EDCs may act via multiple pathways; however one privileged route is through their direct interaction with nuclear receptors (NRs), which leads to perturbation or modulation of downstream gene expression. The thyroid hormone receptors (THR) are important members of the NR family and act as regulators of metabolism, fetal development, bone remodeling, cardiac function, and mental status. Thus, maintenance of normal thyroid function is essential for psychological and physiological human well-being. Long-term exposure to thyroid-disrupting chemicals may potentially result in hypothyroidism and have other significant consequences for human health (Boas et al., 2012).

The majority of THR responses are induced by the thyroid hormone T3 (Harvey and Williams, 2002). There are two main isoforms of THR (THRα and THRβ), and each form can be alternatively spliced and differentially localized across tissue types (Izumo and Mahdavi, 1988; Williams, 2000). THRα_1 is highly expressed in cardiac and skeletal muscles accounting for cardiac responses to the endogenous T3. On the other hand, most of the hormonal effects in the liver (including the influence on the cholesterol metabolism), brain and other tissues are mediated through THRβ_1 (Forrest and Vennstrom, 2000; Takeda et al., 1992). Thus, the ability to recognize environmental chemicals causing THRβ-mediated endocrine disruption is highly important. In addition, agonists and antagonists of THRβ can be used therapeutically for treating several thyroid and non-thyroid disorders. For example, THRβ antagonists serve as therapies for thyrotoxicosis whereas highly selective agonists are used to treat metabolic disorders such as obesity,

Abbreviations: AD, applicability domain; AUC, area under the curve; T3, 3,5,3′-Triiodo-L-thyronine; THR, thyroid hormone receptor; EDCs, endocrine disrupting chemicals; EF, enrichment factor; EPA, US Environmental Protection Agency; PDB, Protein Data Bank; QSAR, Quantitative Structure–Activity Relationships; RF, Random Forest; ROC, receiver operating characteristic; SDF, structure-data file.

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for lowering cholesterol to treat hyperlipidemia, for amelioration of depression, and for stimulation of bone formation in osteoporosis (Grover et al., 2004; Shoemaker et al., 2012). However, development of newer compounds with increased selectivity is required to achieve higher precision of action and avoid adverse effects such as cardiotoxicity mediated by THRs (Forrest and Vennstrom, 2000).

Several functional domains of THR have been identified, which include a DNA binding domain, a ligand binding domain (LBD), a ligand-independent transactivation domain (termed activation function 1 or AF-1), and a ligand-inducible coactivator binding domain (termed activation function 2 or AF-2) (Kumar and Thompson, 1999). In the absence of the ligand, co-repressor proteins are bound to THR preventing transcriptional activation (Chen and Evans, 1995). Ligand binding to the LBD causes dissociation of co-repressors and allows recruitment of co-activator proteins to the AF-2 domain to regulate gene transcription (Ribeiro et al., 1998). Many ligands that bind to the LBD have been reported in the literature; for instance, several selective ligands for THRβ have been identified based on the structural similarity to the endogenous thyroid receptor hormones, T4 and T3 (Carlsson et al., 2002; Garcia Collazo et al., 2006; Garg et al., 2007; Hangeland et al., 2004; Hedfors et al., 2005; Koehler et al., 2006; Li et al., 2006; Malm et al., 2007; Ye et al., 2003).

Recently, a series of THR antagonists have been identified that inhibit THR-coactivator interaction by binding to the AF-2 domain (Arnold et al., 2005, 2006, 2007a,b; Estebanez-Perpina et al., 2007a; Hwang et al., 2009, 2011, 2012, 2013). These compounds may be useful to treat metabolic disorders and hyperthyroidism without affecting thyroid hormone levels; however additional studies revealed significant dose-related cardiotoxicity, suspected to arise from ion-channel inhibition (Arnold et al., 2005, 2006, 2007a,b; Estebanez-Perpina et al., 2007a; Hwang et al., 2009, 2011, 2012, 2013).

Computational methods such as QSAR modeling have been widely used to prioritize chemicals for in vivo or in vitro testing that may pose endocrine disruption hazard (Lo Piparo and Worth, 2010; Tsakovska et al., 2011). Several groups have reported QSAR models for the LBD of THR. These models are summarized in Table 1. Although these previous models were reported to have significant predictive power, all of them were created using relatively small datasets with limited chemical diversity and consequently, these models had a limited applicability domain (AD) (Tropsha, 2010). In addition, these previous models were developed to predict THR binding affinity but none was capable of distinguishing the type of functional activity, or efficacy (i.e., agonism vs. antagonism) of the ligands. Finally, to date no QSAR studies have been reported to predict biological activity of compounds that bind to the AF-2 domain.

