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QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR POLYCHLORINATED HYDROXYBIPHENYL ESTROGEN RECEPTOR BINDING AFFINITY: AN ASSESSMENT OF CONFORMER FLEXIBILITY

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Abstract—A diverse group of xenobiotics has a high binding affinity to the estrogen receptor (ER), suggesting that it can accommodate large variability in ligand structure. Relationships between xenobiotic structure, binding affinity, and estrogenic response have been suggested to be dependent on the conformational structures of the ligands. To explore the influence of conformational flexibility on ER binding affinity, a quantitative structure–activity relationship (QSAR) study was undertaken with estradiol, diethylstilbestrol, and a set of polychlorinated hydroxybiphenyls (PCHBs) of environmental concern. Although the low-energy minima of the PCHB congeners suggested that interconversions among conformers were likely, the electronic parameters associated with the conformer geometries for a specific PCHB congener could vary significantly. The results of the QSAR analysis suggested that among the PCHBs studied, the most polarizable conformers (lower absolute volume polarizability values) were most closely associated with ER binding affinity. Across the set of “polarizable” conformers, which did not include the low-energy gas-phase conformers, the electron donating properties of the hydroxy moiety and the aromatic component of the estradiol A ring analogue in the PCHBs were found to be correlated with higher ER binding affinity.

Keywords—Quantitative structure–activity relationships Conformational searching Polychlorinated hydroxybiphenyls Estradiol Estrogen receptor

INTRODUCTION

The estrogen receptor (ER) has demonstrated considerable structural tolerance for ligands [1–4]. Besides the natural hormone (estradiol, E2) and related steroidal analogues [5,6], many different structural classes of nonsteroidal compounds have high ER binding affinity, such as bibenzyls, stilbenes, triarylethylenes, phenylindoles, phenylindenes, coumarins, and lactones [4]. The broad ligand affinity of the ER is of concern because environmental contaminants could elicit toxicity through their ability to act as agonists or antagonists of estrogen-mediated reproductive and developmental processes. As an initial screen for those chemicals that may interfere with this steroid system through binding to the ER, quantitative structure–activity relationships (QSARs) could prove invaluable. The development of mechanistic QSARs, however, is hampered by a lack of knowledge concerning the three-dimensional (3-D) structure of the ER.

In the absence of a well-defined 3-D structure for the ER, a determination of the electronic requirements for its known ligands can provide a means to assess the receptor’s characteristics. However, the influence of an aqueous environment on ligand and ER geometry and the conformational flexibility of ER ligands [7] create significant challenges to mapping the estrogen binding domain. Ligands can also induce alterations in the tertiary structure of the ER binding complex, which in turn may alter binding to estrogen hormone responsive elements and subsequent transactivational events [8–11].

Recently, force field (MMP2) and quantum chemical (AM1, PM3) modeling methods were used to explore the conformational space of E2 and related steroidal derivatives [5,6] using a “quench” reannealing method [12,13]. Several different steroid ring conformations for each derivative were generated. Not surprisingly, the force field potential energies of predicted conformations of these estrogens and the associated transition barriers were found to be within the range of the receptor binding free energy values. Thus, it appears that ER binding energy is sufficiently large to offset the energy needed for conversion of conformers to a preferred geometry [6]. In a related series of studies on the structural requirements for ER binding and associated estrogenic responses, it was inferred that hydroxylation at specific sites of the estratrien-17β-ol-aromatic A ring is critical [10,11]. Hydroxylation at the 2 or 3 (i.e., E2) positions promoted high affinity of a ligand for the ER, while hydroxylation at the 1 or 4 positions attenuated binding affinity (see Fig. 1 for the location of these positions in E2). It has been hypothesized that the hydroxyl groups at positions 2 and 3 may share, via hydrogen bonding, a common H acceptor/donor site in the receptor cavity [10].

Waller et al. [3] recently described a 3-D modeling effort focused on the binding of a class of important environmental contaminants, polychlorinated hydroxybiphenyls (PCHBs), to the ER. Based on results derived using the comparative field analysis (CoMFA) paradigm with the gas-phase optimized E2 A ring serving as the template (via root-mean-square fit), Waller et al. [3] suggested that ER binding affinity of PCHBs could be related to a steric field. The findings of their study were generally consistent with hypotheses suggesting that conformational restriction of ortho-substituted PCHBs was critical.
for ER binding affinity [14] and that a hydroxyl group on the aromatic A ring was not essential for ER binding [15]. However, the CoMFA [3] showed large differences between conventional ($r^2$ and $s^2$) and cross-validated ($r_{cr}^2$ and $s_{cr}^2$) regression statistics [16]. The limitations in the CoMFA-based model could be related to ligand flexibility, and the possibility that use of the low-energy $E_2$ conformation as the template is inappropriate.

In recent investigations [17; O.G. Mekenyan et al., unpublished manuscript], the role of different polychlorinated biphenyl (PCB) conformations in evaluating QSARs for the binding of PCBs to the aryl hydrocarbon (Ah) receptor has been discussed. The results of these studies showed that considerations of the degree of conformational planarity are critical to the generation of robust structure-affinity models for binding. As discussed previously, the role of ligand conformational flexibility in elucidating the relationship between electronic character, ER binding, and estrogenic responses is also of great interest [4–6,10,11]. To further study the role of PCB conformer flexibility on biological activity, a multiple-conformer QSAR method was employed to model ER binding affinity of $E_2$, diethylstilbestrol (DES), and the series of PCHBs investigated by Korach et al. [14] and Waller et al. [3]. Based on experimental and theoretical findings [5,6,10,11] for the involvement of a negative isopotential surrounding the $E_2$ A ring in ER binding and the assumption that there are cationic sites (protonated amino acid residues) inside the ER [18,19], we hypothesized that PCHBs with greater binding affinity should have higher electron donating abilities associated with their corresponding phenolic ring. Such chemicals should have higher (lower) donor (acceptor) superdelocalizabilities and frontier charges on the HOMO (LUMO) orbital, as well as larger negative charges on the phenolic oxygen and the aromatic carbons in the A ring.

