Prediction of CYP2D6 Drug Interactions from In Vitro Data: Evidence for Substrate-Dependent Inhibition

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ABSTRACT:
Predicting the magnitude of potential drug-drug interactions is important for underwriting patient safety in the clinical setting. Substrate-dependent inhibition of cytochrome P450 enzymes may confound extrapolation of in vitro results to the in vivo situation. However, the potential for substrate-dependent inhibition with CYP2D6 has not been well characterized. The inhibition profiles of 20 known inhibitors of CYP2D6 were characterized in vitro against four clinically relevant CYP2D6 substrates (desipramine, dextromethorphan, metoprolol, and thioridazine) and bufuralol. Dextromethorphan exhibited the highest sensitivity to in vitro inhibition, whereas metoprolol was the least sensitive. In addition, when metoprolol was the substrate, inhibitors with structurally constrained amino moieties (clozapine, debrisoquine, harmine, quinidine, and yohimbine) exhibited at least a 5-fold decrease in inhibition potency when results were compared with those for dextromethorphan. Atypical inhibition kinetics were observed for these and other inhibitor-substrate pairings. In silico docking studies suggested that interactions with Glu216 and an adjacent hydrophobic binding pocket may influence substrate sensitivity and inhibition potency for CYP2D6. The in vivo sensitivities of the clinically relevant CYP2D6 substrates desipramine, dextromethorphan, and metoprolol were determined on the basis of literature drug-drug interaction (DDI) outcomes. Similar to the in vitro results, dextromethorphan exhibited the highest sensitivity to CYP2D6 inhibition in vivo. Finally, the magnitude of in vivo CYP2D6 DDIs caused by quinidine was predicted using desipramine, dextromethorphan, and metoprolol. Comparisons of the predictions with literature results indicated that the marked decrease in inhibition potency observed for the metoprolol-quinidine interaction in vitro translated to the in vivo situation.

Introduction
The metabolism of a majority of currently prescribed drugs and new chemical entities involve the cytochrome P450 (P450) superfamily of drug-metabolizing enzymes (Wienkers and Heath, 2005). Within the P450 superfamily, CYP2D6 accounts for the metabolism of approximately 25% of the marketed drugs (Zhou, 2009a). CYP2D6 expression is polymorphic, with 5 to 10% of whites, approximately 1% of Chinese, and 0 to 19% of African Americans exhibiting the poor metabolizer phenotype (Zhou, 2009a). Administration of a potent CYP2D6 inhibitor is not expected to cause a marked CYP2D6-mediated DDI for individuals exhibiting the poor metabolizer phenotype. However, CYP2D6-mediated DDIs may be observed for individuals exhibiting the ultrapid, extensive, and intermediate metabolizer phenotypes (Zhou, 2009b). Therefore, characterizing new chemical entities for CYP2D6-mediated metabolism and the potential for inhibition is common. The overall objective was to develop a comprehensive understanding of substrate selection for CYP2D6 on the basis of in vitro and in vivo sensitivity analysis (observed magnitude of inhibition).

Bufuralol and dextromethorphan are the U.S. Food and Drug Administration (FDA) recommended in vitro substrates for studying CYP2D6 inhibition (Huang et al., 2007). Approximately 60% of the regulatory submissions from the pharmaceutical industry use bufuralol hydroxylation as the marker reaction to determine CYP2D6 inhibition potency in vitro, whereas approximately 30% use dextromethorphan O-demethylation (Yuan et al., 2002). However, bufuralol does not have marketing approval in the United States, making direct comparisons of predictions from in vitro data to clinical outcomes difficult. Desipramine, dextromethorphan, and atomoxetine are FDA-recommended in vivo substrates for studying CYP2D6 inhibition. Thioridazine is a narrow therapeutic index drug with clearance predominantly modulated by CYP2D6 (Huang et al., 2007). It is not clear how the in vitro and in vivo inhibition profiles of these substrates compare. The first aim was to measure the in vitro inhibition profiles of four clinically relevant CYP2D6 substrates (desipramine, dextromethorphan, metoprolol, and thioridazine) and bufuralol versus a panel of 20 known CYP2D6 inhibitors and to determine the relative in vitro sensitivities.

