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Qualitative and quantitative simulation of androgen receptor antagonists: A case study of polybrominated diphenyl ethers

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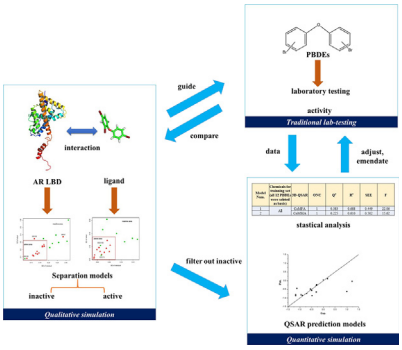
HIGHLIGHTS

- MD-based separation was the most reliable among the three qualitative simulations.
- The MD-based identification model could identify the activeness of 90.5% of PBDEs.
- A new workflow including qualitative and quantitative simulations was generated.
- The workflow could avoid "false positive" that exist in the QSAR prediction.

GRAPHICAL ABSTRACT



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ABSTRACT

Recently, great attention has been paid to the identification and prediction of the androgen disrupting potencies of polybrominated diphenyl ethers (PBDEs). However, few existing models can discriminate active and inactive compounds, which make the quantitative prediction process including the quantitative structure-activity relationship (QSAR) technique unreliable. In this study, different grouping methods were investigated and compared for qualitative identification, including molecular docking and molecular dynamics simulations (MD). The results showed that qualitative identification based on MD, which is lab-independent, accurate and closer to the real transcriptional activation process, could separate 90.5% of active and inactive chemicals and was preferred. The 3D-QSAR models built as the quantitative simulation method showed r^2 and q^2 values of 0.513 and 0.980, respectively. Together, a novel workflow combining qualitative identification and quantitative simulations was generated with processes including activeness discrimination and activity prediction. This workflow, for analyzing the antagonism of androgen receptor (AR) of PBDEs is not only allowing researchers to reduce their intense laboratory experiments but also assisting them in inspecting and adjusting their laboratory systems and results.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of organic flame retardants that were widely used as additives in the production of furniture, textiles, building materials and electronic equipment (Talsness, 2008). The continuous use of PBDEs in commercial products started

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from the late 1960s, and tens of thousands of tons of PBDEs were manufactured globally (Hites, 2004; De Wit, 2002). Although commercial pentabromodiphenyl ethers (pentaBDE) and octabromodiphenyl ethers (octaBDE) have been banned worldwide (W.L. Li et al., 2016; Hites, 2004; Fromme et al., 2016), decabromodiphenyl ethers (decaBDEs) are still manufactured a lot, and their persistence, potential for bioaccumulation and constant leaching from household items result in ubiquitous detection in biotic and abiotic matrices, such as sediments (Piazza et al., 2016; Y.Y. Li et al., 2016), fish (Costa et al., 2016; Widelka et al., 2016), birds (Colabuono et al., 2015), human plasma (Fromme et al., 2016; Wainman et al., 2016; Curren et al., 2014) and even mother's milk (Fromme et al., 2016; Curren et al., 2014). Due to the reported potential endocrine disrupting activities mediated by the thyroid receptor (TR), estrogen receptor (ER) and aryl hydrocarbon receptor (AhR), PBDEs are classified as potential endocrine disrupting chemicals (EDCs) (Jin and Li, 2010; Hamers et al., 2006).

Both *in vitro* and *in vivo* assays have shown that some PBDEs could inhibit the androgen receptor (AR) activity (Hu et al., 2011; Liu et al., 2011; Zhao et al., 2011). Some chemicals have been shown to be able to suppress androgenic effects in lower doses than induce estrogenic effects (Zhang et al., 2011). Due to the 209 theoretical congeners of these chemicals, it is expensive and time-consuming to detect the AR-related activities of all of the chemicals. Meanwhile, the process of hazard assessment of existing industrial chemicals is currently undergoing significant changes. Traditional toxicity testing, which is conducted by the use of live animals, requires large amounts of individuals, uses large numbers of dangerous highly-purified toxic chemicals and is labor intensive, which are unsuitable from an economic, ethical and cultural perspective. As a consequence, there has been increasing interest to develop alternative approaches that could alleviate these limitations while allowing for reliable and objective assessments of toxicities.

The quantitative structure-activity relationship (QSAR) model, which is based on the relationship between chemical structures and their effects, can be conducted to precisely predict the biological activities of untested compounds, and it has been promoted by the U.S. Environmental Protection Agency (U.S. EPA) and OECD (Zeeman et al., 1995). In the last three decades, a number of QSAR models, which were aimed as predictive methods for quantitative assessments of the activities of unknown chemicals, have been built. QSAR models are built on structural similarities between chemical structures. By comparing the tested with untested compounds, the activities of untested chemicals can be predicted. However, as structural similarity does not necessarily equate with functional similarity, chemicals might exhibit wildly different effects while sharing similar structures, and some inactive compounds could be falsely predicted as being active by QSAR models. Moreover, in QSAR studies, almost all authors only choose known active chemicals and ignore inactive chemicals in model building (Xu et al., 2007; Wang et al., 2005), resulting in "false positive" phenomenon. Accordingly, the QSAR techniques have great capability for quantitative simulation, but without proper qualitative simulation to group and identify inactive compounds, the huge number of traditional QSAR models built may be useless in real practice.