**MATERIALS AND METHODS**

The structures of the 12 PCHBs studied, in addition to $E_2$ and DES, are presented in Figure 1 (chemical names provided in Table 1). Cytosolic uterine ER binding affinities from ovariectomized mice, as reported by Korach et al. [14], were expressed as the concentration (molar equivalents [Meq]) of competitor required to displace 50% of ER-bound [$^3$H]estradiol.
The observed binding affinity values, expressed as the negative log of the EC50s (pEC50s), are presented in Table 1 for the PCHBs, E2, and DES.

**Conceptual approach**

With conventional QSAR methods, the molecular structure of each chemical under investigation is represented by a single lowest-energy conformer defined by quantum chemical or force field methods. However, in some instances, the most stable conformations may be the least likely to interact with a solvent or macromolecule [20]. To systematically evaluate conformer flexibility within the context of relevant biological environments and reactions (e.g., partitioning within a complex solution, substrate–receptor complex formation, and subsequent electronic interactions), a “dynamic” QSAR approach was incorporated in the present study [21]. The method assumes that in the complex reaction environments of biological systems a molecule can exist as a variety of conformers, with differences among the conformations. Biological activity is assumed that in the complex reaction environments of biological environments and reactions (e.g., partitioning within a complex solution, substrate–receptor complex formation, and subsequent electronic interactions), a “dynamic” QSAR approach was incorporated in the present study [21]. The method assumes that in the complex reaction environments of biological systems a molecule can exist as a variety of conformers, with differences among the conformations. Biological activity is subsequently modeled as an electronic property for specifically selected conformers (or a set of conformers) rather than as a property derived from the single low-energy gas-phase conformer.

**Conformational generation**

Using the OASIS system [22,23], the dynamic QSAR method combines an exhaustive conformer generation routine [24] with conformer screening algorithms that can be adapted to explore relationships between specific electronic properties and the biological activity under investigation [21]. The conformer generation technique is described by Ivanov et al. [24], and the reader is encouraged to consult this reference for a detailed presentation of the approach. Briefly, the technique is a combinatorial procedure that initiates from molecular topology and generates all conformers in the context of steric constraints (e.g., distances between nonbonded atoms, ring-closure limits, torsional resolution) and expert rules (e.g., likelihood of intramolecular hydrogen bonds, cis/trans or +/- isomers). A unique aspect of the approach involves the initial propagation of an acyclic 3-D model of the molecular skeleton. The “construction” of the skeleton initiates from a specified atom based on its topochemical ranking (a ranking based on connectivity and atom type). A bond “under construction” in this acyclic model is positioned in space by using a recursive procedure based on the 3-D information of previously established bonds, which includes atom type and hybridization of the atoms incident to the bond being constructed as well as the two atoms associated with the previously completed bond. Cyclic fragments incident to the bond being constructed are also addressed. Bond lengths and valence angles are determined through a molecular mechanics parameterization. During the propagation of the acyclic model, cyclic character is gained through defined ring-closure constraints. Rotamers associated with all torsional angles that meet hybridization and specified geometric constraints are retained. With strained molecules the possible violation of imposed geometric constraints are corrected with a strain–relief procedure (“pseudo” molecular mechanics) based on a truncated force field. In summary, the approach used here incorporates the conformational flexibility of saturated cyclic molecular fragments, as opposed to other conformational analysis techniques which explore conformational space formed by rotations around acyclic single bonds only [24]. Finally, the electronic descriptors of interest for a selected set of conformers can be computed either after geometry optimization or by direct single-point (ISCF) calculations of the unoptimized conformations.

For E2 all cyclic and acyclic single bonds were evaluated in deriving conformers, while for DES and the PCHBs all the acyclic single bonds were assessed (there are no saturated cyclic fragments in these compounds). Because of the conformational flexibility of DES and E2, torsion resolution around conformational variables was chosen to be 60°, whereas for the PCHBs it was set at 30°. In addition, less restrictive constraints for nonbonded hydrogens and ring closures (1.1 Å and 2.0 Å, respectively) were imposed for E2 to generate a sufficiently large set of conformers for this compound. Through this analysis, 23, 57, and 72 nonoptimized conformations were derived for E2, DES, and the 12 PCHBs, respectively. During optimization, some of the E2, DES, and PCHB conformations