The substrate-free crystal structure of CYP2D6 has been solved to a resolution of 3.0 Å (Rowland et al., 2006). On the basis of the crystal structure, docking studies, and site-directed mutagenesis experiments, Phe120, Glu216, Asp301, Phe481, and Phe483 have been identified as key residues for substrate interactions within the CYP2D6 active site.
CYP2D6: bufuralol, desipramine, dextromethorphan, metoprolol, and thioridazine (Hayhurst et al., 2001; Guengerich et al., 2003; Paine et al., 2003; Flanagan et al., 2004; de Graaf et al., 2007). The second aim was to perform docking studies of bufuralol, desipramine, dextromethorphan, metoprolol, and select inhibitors to determine whether specific CYP2D6 active site interactions may contribute to the differences in inhibition potency profiles observed in these studies.

Recent guidance from the FDA (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072101.pdf) outlining the design of P450-mediated DDI experiments indicates that sensitive CYP2D6 substrates, such as desipramine or dextromethorphan, may be used for clinical DDI studies (Huang et al., 2007). However, the in vivo sensitivity of CYP2D6 substrates has not been well characterized. The third aim was to mine the literature for clinical CYP2D6 DDI outcomes and to correlate the DDI sensitivity of three CYP2D6 substrates commonly used in the clinical setting: desipramine, dextromethorphan, and metoprolol.

The ability to predict changes in the exposure levels of a given drug in the presence of an inhibitor enables the design of clinical DDI studies (Rostami-Hodjegan and Tucker, 2007). Quinidine, a potent and selective inhibitor of CYP2D6, exhibited substrate-dependent inhibition in vitro. The fourth and final aim was to predict the magnitude of CYP2D6 inhibition expected in vivo using quinidine as the inhibitor to determine whether the substrate dependence observed in vitro translated to the in vivo situation compared with clinical observations.

Materials and Methods

Materials. Pooled human liver microsomes (15 individual donors) were purchased from CelzDirect (Durham, NC). Ammonium formate, HPLC-grade acetonitrile, and HPLC-grade methanol were obtained from Alfa Aesar (Ward Hill, MA). NADPH was purchased from EMD Biosciences (San Diego, CA). The metabolites α-hydroxymetabolite, O-desmethylmetabolite, mesoridazine, and 2-hydroxydesipramine were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). All other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) and were of the highest purity available.

Kᵢ Determination. Incubations were carried out using five substrates of CYP2D6: bufuralol, desipramine, dextromethorphan, metoprolol, and thioridazine. Twenty known inhibitors of CYP2D6 exhibiting a wide range of inhibition potencies were selected for the in vitro studies. Stock solutions of all the inhibitors were made and diluted in acetonitrile-water (50:50) to minimize organic solvent content. Four concentrations of each substrate (0.5 × Kᵢ, 2 × Kᵢ, 5 × Kᵢ, and 10 × Kᵢ) were used for determination of Kᵢ in a 96-well plate format. For inhibitor-substrate pairs that exhibited two-site inhibition kinetics, a follow-on experiment with six concentrations of inhibitor (spanning a 10-fold range of the expected Kᵢ) and substrate (∼0.25 × Kᵢ to 8 × Kᵢ) was used to determine Kᵢ. In brief, each reaction was performed in duplicate, containing 0.1 mg/ml human liver microsomal protein per incubation. The human liver microsomes were pooled from 15 donors but were not genotyped for CYP2D6. Each incubation reaction mixture contained enzyme, substrate, and inhibitor suspended in phosphate buffer (100 mM, pH 7.4) containing 3 mM MgCl₂ and was preincubated for 3 min in an incubator-shaker at 37°C. The reactions were initiated by the addition of NADPH (1 mM final concentration). Total organic solvent concentrations did not exceed 0.5% v/v. Solvent concentrations were the same for all experiments, and turnover rates did not differ significantly from those of solvent controls. The reactions were terminated with 100 μl of acetonitrile containing 0.1 μM tolbutamide (internal standard). Length of the incubations was 5 min. The incubation time and protein concentrations used were within the linear range for each respective P450 reaction.

