



Anti-androgen activity of polybrominated diphenyl ethers determined by comparative molecular similarity indices and molecular docking

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ABSTRACT

Some polybrominated diphenyl ethers (PBDEs) may have endocrine-disrupting (ED) potencies. In this study, molecular docking and three-dimensional quantitative structure–activity relationship (3D-QSAR) were performed to explore the possible anti-androgenicity of PBDEs. Based on the alignment generated by docking conformations, a highly predictive comparative molecular similarity indices analysis (CoMSIA) model was developed with q^2 value of 0.642 and r^2 value of 0.973. The contributions of the steric, electrostatic, hydrophobic fields to the CoMSIA model are 13.1%, 61.0% and 25.9%, respectively. Br substitutions which are at *meta* and *para* positions of PBDEs will be unfavorable for androgen receptor (AR) antagonism and *ortho* Br substitutions for PBDEs are favorable for anti-androgen activity. Mapping the 3D-QSAR models to the active site of the AR provides new insight into the AR–PBDEs interaction. CoMSIA field contributions showed good consistency with structural features of the AR binding site and can be used to predict anti-androgen activities of other PBDE congeners.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been extensively used as flame retardants in my products. While PBDEs are beneficial, they have possible adverse effects on humans and wildlife. Over the last few years, increasing evidence has become available that some PBDEs have the potential to disrupt the endocrine system by mimicking or inhibiting endogenous hormones such as estrogens or androgens. For example, *in vitro* (anti-)estrogenic activities by PBDEs have been reported (Meerts et al., 2001), particularly for the lesser brominated. The estrogen receptor was activated by lesser brominated PBDEs and inactivated by more brominated PBDEs. Exposure to BDE-99 during development affected the regulation of estrogen target genes (Ceccatelli et al., 2006), and impaired spermatogenesis in rats (Kuriyama et al.,

2005). The penta-BDE mixture, DE-71, has weak estrogenic activity *in vivo* and *in vitro* (Mercado-Feliciano and Bigsby, 2008). In addition, DE-71 delayed puberty and suppressed the growth of androgen-dependent tissues in male Wistar rat following a peri-pubertal exposure (Stoker et al., 2004), a result which suggested anti-androgenic properties.

Recently, Hamers et al. (2006) performed a systematic *in vitro* screening of the endocrine-disrupting (ED) potencies of brominated flame retardants (BFRs). A test set of 27 individual BFRs were selected, among them comprising 19 PBDEs. All compounds were tested for their potency to interfere via thyroidal, estrogenic, androgenic, progestagenic and AhR-mediated pathways. Moreover, the potency to interfere with androgenic and progestagenic pathways was tested for the first time for BFRs. However, due to expense and the lack of authentic standards, many PBDEs have not yet been tested for their ED potencies (Chen et al., 2003). Knowledge about the toxicological mechanisms of PBDEs is urgently needed but insufficiently available. Therefore, alternative approaches are needed. Quantitative structure–activity relationship (QSAR) models can be useful in identifying and depicting structural features that contribute to the ability of a chemical to interact with the androgen receptor (AR) (Ai et al., 2003; Bohl et al., 2004; Söderholm et al., 2005, 2006; Tamura et al., 2006).

Abbreviations: BDE, Brominated diphenyl ether; PBDEs, Polybrominated diphenyl ethers; 3D-QSAR, Three-dimensional quantitative structure–activity relationship; CoMSIA, Comparative molecular similarity indices analysis; AR, Androgen receptor; LBD, Ligand-binding domain; PCBs, Polychlorinated biphenyls; BFRs, Brominated flame retardants; ED, Endocrine-disrupting; PLS, Partial least-squares; LOO, Leave-one-out; SEP, Standard error of prediction; SEE, Standard error of estimate.