<table>
<thead>
<tr>
<th>Compound (no.)</th>
<th>Observed</th>
<th>Eqn. 1</th>
<th>Eqn. 2</th>
<th>Eqn. 3</th>
<th>Eqn. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (1)</td>
<td>0.00</td>
<td>–</td>
<td>0.06</td>
<td>–</td>
<td>0.08</td>
</tr>
<tr>
<td>Diethylstibestrol (2)</td>
<td>0.40</td>
<td>–</td>
<td>0.39</td>
<td>–</td>
<td>0.36</td>
</tr>
<tr>
<td>2,4,6-Trichloro-4'-biphenylol (3)</td>
<td>-1.63</td>
<td>-2.10</td>
<td>-2.10</td>
<td>-2.21</td>
<td>-2.21</td>
</tr>
<tr>
<td>2,3,5-Tetrachloro-4'-biphenylol (4)</td>
<td>-1.98</td>
<td>-2.09</td>
<td>-1.91</td>
<td>-1.72</td>
<td>-1.69</td>
</tr>
<tr>
<td>2-Chloro-4,4'-biphenyldiol (5)</td>
<td>-1.95</td>
<td>-3.05</td>
<td>-3.52</td>
<td>-2.95</td>
<td>-2.94</td>
</tr>
<tr>
<td>2,6-Dichloro-4'-biphenylol (6)</td>
<td>-2.59</td>
<td>-2.4</td>
<td>-2.63</td>
<td>-2.46</td>
<td>-2.52</td>
</tr>
<tr>
<td>2,5-Dichloro-4'-biphenylol (7)</td>
<td>-2.70</td>
<td>-2.4</td>
<td>-2.73</td>
<td>-2.41</td>
<td>-2.44</td>
</tr>
<tr>
<td>3',4',5-Trichloro-4'-biphenylol (8)</td>
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<td>-3.06</td>
<td>-3.1</td>
<td>-3.31</td>
<td>-3.26</td>
</tr>
<tr>
<td>3',5',5'-Tetrachloro-4,4'-biphenyldiol (9)</td>
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<td>-3.06</td>
<td>-3.01</td>
<td>-3.02</td>
<td>-2.92</td>
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<tr>
<td>2-Chloro-4-biphenyldiol (10)</td>
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<td>-3.51</td>
<td>-3.46</td>
<td>-3.25</td>
<td>-3.33</td>
</tr>
<tr>
<td>4'-Chloro-4'-biphenyldiol (11)</td>
<td>-3.59</td>
<td>-3.46</td>
<td>-3.32</td>
<td>-3.67</td>
<td>-3.66</td>
</tr>
<tr>
<td>2,3,5,6-Tetrachloro-4,4'-biphenyldiol (12)</td>
<td>-3.70</td>
<td>-3.31</td>
<td>-3.44</td>
<td>-2.99</td>
<td>-2.94</td>
</tr>
<tr>
<td>4,4'-Biphenyldiol (13)</td>
<td>-4.00</td>
<td>-4.23</td>
<td>-4.12</td>
<td>-3.72</td>
<td>-3.71</td>
</tr>
<tr>
<td>4-Biphenyldiol (14)</td>
<td>-4.00</td>
<td>-3.69</td>
<td>-3.73</td>
<td>-4.05</td>
<td>-4.12</td>
</tr>
</tbody>
</table>

* Negative log of the EC50 for displacing [3H]estradiol from mouse cytosolic uterine ER (observed data from Korach et al. [14]).

* Structures depicted in Figure 1.
delocalizability indices are measures of the ability of xenobiotic density through orbital transfers, while acceptor super-delocalizability through charge transfer, i.e., donor super-delocalizability in-fragments were denoted as AF(LEI). Super-delocalizability is restricted to this fragment in the compounds under study. In generation of the above-mentioned local reactivity indices was noted a specific atom in a molecule. To investigate the hypothesis that a negative isopotential surrounding the A ring in compounds, or solvents. Therefore, it is assumed that those compounds with lower absolute and more polarizable (less lipophilic) molecules [31] can be categorized as having potential for PCHB binding to the ER based on nonoptimized conformer geometries were not qualitatively different from those based on optimized structures. To further assess the influence of geometry optimization, two parallel analyses were undertaken. In the first, the electronic parameters associated with the DES and PCHB conformers were calculated using the AM1 Hamiltonian without geometry optimization (i.e., 1SCF calculations), while in the second analysis optimized conformers were evaluated. Assuming that E2 geometries could be decisive for the Q SAR outcome, the 22 optimized conformers for this structure were preserved in both analyses.

In developing and interpreting QSAR models, there is always the challenge of assessing whether statistically significant correlations are based solely on chance. Obviously, the possibility of deriving statistically significant correlations by chance increases as the number of descriptors used in deriving relationships increases. Although it is impossible to ever categorically declare that a QSAR is not based on chance, regardless of the limited number of descriptors evaluated, the likelihood that a relationship is mechanistically reasonable increases if the descriptors chosen for study can be based on a plausible hypothesis or set of hypotheses. In the current study, the possibility of generating biologically insignificant correlations was diminished by restricting the choice of steric, physicochemical, and electronic descriptors to those that were hypothesized to be important for PCHB binding to the ER based on previously published research primarily restricted to E2 derivatives [3,5,6,10,11,18,19]. Another potential means of generating chance correlations using the approach described here concerns the number and nature of the conformer screens. In the current study 10 conformer screens were performed. Two screens, based on GW and Lmax, are associated with issues of steric bulk and interatomic distances, which have been suggested as important criteria for ER binding [3,6]. The possibility that planarity may be associated with active conformers was also assessed based on recent investigations of PCB binding affinities to the Ah receptor [17; O.G. Mekenyan et al., unpublished manuscript]. Five of the six electronic descriptors used to screen the conformers were based on the findings of Waller et al. [3].

Conformer selection and QSAR analyses. In an exploration for ‘‘active’’ conformers, structures were screened based on the steric descriptors GW (a sum of geometric distances [28]), Lmax (the greatest interatomic distance in a molecule), and planarity (the normalized sum of torsional angles in a molecule); the global electronic descriptors \( E_{\text{LUMO}} \), \( E_{\text{HOMO}} \), \( E_{\text{HOMO-LUMO}} \); the local electronic descriptors \( S_1^L \) and \( S_2^L \) (where O3 and 3 correspond to the phenolic oxygen and its neighboring aromatic carbon atom in the E2 A ring, respectively; see Fig. 1); and the physicochemical descriptor VolP. The conformers associated with the prevailing minimum and maximum ranges for these parameters were selected and organized in correlation samples. Within each sample, conformers derived from the same compound were considered as distinct independent variables (i.e., each conformer had different electronic parameters), but each conformer was associated with the pEC50 value (dependent variable) for the ‘‘parent’’ two-dimensional compound. The identification of proposed ‘‘active’’ conformers was based on an evaluation of the regression statistics associated with QSARs derived using the different conformations.