Liquid Chromatography-Tandem Mass Spectral Analysis. All analyses were conducted using HPLC-MS/MS technology. In brief, the HPLC-MS/MS system comprised an Applied Biosystems 4000 QTRAP system (operated in triple quadrupole mode) equipped with an electrospay ionization source (Applied Biosystems, Foster City, CA). The MS/MS was coupled to two LC-20AD pumps with an in-line C18-20A controller and DGU-20As, solvent degasser (Shimadzu, Columbia, MD) and a LEAP CTC HTS PAL autosampler equipped with a dual-solvent self-washing system (CTC Analytics, Carrboro, NC). The injection volume was 10 μl for each sample. For all assays, HPLC separation was achieved using a Gemini C18 column (2.0 × 30 mm, 5 μm; Phenomenex, Torrance, CA). Gradient elution (flow rate = 500 μl/min) was performed using a mobile phase system consisting of (A) 5 mM ammonium formate with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. HPLC flow was diverted from the MS/MS system for the first 20 s to remove any nonvolatile salts. MS/MS conditions were optimized for individual analytes accordingly. Generic mass spectrometry parameters included the curtain gas (10 arbitrary units), collisionally activated dissociation gas (medium), ionspray voltage (4500 V), source temperature (450°C), and ion source gas 1 and gas 2 (40 arbitrary units each). Interface heaters were kept on for all analytes. The limit of detection for each analyte was greater than 10 pmol on column. All analyses were performed using Analyst (version 1.4.2; Applied Biosystems). In general, standard curves were weighted using 1/x. Substrate saturation curves and inhibition data were plotted and analyzed using GraphPad Prism (version 4.01; GraphPad Software Inc., San Diego, CA). Data were then fit to a competitive (eq. 1), noncompetitive (eq. 2), linear-mixed (eq. 3), or two-site inhibition model (eq. 4); the equation for two-site inhibition has been derived previously (Galetin et al., 2002). The mechanism of inhibition was determined by visual inspection of the data using Dixon ([I] versus 1/v) and Lineweaver-Burk (1/[S] versus 1/v) plots and comparative model assessment using the Akaike information criterion:

\[
K_{mi} \left(1 + \frac{[I]}{K_i} \right) + [S] = 0
\]

\[
K_{mi} \left(1 + \frac{[I]}{K_i} \right) + [S] = \frac{V_{max}}{[I] + K_i}
\]

\[
K_{mi} \left(1 + \frac{[I]}{K_i} \right) + [S] = \frac{V_{max}}{[S] \left(1 + \frac{[I]}{K_i} \right)}
\]

\[
1 + \frac{2[S]}{K_c} + \frac{S^2}{K_{c}^2} + \frac{2[S[I]}{S_{c}K_{c}} + \frac{I^2}{K_{c}^2} + \frac{V_{max}}{K_{c}^2} = 0
\]

In the preceding equations, Kᵢᵣ is equal to the substrate concentration at half-maximal reaction velocity, [I]ᵢ is the concentration of inhibitor in the system, Kᵢᵣ is the dissociation constant for the enzyme-inhibitor complex, Kᵢᵣᵣ is the dissociation constant for the enzyme-substrate-inhibitor complex, Kᵢᵣᵣᵣᵣ is the dissociation constant for the enzyme-substrate complex, the interaction factor δ describes the change in binding affinity of the substrate and inhibitor in the presence of each other, and the interaction factor γ describes whether inhibition (γ < 1) or activation (γ > 1) was observed. Note that in the above equations, Kᵢᵣᵣᵣᵣ, Kᵢᵣᵣᵣᵣ, Kᵢᵣᵣᵣᵣᵣ, δ, γ, and Vᵢᵣᵣᵣᵣ were treated as global parameters.

CYP2D6 Docking Studies. The CYP2D6 crystal structure (2P9Q) was input into Maestro and evaluated using Prime (Schrödinger, LLC, New York, NY). In particular, Prime was set to optimize intramolecular hydrogen bonds, cap terminal amino acid residues, and minimize overall protein structure. SiteMap (Schrödinger, LLC) was used to define the CYP2D6 active site and was subsequently used to define the docking grid for all docking experiments. SiteMap was also used to determine the volume of the putative hydrophobic pocket within the CYP2D6 active site and the total active site volume. The grid
parameters were defined by a 14 × 14 × 14 Å box with the default GLIDE settings. Substrates and inhibitors were prepared using LigPrep 2.0 (2006; Schrödinger, LLC). The substrates were generated as a neutral series. Substrate conformations were submitted to pre-energy optimization using the OPLS_2005 force field with a distance-dependent dielectric model. The results from the docking studies were visualized using a PyMOL Molecular Graphics System (version 1.3; Schrödinger, LLC).

