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Molecular docking, molecular dynamics simulation, and structure-based 3D-QSAR studies on estrogenic activity of hydroxylated polychlorinated biphenyls

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HIGHLIGHTS

► The estrogenic activities of HO-PCBs were studied by 3D-QSAR method.
► The binding modes between two HO-PCBs and ER were investigated.
► 3D-QSAR, molecular docking, and molecular dynamics (MD) simulation were performed.

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ABSTRACT

Hydroxylated polychlorinated biphenyls (HO-PCBs), major metabolites of PCBs, have been reported to present agonist or antagonist interactions with estrogen receptor α (ERα) and induce ER-mediated responses. In this work, a multistep framework combining molecular docking, molecular dynamics (MD) simulations, and structure-based three-dimensional quantitative structure–activity relationship (3D-QSAR) studies were performed to explore the influence of structural features on the estrogenic activities of HO-PCBs, and to investigate the molecular mechanism of ERα–ligand interactions. The CoMSIA (comparative molecular similarity indices analysis) model was developed from the conformations obtained from molecular docking. The model exhibited statistically significant results as the cross-validated correlation coefficient q^2 was 0.648, the non-cross-validated correlation coefficient r^2 was 0.968, and the external predictive correlation coefficient r^2_{pred} was 0.625. The key amino acid residues were identified by molecular docking, and the detailed binding modes of the compounds with different activities were determined by MD simulations. The binding free energies correlated well with the experimental activity. An energetic analysis, MM-GBSA energy decomposition, revealed that the van der Waals interaction was the major driving force for the binding of compounds to ERα. The hydrogen bond interactions between the ligands and residue His524 help to stabilize the conformation of ligands at the binding pocket. These results are expected to be beneficial to predict estrogenic activities of other HO-PCB congeners and helpful for understanding the binding mechanism of HO-PCBs and ERα.

1. Introduction

Polychlorinated biphenyls (PCBs) are synthetic organic compounds that have been produced and used for decades. Although production and use of PCBs were banned in most of the countries, these compounds have still been detected in the environment and have received concerns from environmental and ecological perspectives (Buckman et al., 2011; Dirtu et al., 2010; Kodavanti et al., 2011). As a major biological metabolite group of PCBs, hydroxylated PCBs (HO-PCBs) are formed in the biotransformation process of PCBs, which is mediated by cytochrome P450 (CYP) monooxygenase enzymes (Fernandez et al., 2008). To date, several HO-PCBs have been reported to be present in human adipose tissue and serum (Gomara et al., 2012; Nomiyama et al., 2010b).

Adverse effects of estrogenic activity on wildlife, as well as humans, have attracted great attention. HO-PCBs have structural similarities to endogenous compounds, and in vitro and in vivo studies have shown that certain HO-PCBs may have adverse effects on estrogen hormone homeostasis (Meerts et al., 2004; Nomiyama et al., 2010a; Takeuchi et al., 2011). For example, some HO-PCBs showed estrogenic activities in different assay systems, the *Xenopus laevis* vitellogenin (VTG)-assay and yeast two-hybrid assay for estrogen receptor α (ERα) (Nomiyama et al., 2010a). Recently, Takeuchi et al. (2011)

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reported that several HO-PCBs were estrogenic on the basis of reporter gene assay. Consequently, HO-PCBs have attracted great attention.

Concerning the initial step for mode of action is binding to an intracellular receptor (Kavlock et al., 1996), it is supposed that HO-PCBs influence the normal estrogen function and cycle mainly through competing with the endogenous endocrines for the binding to ERα, which is the predominant ER expressed in the uterus, kidney, and ovarian theca cells (Kuiper et al., 1997). Given that the number of HO-PCBs is large, and some purified HO-PCB standards are difficult to be obtained, there is increasing interest to use productive and cost-efficient in silico modeling approaches, such as quantitative structure–activity relationship (QSAR), to predict the biological activities of HO-PCBs (Ekuase et al., 2011; Niu et al., 2007; Ruiz et al., 2008) and affinities of compounds to receptors (Li et al., 2010). Furthermore, to develop a QSAR model on HO-PCBs, it is necessary to understand the mechanisms of interaction between the HO-PCBs and ER. Molecular docking and molecular dynamics (MD) simulations have become an integral part of many structure-based computational simulations of compounds (Gharaghani et al., 2012; Mouchlis et al., 2012b). Molecular docking optimizes the bound ligand into the active site of receptor protein, and investigates protein–ligand interactions. MD simulations investigate the interaction mechanism of protein complex with ligands at the atomic level, and provide dynamic structural information. Combinational use of QSAR, molecular docking, and MD simulations is useful in defining the ligand–receptor binding modes and can provide possible mechanism interpretations (Hao et al., 2011; Liu et al., 2012; Yuan et al., 2011).