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Based on Hamers' experimental data, Harju et al. (2007) constructed a QSAR model for the AR for PBDEs. They correlated structural descriptors derived from quantum-chemical calculation and experiment (ultraviolet absorption spectrum and the relative retention time) with the negative logarithm of half maximal inhibitory concentration ($-\log IC_{50}$) from *in vitro* trans-activation bioassays (AR-CALUX, chemically activated luciferase gene expression). The set of 49 calculated and experimental molecular descriptors was reduced for each QSAR model using a genetic algorithm in the PLS Toolbox within Matlab. Structural descriptors were used since they can be easily obtained and clearly describe defined molecular properties (Xu et al., 2007; Zheng et al., 2007). However, the intermolecular interaction mechanism between PBDEs and the AR is still unclear.

The objective of the present study was to understand the mechanism of intermolecular interaction between PBDEs and the AR by employing 3D QSAR and molecular docking. Molecular docking was used to predict the biologically active conformations of PBDEs and to create the structural alignment of PBDEs for model building. This made it possible to visualize the AR ligand-binding domain (LBD) and interpret the comparative molecular similarity indices analysis (CoMSIA) maps made for the AR LBD, thus understanding interactions that are beneficial or detrimental for the antagonism of PBDEs in their proposed binding mode. The mechanism of toxic action was also elucidated through better understanding of the ligand-receptor interaction so that testable hypotheses of the mechanism of *in vitro* toxicity of PBDEs mediated through the AR could be developed. To the best of our knowledge, this is the first prediction of AR-antagonistic activity of PBDEs by 3D-QSAR CoMSIA method.

2. Materials and methods

2.1. Data sets for analysis

Empirically determined values of the AR-antagonistic potencies for 19 PBDEs were obtained from literature (Hamers et al., 2006). For all test compounds, the potency to interact as an agonist or antagonist with the AR was determined in the AR-CALUX bioassay. The AR-CALUX bioassay uses trans-activation reporter systems in cells carrying a luciferase gene under the transcriptional control

of response elements for activated receptors. The results of the AR-CALUX assays show that several PBDEs have a peak activity at concentration greater than 10 μM (Table 1). In these cases, an extrapolation was made up to a maximum concentration of 15 μM . For modeling purposes, PBDEs for which activity was observed but with IC_{50} values greater than 15 μM (determined by extrapolation) were given a response of 15 μM . Inactive compounds were given an IC_{50} value one log unit greater (100 μM) than the greatest tested concentration of 10 μM (Harju et al., 2007).

2.2. Molecular modeling and docking

To select the initial conformation of compounds, the coordinates of X-ray crystallographic data for each PBDE compound was obtained from the Cambridge Structure Database, if available. Compounds not contained in the Database were constructed from the structures of similar compounds (Tamura et al., 2006). The geometries of these compounds were subsequently optimized in SYBYL7.3. The minimization process used the Powell method with the Tripos force field (distance-dependent dielectric) to reach a final energy convergence gradient value of 0.001 kcal/mol. Gasteiger-Hückel charges were assigned to each compound. The minimized structures offered reasonable starting conformations for docking. The Surflex-Dock program interfaced with SYBYL 7.3 was used to dock the compounds to the LBD of human AR. The crystal structure of human AR LBD complexed with hydroxyflutamide (PDB code: 2AX6) that was used in Surflex-Dock was obtained from the Protein Data Bank (<http://www.rcsb.org/pdb/>). Prior to docking, hydroxyflutamide was extracted from the crystal structure, the structural water molecules were removed and hydrogen atoms were added in standard geometry using the Biopolymer modulators. The Kollman-all atom charges were assigned to protein atoms using SYBYL 7.3. Default parameters were employed in all runs.

2.3. Molecular alignment

Generation of the correct spatial alignment of the investigated compounds for 3D QSAR analysis is of vital importance, since the correctness of the analysis is dependent on the quality of the

Table 1
Structure and biological activities of PBDEs.