Recent dynamic QSAR studies on the toxicity of unsaturated alcohols [21], semicarbazides [33], and \( \alpha \)-terthienyls [34], as well as the binding of PCBs to the Ah receptor (O.G. Mekenyan et al., unpublished manuscript), indicated that models based on nonoptimized conformer geometries were not qualitatively different from those based on optimized structures. To further assess the influence of geometry optimization, two parallel analyses were undertaken. In the first, the electronic parameters associated with the DES and PCHB conformers were calculated using the AM1 Hamiltonian without geometry optimization (i.e., 1SCF calculations), while in the second analysis optimized conformers were evaluated. Assuming that E2 geometries could be decisive for the Q SAR outcome, the 22 optimized conformers for this structure were preserved in both analyses.

Conformer selection and QSAR analyses. In an exploration for ‘‘active’’ conformers, structures were screened based on the steric descriptors GW (a sum of geometric distances [28]), Lmax (the greatest interatomic distance in a molecule), and planarity (the normalized sum of torsional angles in a molecule); the global electronic descriptors \( E_{\text{LUMO}} \), \( E_{\text{HOMO}} \), \( E_{\text{HOMO-LUMO}} \); the local electronic descriptors \( S_1^L \) and \( S_2^L \) (where O3 and 3 correspond to the phenolic oxygen and its neighboring aromatic carbon atom in the E2 A ring, respectively; see Fig. 1); and the physicochemical descriptor VolP. The conformers associated with the prevailing minimum and maximum ranges for these parameters were selected and organized in correlation samples. Within each sample, conformers derived from the same compound were considered as distinct independent variables (i.e., each conformer had different electronic parameters), but each conformer was associated with the pEC50 value (dependent variable) for the ‘‘parent’’ two-dimensional compound. The identification of proposed ‘‘active’’ conformers was based on an evaluation of the regression statistics associated with QSARs derived using the different conformations.
Finally, VoIP was employed as a screening descriptor in an attempt to assess whether the polar characteristics of the media surrounding the ER could play a role in binding. Thus, it is important to note that an initial data set based on all possible conformers and all possible descriptors was not employed. Rather, specific conformer sets (selected using the 10 descriptors described above) were evaluated individually with QSARs derived from a restricted set of mechanistically plausible descriptors that were identified based on previously published research.

RESULTS

In the present study, the range of \( \Delta H^f \) values for the energy minima of the 22 optimized E\(_2\) conformers (–108.50 to –95.08 kcal/mol) was found to be comparable with the free energy of binding for E\(_2\) to the ER (–12.1 kcal/mol; see Wiese and Brooks [6]). The range of \( \Delta H^f \) values for the energy minima of the 45 optimized DES conformers (–47.35 to –44.74 kcal/mol) was even smaller than that observed for E\(_2\). Assuming the activation energy barriers between the conformers are less than 12.1 kcal/mol, binding to the ER could offset the energy necessary for E\(_2\) and DES conformer interconversion. The ranges for some potentially relevant electronic descriptors for these conformers can be significant. For example, with the optimized DES conformers, ranges of 0.29 eV for \( E_{\text{HOMO}} \), 0.23 eV for \( E_{\text{LUMO}} \), 0.52 eV for \( E_{\text{HOMO-LUMO}} \); and 0.021 and 0.036 for \( q_3 \) and \( q_4 \), respectively, were observed. The ranges of values for representative electronic descriptors across all the optimized conformers are presented in Table 2. In some cases, the variation of values across conformers of the same compound can be comparable to the range of values for all molecules under investigation. For example, the charge on C3 in the A ring (\( q_3 \)) has a range of 0.036 for DES, while the range across \( q_3 \) for all the compounds in the series was 0.063. Similarly, the variation in \( E_{\text{HOMO-LUMO}} \) for the conformers of DES was 0.52 eV, while the variation across all 14 compounds was 1.34 eV. The range of \( AF(f^{\text{HOMO}}) \) was from 0.003 to 0.230 for optimized DES conformers and from 0.003 to 0.294 across all the conformers in the data set. Generally, variations in descriptor values for the nonoptimized conformers were larger. These results illustrate that the selection of specific geometries can lead to the use of electronic values that could significantly influence the outcome of a QSAR analysis and associated mechanistic interpretation.

As discussed previously, we hypothesized that PCHBs with greater binding affinity should have higher electron donating abilities associated with their corresponding phenolic ring. Such chemicals should have higher (lower) donor (acceptor) superdelocalizabilities and frontier charges on the HOMO (LUMO) orbital, as well as larger negative charges on the phenolic oxygen and the aromatic carbons in the A ring. This hypothesis is based on experimental and theoretical findings [5,6,10,11] for the involvement of a negative isopotential surrounding the E\(_2\) A ring in ER binding and the assumption that there are cationic sites (protonated amino acid residues) inside the estrogen receptor [18,19].

Because E\(_2\) and DES are significantly different from the PCHBs in terms of size, incorporation of these structures in the same QSAR reaction series raises the possibility of a size bias that could obscure the ability to assess the involvement of reactivity in ER binding. This concern was confirmed in that significant correlations were found between ER binding and volumetric parameters. For example, a regression between pEC50 and VoIP, for the lowest-energy gas-phase conformers, yielded a correlation with an \( r^2 \) of 0.73. Due to this significant influence of volume, analyses were first undertaken with the PCHBs separately to investigate those reactivity parameters associated with ER binding. Based on the assumption that the chemicals under investigation bind with the ER through a similar molecular mechanism, it was anticipated that reactivity descriptors in QSARs for the combined set of PCHBs, E\(_2\), and DES would be mechanistically related, if not identical, to those used in regressions based on the PCHBs alone. It was anticipated, however, that regressions based on the entire data set would likely require the inclusion of a volumetric (size and/or shape) parameter.