In this study, a multistep framework by combining molecular docking, MD simulations, and 3D-QSAR (comparative molecular similarity indices analysis, CoMSIA) was performed to investigate the detailed binding mode between ERα with HO-PCBs and also to develop a rational estrogenic activity predictive model. Molecular docking was applied to dock all the compounds into the active site of ERα and the docked pose of each compound was subsequently used in a receptor-based alignment for the generation of the CoMSIA fields. A 3D-QSAR CoMSIA model was then developed. Moreover, the probable binding modes of two compounds with much difference in their activity and ERα were analyzed based on the results from molecular docking and MD simulations. This computational workflow could provide some insights into the structural characteristics that affect the estrogenic activity of HO-PCBs and help to understand the binding process.

2. Materials and methods

2.1. Data set

For the present molecular modeling study, a set of 44 HO-PCBs with reported estrogenic potency was obtained from published literature (Takeuchi et al., 2011). The estrogenic potency to the ERα was estimated by in vitro ERα dependent reporter gene assays. The estrogenic activity in terms of 20% relative effective concentration (REC_{20}, M) was gained from the dose–response curve of the luminescence intensity. In this study, the REC_{20} values were converted to the corresponding $pREC_{20}$ ($-\log REC_{20}$) and used as dependent variables in the current 3D-QSAR analyses. The whole data set was divided, in the ratio of 4:1, into training set (containing 35 compounds) for 3D-QSAR model generation and test set (containing 9 compounds) for model validation, respectively. The test set compounds were selected randomly by considering if they can appropriately represent the structural diversity and the distribution of activity. All the compounds and associated estrogenic activity are listed in Table 1.

The 3D-structures of HO-PCBs were initially constructed by the sketch molecule module of Sybyl 7.3 molecular modeling package (Tripos Inc., St. Louis, MO, USA). Structural energy minimization was performed using Powell gradient algorithm and the Tripos force field (Clark et al., 1989) with a convergence criterion of 0.001 kcal/mol·Å and a maximum of 1000 iterations. MMFF94 charges were assigned to each compound. The minimized structure was used as the initial conformation for molecular docking.

2.2. Molecular docking

To identify the probable bioactive conformations of these compounds and investigate the protein–ligand interactions, the Surflex-Dock program (Jain, 2003, 2007) interfaced with Sybyl 7.3 was used to dock all the compounds into the ligand-binding domain (LBD) of ERα. There were over 60 crystal structures containing ERα or ERα and ERβ in the Protein Data Bank (http://www.rcsb.org/pdb/). Considering the structural similarity of the native ligand and target compounds in this study, and the stability of the active pocket, the crystal structure of ERα LBD bound to 17β-estradiol (E2) (PDB code: 1ERE) (Brzozowski et al., 1997) was selected and used in the docking process. Prior to docking, all the native ligands (E2) and water molecules were removed from the protein structure, polar hydrogen atoms were added and Kollman All atom charges were assigned to protein atoms. The other parameters were set to default values. The Surflex-Dock supports a fully automated flexible docking procedure for the ligand and relies on the rigid-receptor approximation to treat ligand–receptor binding (Jain, 2003). In this program, ligands were automatically docked into the binding site of protein using a ProtoMol based approach (Zhang et al., 2011) with an empirical scoring function and a patented search engine. ProtoMol, a computational representation of the intended binding site with which putative ligands make potential interaction, can be generated by three modes: automatic, ligand, and residues modes (Tripos Inc., 2006). In this study, the automatic-based mode was adopted to generate the ProtoMol, with two important parameters, i.e., ProtoMol bloat and ProtoMol threshold at their default values of 0 and 0.50 Å, respectively. With the other defaulted parameters used and the option of ring flexibility selected, 10 conformations per ligand were obtained. The final conformation assumed to represent the bioactive conformation of ligands and the probable protein–ligand interactions was selected based on the following two criteria: (i) the conformation possessed the highest docking score, and (ii) the orientation of the conformation is similar with that of the cocrystallized ligands. In the molecular docking, the structure of host protein is rigid and the structures of ligands are flexible.

2.3. 3D-QSAR studies

Molecular alignment of compounds was one of the most important steps in 3D-QSAR analysis (Ai et al., 2011). To derive the optimal 3D-QSAR statistical model, two different alignment rules were adopted. One method is the ligand-based alignment. In this approach, all compounds were aligned to the most potent compound 4′-OH-CB50 by the Align Database command in Sybyl. The other method is the receptor-based alignment. By this process, the bioactive conformations of all compounds firstly derived from molecular docking were assigned MMFF94 charges and imported into a SYBYL molecular database for 3D-QSAR studies without further energy minimization.

The CoMSIA models were developed for the aligned molecular data set. In CoMSIA, five molecular fields were calculated: steric, electrostatic, hydrophobic, hydrogen bond donor, and hydrogen bond acceptor. The CoMSIA descriptors were calculated by a 3D cubic lattice with grid spacing of 2 Å and extending 4 Å units beyond the aligned molecules in all directions. The default value of 0.3 was used as the attenuation factor α (Yang et al., 2011a).

In order to generate statistically significant 3D-QSAR models, the partial least squares (PLS) analysis was employed to correlate the CoMSIA fields to the $pREC_{20}$ values. PLS was performed in two stages. First, a leave-one-out (LOO) cross-validation analysis was performed to determine the optimum number of components (ONC) and cross-validated correlation coefficient (q^2). Second, non-cross-validation analysis was