No.	Congener	Substitution order	IC_{50} (μM)	$-\log IC_{50}$ (μM)				
				Exp.	Pred. 1	Resid.1	Pred. 2	Resid.2
1	BDE169*	3,3',4,4',5,5'	100	-2	-2.102	0.102	-1.228	-0.772
2	BDE206	2,2',3,3',4,4',5,5',6	100	-2	-2.046	0.046	-2.109	0.109
3	BDE209	2,2',3,3',4,4',5,5',6,6'	100	-2	-2.025	0.025	-2.027	0.027
4	BDE183	2,2',3,4,4',5',6	15	-1.176	-1.198	0.022	-1.074	-0.102
5	BDE185	2,2',3,3',5,5',6	15	-1.176	-1.045	-0.131	-1.081	-0.095
6	BDE153*	2,2',4,4',5,5'	13	-1.114	-1.028	-0.086	-0.727	-0.387
7	BDE190	2,3,3',4,4',5,6	8.8	-0.944	-0.905	-0.039	-0.987	0.043
8	BDE99	2,2',4,4',5	7.8	-0.892	-0.750	-0.142	-0.772	-0.120
9	BDE127	3,3',4,5,5'	5.1	-0.708	-0.712	0.004	-0.630	-0.078
10	BDE39	3,4',5	3.5	-0.544	-0.567	0.023	-0.627	0.083
11	BDE28*	2,4,4'	3.1	-0.491	-0.516	0.025	-0.264	-0.227
12	BDE181	2,2',3,4,4',5,6	3.0	-0.477	-0.462	-0.015	-0.476	-0.001
13	BDE79	3,3',4,5'	2.0	-0.301	-0.452	0.151	-0.498	0.197
14	BDE155	2,2',4,4',6,6'	2.0	-0.301	-0.222	-0.079	-0.193	-0.108
15	BDE38	3,4,5	1.9	-0.279	-0.108	-0.171	-0.237	-0.042
16	BDE47*	2,2',4,4'	1.0	0	-0.169	0.169	-0.443	0.443
17	BDE49	2,2',4,5'	0.67	0.174	0.454	-0.280	0.381	-0.207
18	BDE100	2,2',4,4',6	0.097	1.013	0.624	0.389	0.672	0.341
19	BDE19	2,2',6	0.06	1.222	1.235	-0.013	1.269	-0.047

Pred. 1 presents the predictive values of the whole compounds as a training set.

Pred. 2 presents the predictive values from data set which was divided into training set (for model development) and test set (for model predictive assessment).

* The compounds in the test set for model validation.

alignment. This challenging step is often impeded by the lack of data on biologically active conformations of the compounds in complex with their target protein. Docking provides a powerful way to screen the conformational space of the ligands in search of the preferred binding conformation, while taking into account the structure and the chemical environment of the LBD. In present study, the alignment was generated by the docking protocol implemented in Surflex-Dock. The aligned molecules were then exported to SYBYL and MMFF94 charges were assigned before CoMSIA field calculations (Castilho et al., 2006).

2.4. 3D-QSAR analysis

2.4.1. CoMSIA modeling

CoMSIA (Klebe et al., 1994) can avoid some inherent deficiencies arising from the functional form of Lennard-Jones and Coulomb potentials used in CoMFA. In CoMSIA, a distance-dependent Gaussian-type functional form has been introduced, which can avoid singularities at the atomic positions and the dramatic changes of potential energy for threshold grids in the proximity of the surface. In deriving the CoMSIA descriptor fields, a 3D cubic lattice with grid spacing of 2 Å and extending 4 Å units beyond the aligned molecules in all directions was created to encompass the aligned molecules. The default value of 0.3 was used as the attenuation factor. In CoMSIA, similarity is expressed in terms of different physicochemical properties: steric occupancy, partial atomic charges, local hydrophobicity, hydrogen bond donor and acceptor properties.