**Analysis of In Vivo Drug Interaction Potential.** Literature results for AUC<sub>i</sub>/AUC were obtained using the University of Washington Metabolism and Transport Drug Interaction Database, where AUC<sub>i</sub> is defined as the area under the plasma concentration-time curve for a given substrate in the absence of inhibitor. Studies were considered comparable if they had a similar dose regimen for the substrate with the same inhibitor. For instances in which multiple AUC<sub>i</sub>/AUC values were determined using eq. 6 (Ito et al., 2005):

\[
\frac{AUC_i}{AUC} = \frac{1}{1 + \frac{f_{ucc} \cdot K_i}{I_{Bu}}} \tag{5}
\]

In the preceding equation \( f_{ucc} \) is the fraction of substrate cleared by the indicated P450, \( K_i \) is the inhibition constant, and \( I_{Bu} \) is the maximum unbound hepatic input concentration. The \( f_{ucc} \) of 0.96 for dextromethorphan (Nakashima et al., 2007) and 0.78 for thioridazine (von Moltke et al., 1998) was determined using eq. 6 (Ito et al., 2005):

\[
f_{ucc} = 1 - \frac{AUC_{EM}}{AUC_{PM}} \tag{6}
\]

In the preceding equation, AUC<sub>EM</sub> is the area under the plasma concentration-time curve for extensive metabolizers and AUC<sub>PM</sub> is the area under the plasma concentration-time curve for poor metabolizers. For the predictions using quinidine as inhibitor, the following inputs were used: \( f_{ucc} \) values of 0.88, 0.96, and 0.83 for desipramine, dextromethorphan, and metoprolol, respectively (Brown et al., 2005; Nakashima et al., 2007); \( I_{Bu} \) of 1.81, 0.9, and 0.45 μM at 200-, 100-, and 50-mg doses of quinidine, respectively (McGinnity et al., 2008); and the fraction unbound (\( I_{Bu} \)) for quinidine of 0.8 for in vitro incubations (Margolis and Obach, 2003).

**Results**

A set of 20 inhibitors and 5 substrates (bufuralol, desipramine, dextromethorphan, metoprolol, and thioridazine) were used to determine the inhibition constants (\( K_i \)) for CYP2D6 (Table 1). The structures of the substrates are shown in Fig. 1. Competitive or two-site inhibition profiles were observed in all cases. Tramadol was the only inhibitor to exhibit two-site inhibition profiles for all of the substrates tested. Dextromethorphan was the most sensitive substrate except for the cases of inhibition by bupropion, desipramine, dextromethorphan, tramadol, and verapamil, in which desipramine or thioridazine exhibited greater sensitivity. Metoprolol exhibited marked reductions in observed inhibition sensitivity from dextromethorphan for five inhibitors: clozapine (5-fold), debrisoquine (15-fold), harmine (19-fold), quinidine (17-fold), and yohimbine (18-fold). Relative to dextromethorphan, the average fold decrease in inhibition sensitivity with S.E. for each substrate was as follows: bufuralol, 2 ± 1; desipramine, 2 ± 1; metoprolol, 5 ± 6; and thioridazine, 3 ± 3.

Docking studies were performed to distinguish interactions between the substrates or inhibitors and the CYP2D6 active site that may contribute to the observed differences in inhibition potency. The total active site volume of CYP2D6 was estimated to be 580 Å³. On the basis of the docking studies, a hydrophobic binding pocket was defined in the active site of CYP2D6 by residues Phe110, Gly244, Leu248, Ile297, and Ala300 (Fig. 2). The side chain of metoprolol was positioned into the hydrophobic binding pocket with the primary site of metabolism positioned 4.1 Å away from the heme iron. In contrast, dextromethorphan did not interact with the hydrophobic