To provide a point of reference for the following presentation of modeling results based on the conformer screening method, the best regression derived with the lowest-energy gas-phase PCHB conformers was based on log \( P \) and \( S_{\text{HOMO}}^\text{H} \) and had an \( r^2 \) of 0.60. The best regression obtained when the lowest-energy gas-phase conformers of E\(_2\) and DES were combined with the lowest-energy gas-phase PCHB conformers had an \( r^2 \) of 0.90.

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Table 2. Ranges of values for representative electronic descriptors for optimized estradiol, diethylstibestrol, and polychlorinated hydroxybiphenyl conformers

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>No. of optimized conformers</th>
<th>( AF(f^{\text{HOMO}}) ) (a.u.)</th>
<th>VoIP (a.u.)</th>
<th>( S_{\text{HOMO}}^\text{H} ) (a.u.)</th>
<th>( E_{\text{HOMO-LUMO}} ) (eV)</th>
<th>( q_3 ) (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>0.266 to 0.271</td>
<td>–1.805 to –1.796</td>
<td>0.299 to 0.301</td>
<td>9.202 to 9.270</td>
<td>0.075 to 0.079</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>0.003 to 0.230</td>
<td>–1.765 to –1.758</td>
<td>0.297 to 0.302</td>
<td>8.743 to 9.262</td>
<td>0.042 to 0.078</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.264 to 0.277</td>
<td>–1.181 to –1.180</td>
<td>0.304</td>
<td>8.858 to 8.866</td>
<td>0.086 to 0.088</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.151 to 0.215</td>
<td>–1.188 to –1.179</td>
<td>0.304 to 0.310</td>
<td>8.440 to 8.889</td>
<td>0.056 to 0.092</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.121 to 0.168</td>
<td>–1.166 to –1.165</td>
<td>0.299 to 0.304</td>
<td>8.635 to 8.746</td>
<td>0.047 to 0.085</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.279</td>
<td>–1.149</td>
<td>0.304</td>
<td>8.995</td>
<td>0.088</td>
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<tr>
<td>7</td>
<td>3</td>
<td>0.216 to 0.251</td>
<td>–1.154 to –1.149</td>
<td>0.306</td>
<td>8.648 to 8.910</td>
<td>0.087 to 0.089</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0.135 to 0.165</td>
<td>–1.146 to –1.145</td>
<td>0.317 to 0.324</td>
<td>8.389 to 8.527</td>
<td>0.060 to 0.104</td>
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<tr>
<td>9</td>
<td>2</td>
<td>0.135 to 0.136</td>
<td>–1.122</td>
<td>0.325</td>
<td>8.294 to 8.302</td>
<td>0.105</td>
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<tr>
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<td>8.844 to 9.259</td>
<td>0.061 to 0.090</td>
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<tr>
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<td>8.444 to 9.449</td>
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<tr>
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<td>8.504</td>
<td>0.125</td>
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<td>13</td>
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<td>8.427 to 8.430</td>
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<tr>
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<td>3</td>
<td>0.196 to 0.294</td>
<td>–1.117 to –1.108</td>
<td>0.297 to 0.303</td>
<td>8.611 to 9.631</td>
<td>0.119 to 0.123</td>
</tr>
</tbody>
</table>

\( ^a \) See Table 1 for compound names.
and was based on log $P$ and $S_r^r$ in addition to VolP. However, as presented below, these correlations were lower than those derived through an analysis of specific conformers.

Using the dynamic QSAR method to isolate the most active PCHB conformers, the most statistically robust models (confidence level of 99%) were obtained when conformers having the lowest absolute values for VolP were incorporated into the correlation samples. As discussed previously, lower absolute values of VolP for conformers are typically associated with more polarizable 3-D structures. In general, the most polarizable unoptimized conformers of DES and PCHBs tended to have lower conjugation and lower planarity than the lowest-energy gas-phase conformers. As indicated in Table 3, the torsional angle for the C-1±C-10±C-13±C-1 bond in the most polarizable optimzied conformer and the lowest-energy gas phase conformer, respectively. The length from O3 to O17 was 10.2 and 10.4 Å in the most polarizable optimized conformer and the lowest-energy gas-phase conformer, respectively. See Figure 1 for the location of specific atoms.

The torsional angle for C-1 in the PCHBs. See Figure 1 for the location of specific atoms.

Regressions for the most polarizable nonoptimized PCHB conformers a

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Most polarizable unoptimized conformer</th>
<th>Most polarizable optimized conformer</th>
<th>Lowest energy gas-phased conformer</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>95.1</td>
<td>60.1</td>
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<td>120</td>
<td>109.4</td>
<td>114.3</td>
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<td>40.6</td>
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</tr>
</tbody>
</table>

* Upon request, the X, Y, Z coordinates for all conformers are available from the authors in MOL2 format.

See Table 1 for compound names.

The twist bond in E$_2$, which is a measure of the C-1–C-10–C-13–C-18 torsion angle [6], is reported in the table. In addition, the ring bowing angle, a measure of the plane angle difference of the A ring plane (C-1, C-2, C-4, C-5) in relation to the B–C–D ring plane (C-6–C-12, C-14–C-17) [6], was 65.9° and 38.8° in the most polarizable optimized conformer and the lowest-energy gas phase conformer, respectively. The length from O3 to O17 was 10.2 and 10.4 Å in the most polarizable optimized conformer and the lowest-energy gas-phase conformer, respectively. See Figure 1 for the location of specific atoms.