2.4.2. Statistical regression methods

The biological activities for 19 PBDEs were correlated with the CoMSIA generated steric, electrostatic and hydrophobic fields using the statistical method of partial least-squares (PLS) regression (He et al., 2007). Using PLS, the large number of steric–electrostatic descriptors was reduced to a few principal components that are linear combinations of the original descriptors. The optimum number of principal components was determined by the Leave-One-Out (LOO) cross-validation procedure. In this method, each compound is systematically excluded once from the training set, after which its activity is predicted by a model derived from the remaining compounds. Cross-validation with LOO option and a column filtering of 2.0 kcal/mol was conducted to obtain the optimum number of components to be used in the final analysis. The number of components used was not greater than one-third of the number of rows in the training set. After the optimum number of components was determined, a non-cross-validated analysis was performed with a column filtering of 2.0 kcal/mol and generated a predictive QSAR model with a conventional correlation coefficient (Cramer et al., 1988). The cross-validated correlation coefficient q^2

$(q^2 = 1 - \frac{\sum(Y_{pred} - Y_{actual})^2}{\sum(Y_{actual} - Y_{mean})^2})$, standard error of prediction (SEP) values $(SEP = \sqrt{\frac{PRESS}{n-c-1}})$, non-cross-validated correlation coefficient r^2 , F

values and standard error of estimate (SEE) values $(SEE = \sqrt{\frac{PRESS}{n}})$ were computed according to the definitions in SYBYL, where $Y_{pred.}$, Y_{actual} , and Y_{mean} are predicted, actual, and mean values of the target property $\text{pIC}_{50}(-\log\text{IC}_{50})$, respectively, $PRESS = \sum(Y_{pred} - Y_{actual})^2$, n is the number of compounds, c is the optimum number of components.

2.4.3. Validation of models

Compared with cross-validation, external validation can provide a more rigorous evaluation of the model's predictive capability for untested chemicals (Liu et al., 2006). When a sufficiently large number of new (i.e., obtained after the model development)

and reliable experimental data is available, the best proof of already developed model accuracy is to test model performance on these additional data. This is the best way to carry out external validation after model development. However, in the absence of available additional data (in useful quantity and quality), statistical external validation can be done by adequately splitting the available input data set, before model development, into training set (for model development) and prediction set (for model predictive assessment) by different procedures. In this investigation, the model was validated by splitting the original data set into training set and prediction set. The method for selection of test set in this study was fixed interval-sampling method. The chemicals were ordered according to their ascending experimental activity values $(-\log\text{IC}_{50})$ (see Table 1), then any molecules with the number of $5n + 1$ ($n = 0, 1, 2$ and 3) would be included in the test set, the activities of the chemicals in the omitted group are predicted by a model derived from the remaining chemicals in the data set. The most active chemicals were kept in the training set (Liu et al., 2008).

3. Results

3.1. Alignment generation

Since the structure of AR LBD in complex with PBDE ligands has not yet been experimentally resolved, we used molecular docking to predict the biologically active conformations of the PBDEs and to create the structural alignment of AR ligands for model building (Söderholm et al., 2005, 2006). Docking with program Surflex-Dock could successfully reproduce the X-ray pose of natural ligand hydroxyflutamide with a root-mean-square deviation (RMSD) of 0.34 Å. Such proximity can be regarded as a good reproduction of the crystal structure (Söderholm et al., 2006). We therefore believe that the binding conformations of the PBDE ligands analyzed here are reasonably well predicted by Surflex-Dock. Docking generated 10 conformations for each PBDE ligand. The alignment was determined as automatically and objectively as possible based on a top-ranked docking pose from all the poses generated for each ligand. To obtain a well-superposed set of ligands, the alignment based on top ranked pose was improved by manually selecting docking poses for ligands (BDE100 in this study) that deviated from the alignment.