Till nowadays, the identification methods are mainly docking-based, including ligand- and receptor-based approaches. In recent years, the rapid accumulation of high-resolution 3D structures and homology modeling techniques accompanied by the improvements in docking and scoring technologies are making the identification methods an attractive possibility for the qualitative simulation of the activity division (Tuccinardi, 2009; Mouchlis et al., 2012). However, the inflexibility of the backbone or even all of the atoms of the receptor in molecular docking could not describe the actual movement and interactions between ligands and receptors, which weaken the authenticity and accuracy of these qualitative simulations. Results of our previous studies have shown that molecular dynamics (MD) simulations, which could mimic the actual interactions between ligands and receptors, are an effective method for qualitative simulation (Wang et al., 2013). Moreover, our previous research also showed that by analyzing the fluctuation of H12 and ligands generated by MD simulations, the AR-antagonism could be recognized from flavonoids (Wu et al., 2016). However, the qualitative simulation has never been conducted on PBDEs.

The main purpose of this study is to set up a reliable qualitative grouping protocol to discriminate active and inactive AR-antagonists of PBDEs, build quantitative models to predict the activities of these known active PBDEs, and build a novel workflow by combining qualitative simulation and quantitative prediction. As the inactive chemicals could be identified by the workflow, the high throughput protocol can help researchers estimate the AR-antagonism of PBDEs more conveniently and accurately.

2. Materials and methods

2.1. Chemicals and activities

For the present study, a set of 21 PBDEs was obtained from two published works in the literature (Table S1) (Hamers et al., 2006; Kojima et al., 2009). Their anti-androgenic potencies to AR were determined by the AR chemically-activated luciferase gene expression (AR-CALUX) and human mammary carcinoma cell line MDA-kb2 bioassays. Thirteen of them had anti-androgenic activities, and eight of them had no activity (4 of them with n = 1 in AR-CALUX were considered as inactive). In this study, the IC_{50} and RIC_{20} values were converted to the corresponding pIC_{50} ($-\log IC_{50}$) and $pRIC_{20}$ ($-\log RIC_{20}$) and used as dependent variables. The structural features of all of the chemicals are listed in Table S1.

2.2. Docking and structural model preparation

The 3D-structures of compounds were initially constructed by the sketch molecular module of the Sybyl 7.3 molecular modeling package (Tripos Inc. St. Louis, MO, USA). All of the hydrogen atoms were added, and the geometries of these compounds was subsequently optimized using the Tripos force field with Gasteiger-Hückel charges and minimized by the Powell method with a maximum iteration of 1000 to reach an energy convergence gradient value of 0.001 kcal mol^{-1} Å (Clark et al., 1989). The minimized structures were used as the initial conformations for molecular docking and MD simulations.

The structural model of the apo form of the ligand binding domain (LBD) of AR (AR-LBD) has been built using homology modeling in SwissModel workspace (http://swissmodel.expasy.org/workspace/) (Arnold et al., 2006; Kiefer et al., 2009) in our previous research, and the processes have been described in detail previously (Wang et al., 2013). In brief, the crystal structure of AR-LBD in a complex with DHT (PDB entry: 1T7T; http://www.rcsb.org/pdb/) was used as a template for the main body of the AR-LBD (residues 669–882 and residues 892–916). The H11-H12 loop (residues 883–891) was built based on the structure of apo ER-LBD (PDB entry: 1A52). Then, all minimized structures were docked into the apo AR-LBD by the Surflex-Dock program of Sybyl 7.3. The docking scores (DS) were generated automatically, and the top DS conformation of each ligand was selected as the bioactive conformation. The details and evaluation of our docking process have been described previously (Li et al., 2013; Li et al., 2012).

2.3. Molecular dynamics (MD) simulations and data analysis

The MD simulations were carried out using GROMACS 4 (Hess et al., 2008) package on an International Business Machines (IBM) blade cluster system. The CHARMM 27 force field was applied to all structural models by using the GROMACS 4 package and SwissParam (http://www.swissparam.ch/) (Zoete et al., 2011). The model was solvated in a box with TIP3P water molecules (Jorgensen et al., 1983), keeping the boundary of box at least 10 Å away from any protein atoms. Five chloride ions were subsequently added for charge neutralization. The whole system was energetically minimized by the steepest-descent method (Garrett et al., 1988), and the minimized systems were gradually heated from