The torsional angle for C-8±C-7±C-3±C-4 in DES. See Figure 1 for the location of specific atoms.

The torsional angle for C-1±C-1 in the PCHBs. See Figure 1 for the location of specific atoms.

VolP values) can be summarized by the following equations (see Fig. 2a and Tables 1 and 4 for Eqn. 1):

$$pE_{C50} = -6.46 (\pm 0.32) + 0.51 (\pm 0.063) \log P + 5.65 (\pm 0.14) \alpha f^{HOMO}$$

$$r^2 = 0.79; \quad s^2 = 0.11; \quad F = 51.58; \quad r_n^2 = 0.79; \quad s_n^2 = 0.18$$

where $n$ is the cardinality of the correlation sample (i.e., the number of conformers associated with the 12 PCHBs);

$r^2$ is the variance;

$s^2$ is the standard error of the estimate;

$F$ is the Fisher criterion; and

$r_n^2$ and $s_n^2$ is a “leave-one-out” cross-validation variance and standard error estimate [16], respectively.

Significant regressions also were obtained when log $P$ was combined with $\alpha f^{HOMO}$ ($r^2 = 0.79, s^2 = 0.12$), $\alpha f^{LUMO}$ ($r^2 = 0.78, s^2 = 0.12$), $-f^{HOMO}$ ($r^2 = 0.75, s^2 = 0.13$), $-f^{LUMO}$ ($r^2 = 0.74, s^2 = 0.13$, $\alpha F(f^{LUMO})$ ($r^2 = 0.74, s^2 = 0.13$), and $-\alpha F(S^t)$ ($r^2 = 0.67, s^2 = 0.17$), where a negatively correlated parameter is denoted by “-$-$” and a positively indicates a positive correlation.
on HOMO and LUMO orbitals, related to the ability of the phenolic fragment to exchange (donate) electrons, appeared in most of the regressions.

Incorporation of the most polarizable E2 and DES conformers (nonoptimized for DES) with the polarizable but nonoptimized PCHB conformers in the correlation sample resulted in regressions with a similar mechanistic interpretation. However, as expected, the inclusion of a volumetric parameter (e.g., VolP or related geometric and topological indices) was required. A regression that includes VolP is described by Equation 2 (see Fig. 2b and Tables 1 and 4 for Eqn. 2):

$$pEC50 = 3.29(\pm 2.39) + 0.74(\pm 0.064)\log P - 47.8(\pm 7.91)S_N - 4.18(\pm 0.20)\text{VolP}$$

$$n = 54(14); \quad r^2 = 0.98; \quad s^2 = 0.072; \quad F = 860.1 \quad r_{ca}^2 = 0.98, \quad s_{ca}^2 = 0.096$$

Other significant models were obtained with $$+S_{ab}^N$$ ($$r^2 = 0.98; \quad s^2 = 0.073$$), $$+AF(\text{LUMO})$$ ($$r^2 = 0.98, \quad s^2 = 0.077$$), $$+S_{ab}^N$$ ($$r^2 = 0.98, \quad s^2 = 0.075$$), $$-g_{D1}$$ ($$r^2 = 0.98, \quad s^2 = 0.083$$), $$-\text{AF}(S^N)$$ ($$r^2 = 0.98, \quad s^2 = 0.092$$), $$-\text{AF}(S^N)$$ ($$r^2 = 0.98, \quad s^2 = 0.084$$), and $$+\text{AF}(S^N)$$ ($$r^2 = 0.98, \quad s^2 = 0.089$$).

Interestingly, optimization of the PCHB conformers resulted in a set of 3-D structures that was inherently more polarizable than the original set of nonoptimized compounds. Thus, after geometry optimization, the range of VolP values for the conformers of a given PCHB congener tended to shift toward less negative values (e.g., with 2-chloro-4,4’-biphenyldiol, VolP values ranged from −1.174 to −1.170 for the nonoptimized conformers, but for the optimized conformers VolP values ranged from −1.166 to −1.165). Consistent with this observation, the differences in specific torsional angles for the most polarizable optimized conformers compared to the lowest-energy gas-phase conformers were not as large as those noted previously with the unoptimized conformers (see Table 3).

However, a general trend of increased polarizability and decreased planarity was noted when examining the lowest-energy gas-phase and most polarizable optimized conformers (a notable exception being compound 10, 2-chloro-4-biphenyldiol). The largest differences in the C-1’-C-1 torsional angles for the different conformers were noted with the 2,3,4,5-tetrachloro-4’-biphenyldiol and 2,5-dichloro-4’-biphenyldiol (compounds 4 and 7). The “twist” torsional angle, the ring bowing angle, and the distance from O-3 to O-17 for E2 are also provided in Table 3. Based on MMP2 molecular modeling optimization, Wiese and Brooks [6] reported two unique optimized E2 conformers, with the lowest-energy gas-phase conformer and the higher-energy conformer having twist angles, ring bowing angles, and O-3 to O-17 distances of 86.3 and 54.3°, 12.6 and 28.1°, and 10.9 Å, respectively.