3.2. 3D-QSAR analysis

To examine the structural and chemical features contributing to the biological activity of the studied ligands, the alignment derived from docking simulations was quantitatively analyzed using the CoMSIA procedures. The statistical quality and the robustness of the model were determined with internal cross-validation procedures. Internal validation using LOO cross-validation gave a correlation coefficient q_{LOO}^2 of 0.642 using three optimum numbers of components with a SEP of 0.582. The non-validated PLS analysis gave a correlation coefficient r^2 of 0.973, F value of 182.627 and a SEE of 0.159. These values are typical of such models that are regarded as self-consistent and internally predictive. The contributions of the steric, electrostatic, hydrophobic fields to the CoMSIA model are 13.1%, 61.0% and 25.9%, respectively, i.e. electrostatic field constitutes the most important descriptors to the information content of the CoMSIA model, while the hydrophobic field plays a second important role. The predicted activities indices (pred.1) and the residual values (resid.1) of 19 PBDEs were compared (see Table 1).

To test the predictive power of the CoMSIA model, further calculations were performed on the training set, and the model obtained was tested on the test set. A good q^2 ($q^2 = 0.546$) was

Table 2
Statistics of CoMSIA model for the training and test sets.

Parameter	CoMSIA
<i>Training set</i>	
q^2	0.546
c	3
SEP	0.692
r^2	0.976
SEE	0.158
F	151.301
No. of compounds	15
<i>Test set</i>	
r^2	0.837
r^2_{pred}	0.555
No. of compounds	4

q^2 is the LOO cross-validated correlation coefficient, c is the number of the principal components, SEP is cross-validated standard error of prediction, r^2 is non-cross-validated correlation coefficient, SEE is standard error of estimate, r^2_{pred} is predictive r^2 .

obtained for the training set. Predictive r^2 is 0.555 for the test set (see Table 2). Comparison of the CoMSIA predicted (pred.2 in Table 1) and experimentally observed $-\log IC_{50}$ values for each compound when it belonged to the test set revealed residual values ranging from -0.772 to 0.443 log units (resid.2 in Table 1). The average absolute error of prediction for the molecules of the external test set was 0.457 log units.

4. Discussion

Levels of PBDEs are increasing in the environment due to their use as flame retardants. The similarities of structure to polychlorinated biphenyl (PCB) congeners suggest that they may share similar toxicological properties (Wang et al., 2006). Previous study (Portigal et al., 2002) found four Aroclor PCB mixtures were able to antagonize AR-mediated transcription in the presence of the natural AR ligand dihydrotestosterone (DHT). The antagonistic activity of Aroclor mixtures increased in the following order: Aroclor 1260 < Aroclor 1242 < Aroclor 1254 < Aroclor 1248. Within a series of individual congeners, congeners PCB 42, PCB 128, and PCB 138 are shown to antagonize AR activity. Ligand-binding studies demonstrate that endocrine activities of PCB mixtures and congeners on AR are likely due to direct and specific binding to AR LBD.

One important feature of 3D-QSAR analysis is the graphic representation of the model, usually used to make its interpretation easier. PBDEs can not form hydrogen-bonding with amino acid residues in AR LBD (Harju et al., 2007), the contour maps of our CoMSIA model based on electrostatic and hydrophobic fields are displayed as PLS stdev* coeff maps. Since the superposition of PBDE ligands for the analysis was done using the receptor structure and ligand docking, the CoMSIA maps can be drawn inside the AR binding cavity. The maps of the 3D QSAR model based on the chemical properties and molecular interaction fields of ligands should correlate with the features of the LBD. Also, the ligand interactions with the residues of the binding cavity should provide an explanation of the variation of the experimental activity. All residues within a 6 Å radius from the centroid of hydroxyflutamide defined the active site.