In this study, we have assembled the largest dataset (as compared to all data reported in the open literature) of ligands tested for their interaction with the THR, including data on the THRβ$_1$ binding affinity (129 compounds binding at the LBD and 181 compounds binding at the AF-2 domain) and functional activity (57 agonists/15 antagonists binding at the LBD, 210 antagonists binding at the AF-2 domain). Using OECD (Organization for Economic Co-operation and Development)-compliant predictive QSAR modeling workflow (Tropsha, 2010), we have developed both continuous and categorical QSAR models for ligands of both the LBD and the AF-2 domain. Furthermore, we have identified co-crystalized complexes between THR and several ligands in the Protein Data Bank that allowed the use of molecular docking to classify ligands binding at the LBD as either agonists or antagonists. The predictive models developed in this study are suitable to use in virtual screening to identify putative THR binders and non-binders in environmental chemical libraries and classify them into agonists or antagonists. Here we present an example of such a study using the EPA Tox21 database of suspected endocrine disrupting chemicals. In addition, these models can be employed to exclude THR-mediated mechanism of endocrine disruption for environmental chemicals of concern.

Materials and methods

Datasets

THRβ binding affinity. 129 unique organic compounds with known binding affinity to the LBD of the THRβ were collected from ChEMBL (Gaulton et al., 2012). Their binding affinities and structures were verified against published literature (Du et al., 2008; Hedfors et al., 2005; Liu and Gramatica, 2007; Malm et al., 2007; Valadares et al., 2007; Vedani et al., 2007). The affinity data were reported as IC_{50} values determined from the radioligand binding assay as described by Ye et al. (2003). For the AF-2 domain, 210 ligands were found; however, only 181 had well-defined IC_{50} values (Arnold et al., 2007b; Hwang et al., 2009, 2012) (see Table 2). The IC_{50} values associated with inhibition of co-regulatory peptide SRC2-2 binding to THRβ were determined using fluorescence polarization (Arnold et al., 2007b; Hwang et al., 2009, 2012).

Collected IC_{50} values varied from 0.0191 nM to 32 μM and from 0.310 μM to 100 μM for LBD and AF-2 domains, respectively. The IC_{50} values were converted to $-\log IC_{50}$ (pIC_{50}). As can be seen in the Supplemental Fig. 1, pIC_{50} values of ligands for both domains showed normal distribution; however, most of the AF-2 domain ligands were not very potent. The chemical structures for 129 and 181 compounds able to bind at the LBD and AF-2 domains, respectively, are included in the Supplemental Table 1.

THRβ functional activity. 57 known agonists and 15 antagonists for the LBD were obtained from ChEMBL (Gaulton et al., 2012) and their functional annotation was verified using published literature (Carlsson et al., 2002; Garcia Collazo et al., 2006; Garg et al., 2007; Hedfors et al., 2005; Ye et al., 2003). In addition, 5101 presumed decoys were obtained from the DUD-E (Database of Useful Decoys: Enhanced) database (Mysinger et al., 2012). Presumed decoys are defined as chemicals that have similar physical properties but are topologically dissimilar from the known ligand structures and are expected not to bind to the respective receptor. For the AF-2 domain, 210 known antagonists were obtained from the published literature (Arnold et al., 2005, 2006, 2007a,b; Estebanez-Perpina et al., 2007a; Hwang et al., 2009, 2011,

Table 1
Previous QSAR studies of THRβ$_1$.

Reference	Modeling method description	Number of compounds in dataset[a]	Reported prediction accuracy for test sets (R^2)
Liu and Gramatica (2007)	MLR with variable selection. (0–3)D Dragon descriptors	85 (21)	0.73
Vedani et al. (2007)	Multi-dimensional QSAR (Quasar and Raptor software)	82 (18)	0.796
Valadares et al. (2007)	Classical QSAR with 2D Dragon descriptors and Hologram QSAR (specialized fragment fingerprints)	68 (13)	0.84
Du et al. (2008)	3D QSAR (CoMFA and CoMSIA)	61 (12)	0.68
Ren et al. (2007)	Projection Pursuit Regression (PPR) with variable selection. CODESSA descriptors	80 (13)	0.893

[a] Number of compounds in test set is given in parentheses.