Based on the results obtained with nonoptimized conformers, the optimized PCHB conformers were subsequently screened to select those 3-D geometries associated with the most polarizable structures. The regression with the highest correlation for the “polarizable” subset of optimized PCHB conformers is summarized in Equation 3 (see Tables 1 and 4):

$$pEC50 = -43.6(\pm 6.2) + 3.13(\pm 0.58)D^2 + 3.25(\pm 0.52)E_{\text{HOMO-LUMO}}$$

$$n = 18(12); \quad r^2 = 0.74; \quad s^2 = 0.16; \quad F = 21.42; \quad r_{ca}^2 = 0.74, \quad s_{ca}^2 = 0.22$$

where

$$D^2 = \text{the Balaban mean-square distance topological index. This index reflects structural characteristics such as molecular size and branching; high values of } D^2 \text{ correspond to large molecular size and low degree of branching [25]}

As discussed previously for the nonoptimized conformers, inclusion of E2 and DES with the PCHB series in the correlation sample required the incorporation of a volumetric parameter (VolP) in the regression (see Eqn. 2). However, as summarized in Equation 4 (see Fig. 3 and Tables 1 and 4), the topological index, $$D^2$$, sufficiently incorporated the size bias associated with the addition of the optimized E2 and DES conformers to the PCHB conformer set:
\[ pEC_{50} = -44.3(\pm 1.8) + 3.38(\pm 0.12)D^2 \\
+ 3.22(\pm 0.20)E_{\text{HOMO-LUMO}} \]

\[ n = 33(14); \quad r^2 = 0.98; \quad s^2 = 0.088; \quad F = 595.02, \quad r^2_0 = 0.97, \quad s^2_0 = 0.13 \]

Although the descriptors used in the regressions for the optimized conformers (Eqns. 3 and 4) were different from those found in the regressions based on the most polarizable nonoptimized conformers (Eqns. 1 and 2), they are related. For example, the topological index, \( D^2 \), was correlated with \( \text{VoIP} \) \( (r^2 = 0.77) \) and \( \log P \) \( (r^2 = 0.55) \), which is consistent with commonly acknowledged relationships between size, degree of branching, and hydrophobicity. With this data set, it appears that the HOMO–LUMO gap is representing an integral descriptor associated with the electronic donating character of the conformers. Thus, the HOMO–LUMO gap was regressed satisfactorily by two parameter linear correlations that included \( -\text{AF}(S^N) \) and \( -\text{AF}(q) \) \( (r^2 = 0.75) \) or \( +\text{AF}(S^P) \) and \( -\text{AF}(q) \) \( (r^2 = 0.74) \). These parameters are related to the descriptors associated with Equations 1 and 2 and are consistent with our basic hypothesis for the electronic requirements for ER binding. However, inclusion of \( D^2 \) with \( -\text{AF}(S^N) \) and \( -\text{AF}(q) \) or \( +\text{AF}(S^P) \) and \( -\text{AF}(q) \) in three parameter regressions resulted in lower correlations than those obtained in Equations 3 and 4, which were based on \( D^2 \) and \( E_{\text{HOMO-LUMO}} \).

Because the optimized conformers were generally more polarizable to begin with, an exploratory screening was performed to determine whether a different subset of the optimized conformers were more closely related to \( pEC_{50} \). Based on a screening that selected those optimized conformers with minimum \( E_{\text{LUMO}} \) values, QSARs similar to Equations 1 and 2 were obtained. For example, with the series of PCHBs alone, significant equations were obtained by combining \( \log P \) with \( S^E_{\text{DO}} \) \( (r^2 = 0.75, s^2 = 0.16) \), \( -q_{\text{DO}} \) \( (r^2 = 0.73, s^2 = 0.18) \), and \( -S^D_{\text{DO}} \) \( (r^2 = 0.72, s^2 = 0.19) \). Consistent with the previously reported regressions, the inclusion of \( E_2 \) and DES with the PCHB series required the incorporation of a volumetric parameter. For example, significant models were obtained by combining \( \log P \) and \( \text{VoIP} \) with \( -q_{\text{DO}} \) \( (r^2 = 0.98, s^2 = 0.066) \), \( S^E_{\text{DO}} \) \( (r^2 = 0.98, s^2 = 0.070) \), and \( -\text{AF}(S^P) \) \( (r^2 = 0.97, s^2 = 0.10) \).

**DISCUSSION**

Based on the assumption that conformational flexibility is of critical importance in ligand–receptor interactions, we employed a dynamic QSAR method [21] to model the relative ER binding affinity of a series of PCHBs. As opposed to conventional QSAR methods, the approach used in this study does not assume a priori that 3-D molecular structures represented by single lowest-energy gas-phase conformers are appropriate to model physicochemical properties, toxicological end points, or the interactions of xenobiotics with receptors in a solvated environment [21,33,34]. Use of this method permits a systematic assessment of the conformational space associated with the compounds of interest, either in the context of testing specific hypotheses or as a means of exploring possible ligand–receptor interactions.

In the context of ER binding, the results of the current study documented that the use of a single low-energy gas-phase conformer may be problematic. An assessment of the 22 optimized \( E_2 \) conformers indicated that the free energy of binding to the ER complex was likely sufficient to facilitate interconformational variations for complex structures. An explanation for the finding that a subset of optimized conformers most closely associated with ER binding affinity had the highest electron acceptor ability (minimum \( E_{\text{LUMO}} \) values) is not readily apparent. Consistent with the QSARs reported for the more polarizable conformers, correlations for ER binding obtained using the subset of optimized conformers based on minimum \( E_{\text{LUMO}} \) values were still highly dependent on the electron donating properties of the A ring. The observation that the electron acceptor character of the entire structure was associated with high ER binding may be reflective of an additional reaction step that is not related to the required electron donating character of the A ring.