4.1. Electrostatic field analysis

Electrostatic field is the major contributor for PBDEs anti-androgen activity and the contribution of the electrostatic field to the CoMSIA model is 61.0%. To interpret the field, the electrostatic complementarity with the binding site structure and the electro-

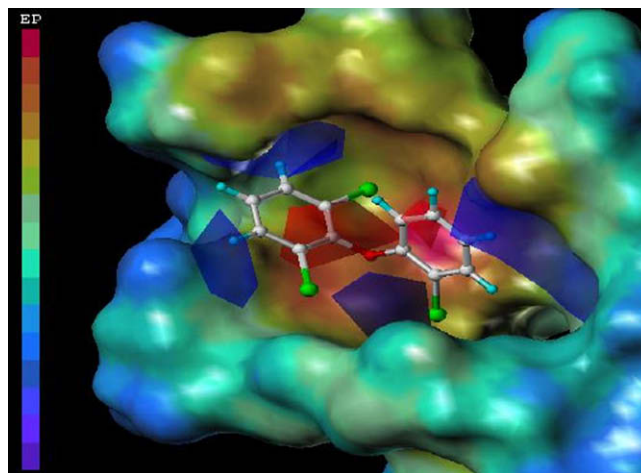


Fig. 1. Probable binding conformation of BDE19 compound displayed inside the active site of the AR. This image was generated with the MOLCAD program in SYBYL 7.3, with some residues removed for clear visualization. The AR surface was rendered with electrostatic potential (red/brown, positive potential; blue, negative potential). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

static properties (electron distribution) of the molecules within the ligand set need to be considered.

The projection of the CoMSIA electrostatic contour map onto the electrostatic potential surface map of the binding site generated by the MOLCAD showed a general complementarity (Fig. 1). Red regions on the binding site surface map represent positive electrostatic potential, whereas blue regions on the surface represent negative electrostatic potential. Electrostatically positive blue polyhedra from contour complement with the negative potential surface inside the active site of the AR, while the red CoMSIA contour (electronegative) match well with the red, highly electropositive surface of the binding site of AR (Fig. 1).

There are several blue volumes that are indicated by the CoMSIA model to be favorable for positive partial charge (Fig. 2). The first blue polyhedron is around the *para* position of a phenyl ring. This is consistent with the experimental results, e.g. negative charged Br substituent in *para* position will decrease the activity, such as the anti-androgen activity of 2,2',4,4',6-penta-BDE100 ($IC_{50} = 0.097 \mu M$) is less than that of 2,2',6-tri-BDE19 ($IC_{50} = 0.060 \mu M$). Carbonyl oxygen atoms of residues Leu701 and Asn705 support the presence of the positively charged blue polyhedron. Meanwhile, two blue isopleth are located in 3-, 5-position on another phenyl ring of PBDEs. This indicates that negative charged Br substituents herein will also be unfavorable for anti-androgen activity, for example, 3,3',4,5,5'-penta-BDE127 ($IC_{50} = 5.1 \mu M$) exhibits weaker anti-androgen activity than 3,3',4,5'-tetra-BDE79 ($IC_{50} = 2.0 \mu M$). Negative charged oxygen atoms on residues Val746, Phe764 and Gln711 are in the vicinity of the two blue isopleth. The two blue volumes are therefore complementary with the receptor structure. In addition, there are two red volumes favoring negative partial charge in the middle of the binding pocket, i.e. Br substituents around them are favorable for activity. These findings imply that *ortho* position Br substituents were favorable for AR antagonism and *meta* and *para* position Br substituents were unfavorable for AR antagonism by PBDEs. These results are basically in accordance with experimental data that BDE19 with bromine substitutions in *ortho* positions and bromine-free *meta* and *para* positions exhibited the greatest AR antagonism.

4.2. Hydrophobic field analysis

The CoMSIA model indicated that the hydrophobic field was also an important factor for the AR antagonism activity of PBDEs,

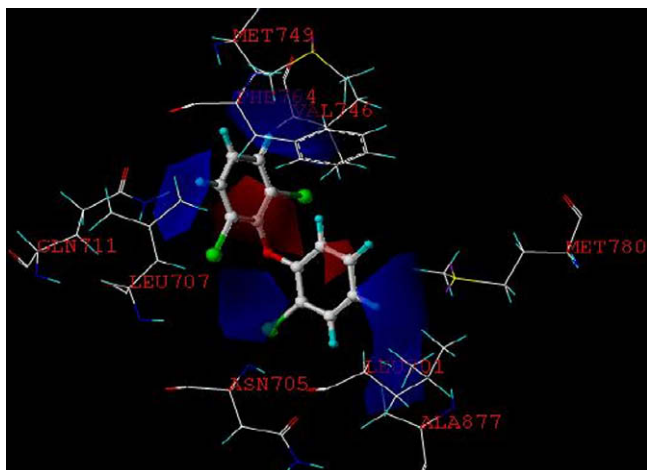


Fig. 2. Electrostatic contour plots from CoMSIA analysis mapped onto the binding site of AR. Positive-charge-favored areas are represented by blue contours. Negative-charge-favored areas are represented by red contours. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

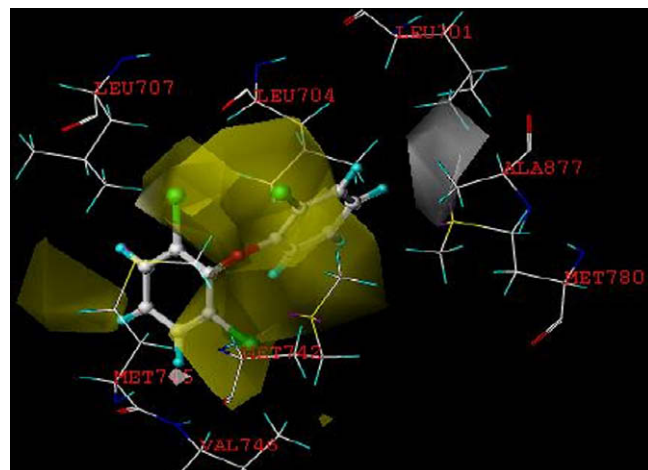


Fig. 4. Hydrophobic contour plots from the CoMSIA analysis mapped onto the binding site of AR. Yellow regions indicate areas where hydrophobic groups increase activity and gray regions indicate areas where hydrophilic groups will increase activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

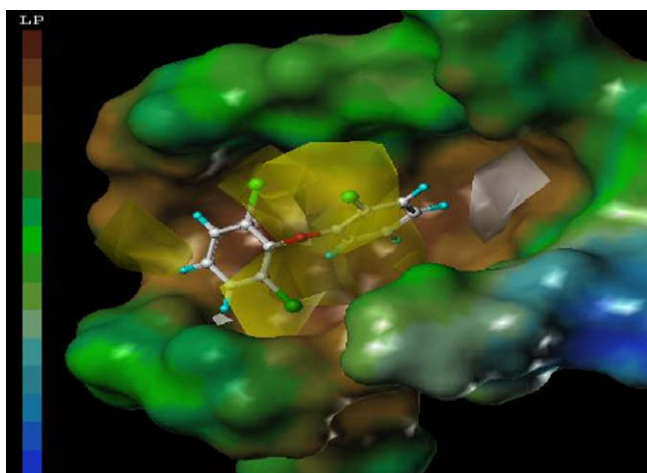


Fig. 3. Probable binding conformation of BDE19 compound displayed inside the active site of the AR. This image was generated with the MOLCAD program in SYBYL 7.3, with some residues removed for clear visualization. The AR surface was rendered with hydrophobic potential (brown, hydrophobic; blue, hydrophilic).