As mentioned above, trends of the reactivity parameters in the QSARs derived in this study supported the hypothesis that PCHBs with greater binding affinity should have higher electron donating abilities associated with their corresponding phenolic ring. As indicated in Equations 1 through 4 and related correlations, the increase (decrease) of electron donor (acceptor) properties of the phenolic oxygen and those of the aromatic fragment of the A ring analogue in the PCHBs was associated with enhanced ER binding (e.g., positive correlations with \( S^E \) and negative correlations with \( S^N \) and \( q \) on the oxygen atom and the neighboring aromatic carbon atom). Increases in hydrophobicity (positive correlations with \( \log P \) and volume (positive correlations with VoIP, GW, \( I_{\text{max}} \), \( D^2 \)) also were associated with higher affinity for the ER.
In a previously reported CoMFA-based analysis of the PCHB, DES, and E2 data set [3], ER binding affinities were related to a steric field; however, there were substantial differences between conventional and cross-validated regression statistics for the associated models \( (r^2 = 0.960-0.974 \text{ and } r^2_c = 0.345-0.544) \). The findings of this previous study also suggested that a CoMFA model based solely on an electrostatic field was not predictive \( (r^2 = 0.979 \text{ and } r^2_c = -0.0548) \), as was a CoMFA model that combined steric and electrostatic fields \( (r^2 = 0.957 \text{ and } r^2_c = 0.276) \). In the present study it was noted that E2 and DES contribute a significant size bias when included with the PCHBs. This size bias may have contributed to the discrepancies observed between the conventional and cross-validated statistics in the CoMFA models. To better interpret the predictive capability of CoMFA-based models for ER binding affinity of PCHBs, additional analyses with and without E2 and DES in the correlation samples are required to more completely assess the influence of template flexibility, as well as steric and electrostatic fields. The influence of template (i.e., E2) flexibility also could be analyzed by using specific conformers.

The results of the current study, which illustrate the importance of the electron donating capability of the A ring in the PCHB structure, are consistent with an electrostatic modeling study of E2 and related derivatives by VanderKuur et al. [10]. Our findings also are consistent with those of Lewis et al. [35], who used \( \alpha \)-antitrypsin to develop a proposed molecular structure of the ligand-bound ER. The investigation by VanderKuur et al. [10] suggested a requirement for a distinct negative isopotential pattern around C-2 and C-3 oxygens of estratrienes and the C-3 oxygen of androstan. Binding to the ER is completely eliminated in the absence of the electronegative isopotential above the nonaromatic ring of androstanes. The negative isopotential associated with the unpaired electrons of the hydroxy group is presumed to be associated with hydrogen bonding to a H acceptor/donor on the receptor molecule. This proposal is consistent with a hypothesis for the existence of electrophilic sites (protonated amino acid residues) in the ER receptor cavity [18,19]. In keeping with these conclusions, Lewis et al. [35] suggested that a tyrosine residue in the hydrophobic ligand binding site likely interacts with the A ring of estradiol through possible hydrogen bonding and \( \pi-\pi \) stacking interactions between the two phenolic structures. These observations also are consistent with the current results which indicated that hydrophobicity, size, and electron donating character of the A ring in PCHBs are significantly related to binding affinity. Based on the similar results among these studies it is reasonable to presume that any nucleophilic substituent on the A ring of an E2 analogue, such as \( \pm \)NH or \( \pm \)SH, could elicit similar binding characteristics by acting as H bond donors.

In the current study high intercorrelations were found between reactivity parameters for the hydroxyl oxygen and those of the aromatic ring. Thus, donor superdelocalizability of the phenolic oxygen \( (S^0_{\text{el}}) \) was correlated \( (0.90 \leq r^2 \leq 0.98 \) for different conformer screens) with the average donor superdelocalizability of the aromatic ring, \( \Delta F(S^0) \), which suggests that the electronic structures of the phenolic hydroxy group and the aromatic ring may be interrelated through conjugation and inductive effects. However, the systematic presence of the hydroxy moiety in all the chemicals examined in the current study and the significance of the atom-averaged aromatic ring indices in the associated QSARs suggests that the hydroxy moiety in the aromatic A ring is not an obligatory requirement for ER affinity. The possibility that the aromatic nature of the A ring may be sufficient for ER binding has been previously reported [10,15]. For example, estratrien-17\( \beta \)-ol has a binding affinity similar to that of 4-hydroxyestratrien-17\( \beta \)-ol, which is approx. 10-fold lower than that of E2 [9]. These findings suggested that the hydroxy group increases binding affinity by acting as a promoter for the aromatic ring and by undergoing hydrogen bonding at a distinct reaction site. This suggestion is consistent with the observations of Lewis et al. [35], who indicated in their model that a specific tyrosine residue in the ligand binding site is involved.

In conclusion, the results of this investigation are consistent with our previous studies and suggest that the use of low-energy gas-phase conformers to model complex biological or toxicological responses may be inappropriate in some cases [20,24]. Because the energy for transitions among low-energy conformations of a ligand can be less than the free energy of binding to a receptor and because the electronic characteristics across conformers of a molecule can vary widely, hypotheses concerning the role of specific electronic properties in predicting binding affinities of xenobiotics must be tested with careful attention paid to the appropriate use of specific geometries. In addition, ligand binding to a receptor can alter the geometry/energy of the resulting activated complex and modulate subsequent effects. Thus, numerous reports that the regulation of estrogenic responses by E2 derivatives is not directly related to ER binding affinity may reflect, at least in part, the role of conformer flexibility [6,9–11], the geometry and energy of the activated ER–ligand complex, and associated cooperative conformational changes within the DNA binding domain [35]. In the context of developing estrogenic and antiestrogenic structure–activity relationships for non-steroidal compounds, such as PCHBs, the ability to assess conformational flexibility also appears to be critical, not only for predicting ER binding affinity but also for predicting nuclear binding to the ER complex and the subsequent induction of products from responsive genes and associated in vivo effects.

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