which accounted for 25.9% of the actual variance in activity (Figs. 3 and 4). With regard to the lipophilicity potential surface, brown is hydrophobic whereas blue is hydrophilic. In CoMSIA contour maps, Gray and yellow volumes indicated the areas where hydrophilic and hydrophobic properties were preferred, respectively. In Fig. 3, hydrophobic surfaces in the middle of the AR LBD match well with the favored hydrophobic volume (yellow volume). A large yellow region appeared around the *ortho* positions, which indicated that a group with an optimal hydrophobicity at *ortho* positions of PBDEs resulted in a positive effect on the potency, i.e. is favorable for anti-androgen activities (In Fig. 4). The yellow polyhedron is surrounded by hydrophobic amino acid residues Leu707, Leu704, Met742 and Met745. This volume partly overlaps with the electrostatic volume favoring partial negative charge, suggesting that electron-withdrawing moieties with hydrophobic property are preferred at this site, i.e. *ortho* Br substitutions for PBDEs are favorable for AR antagonism. This is in line with the experimental anti-androgen activity measurements (Hamers et al., 2006), e.g. the anti-androgen activity order is

2,2',4,4',6-penta-BDE100($IC_{50} = 0.097 \mu M$) > 2,2',4,4'-tetra-BDE47- ($IC_{50} = 1.0 \mu M$) > 2,4,4'-tri-BDE28($IC_{50} = 3.1 \mu M$). In addition, a very distinct hydrophilic site (gray polyhedron) can be observed near the *para* position, which suggest that a hydrophilic group at the *para* substituent might improve the anti-androgen activity. In contrast, a blue volume favoring partial positive charge is located at almost same position in electrostatic field contour. Two contours showed that positive charged and hydrophilic group is preferred at *para* position whereas electron-withdrawing and hydrophobic Br substitution is not favorable at this site. In general, hydrophobic field is consistent with the electrostatic field in elucidating Br positions' effect on PBDEs' anti-androgen activity, i.e. *ortho* Br substitutions for PBDEs are favorable for the AR antagonism whereas *meta* and *para* Br substitutions for PBDEs are unfavorable for anti-androgen activity. However, why there is a blue polyhedra near ether bond is not clear. Further study about PBDE-AR interaction mechanism is needed.

Recently, to elucidate the potential anti-androgenic effects of DE-71 mixture, Stoker et al. (2005) conducted AR binding in a competitive binding assay and evaluated gene activation in a transcriptional activation assay. The results suggested that the delay in puberty in the male rat and decreased growth of androgen-dependent tissues observed previously following exposure to DE-71 were likely due to the inhibition of AR binding by several of the congeners which make up this mixture. In the rat VP binding assay, BDE-47, -99 and -100 displayed antagonistic properties, with IC_{50} of 16.7, 33 and 3 μM , respectively, i.e. anti-androgenic activity order is BDE100 (2,2',4,4',6) > BDE47 (2,2',4,4') > BDE99 (2,2',4,4',5). This order of relative potency is consistent with our results in this study, i.e. Br substitutions which are at *ortho* positions (2,2',6,6') are beneficial to AR antagonism whereas Br substitutions located at *meta* positions (3,3',5,5') of PBDEs will be unfavorable for AR antagonism.

5. Conclusions

In this study, we have utilized molecular docking and CoMSIA model to explore the interactions between the AR and PBDEs. The results obtained from the CoMSIA model reveal the importance of electrostatic and hydrophobic fields for PBDEs anti-androgen activity. The CoMSIA contour maps, which visualize the regions of structural features explaining the variance in the anti-androgen

activities, nicely complement the structural elements of AR LBD. Research found that Br substitutions which are at *meta* and *para* positions of PBDEs will be unfavorable for AR antagonism and *ortho* Br substitutions for PBDEs are favorable for anti-androgen activity.

Interpretation of the CoMSIA contour maps combining with the binding site of the AR provided a better understanding of the PBDEs–AR interactions. The results obtained from 3D QSAR models were found to accurately predict the PBDEs anti-androgen activity and to yield reliable clues for further mechanism elucidation of the ligand–receptor interaction.

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