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**Table 1**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>BUF</th>
<th>DES</th>
<th>DEX</th>
<th>MET</th>
<th>TRZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiodarone</td>
<td>26.8</td>
<td>26.3</td>
<td>11.8</td>
<td>18.5</td>
<td>25.8</td>
</tr>
<tr>
<td>Bupropion</td>
<td>7.9</td>
<td>3.9</td>
<td>7.1</td>
<td>12.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Cinemidrine</td>
<td>9.4</td>
<td>23.2</td>
<td>7.7</td>
<td>7.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Clozapine</td>
<td>3.1</td>
<td>2.2</td>
<td>1.4</td>
<td>7.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4</td>
</tr>
<tr>
<td>Debrisoquine</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1</td>
</tr>
<tr>
<td>Desipramine</td>
<td>5.1</td>
<td>2.2</td>
<td>5.5</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>4.1</td>
<td>4.4</td>
<td>7.5</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>5.6</td>
<td>4.4</td>
<td>7.4</td>
<td>3.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Fluoxetine</td>
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<td>0.12</td>
<td>0.056</td>
<td>0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>2.4</td>
<td>4.1</td>
<td>0.83</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Harmine</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50</td>
<td>9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>17.0</td>
<td>14.1</td>
<td>11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Paroxetine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23</td>
<td>0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38</td>
</tr>
<tr>
<td>Propafenone</td>
<td>0.080</td>
<td>0.036</td>
<td>0.031</td>
<td>0.090</td>
<td>0.098</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.019</td>
<td>0.022</td>
<td>0.010</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.047</td>
</tr>
<tr>
<td>Quinine</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5.8</td>
</tr>
<tr>
<td>Sertraline</td>
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<td>0.93</td>
<td>0.47</td>
<td>0.74</td>
<td>0.94</td>
</tr>
<tr>
<td>Tramadol</td>
<td>1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Verapamil</td>
<td>33.9</td>
<td>8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7</td>
<td>61.7</td>
<td>38.2</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>0.078</td>
<td>0.075</td>
<td>0.062</td>
<td>0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<sup>a</sup>FIT using a two-site model; δ and θ values ranged between 0.05 and 0.5.

<sup>a</sup>A known time-dependent inhibitor of CYP2D6.
binding pocket with the site of metabolism positioned 3.6 Å away from the heme iron.

Desipramine, dextromethorphan, and metoprolol are three commonly used substrates for CYP2D6 in vivo DDI studies. The magnitude of clinical DDI observations for those CYP2D6 substrates were collected from the literature and compiled under similar study conditions (Table 2). Dextromethorphan exhibited markedly enhanced in vivo sensitivity relative to that of desipramine, whereas metoprolol exhibited similar or reduced sensitivity.

The magnitude of in vivo DDIs caused by quinidine was predicted using desipramine, dextromethorphan, and metoprolol as substrates (Table 3). Quinidine doses of 50, 100, and 200 mg were chosen on the basis of the availability of clinical DDI results from the literature. All of the predictions were within 2-fold of the experimentally determined values. For all but the low-dose desipramine-quinidine interaction, actual and predicted values were within 15% difference of each other.

Discussion

Screening for and predicting the magnitude of P450-mediated DDIs is a crucial part of the drug discovery and development paradigm with the potential to influence both patient safety and product differentiation. However, a comprehensive understanding of substrate selection for CYP2D6 on the basis of in vitro and in vivo sensitivity analysis has been lacking. In addition, several P450s exhibit substrate-dependent inhibition profiles in vitro, which may confound predictions of the in vivo situation (Kenworthy et al., 1999; Stresser et al., 2000; Kumar et al., 2006; Foti and Wahlstrom, 2008; Foti et al., 2010). Predictions of CYP2D6 inhibition using quinidine, which exhibited substrate-dependent inhibition in vitro, were used to determine whether the substrate dependence translated to the in vivo situation by comparison with observed clinical outcomes.

Our selection of in vitro substrates was based on recommendations from the draft FDA guidance and the availability of clinical DDI data. In general, bufuralol or dextromethorphan has been used as an in vitro CYP2D6 substrate (Kronbach, 1991; Huang et al., 2007). Desipramine and metoprolol were selected because of the availability of clinical DDI results. Other CYP2D6 substrates with available clinical DDI literature were excluded from consideration for the in vitro portion of this study because a significant contribution to their clearance is mediated by enzymes other than CYP2D6 (Ito et al., 2005). Thoridiazine was chosen because of its narrow therapeutic index designation from the FDA and the importance of assessing potential DDIs due to safety margins. As demonstrated by the 2-, 2-, 5-, or 3-fold average decrease in observed inhibition potency for bufuralol, desipramine, metoprolol, or thoridiazine relative to that for dextromethorphan, CYP2D6 exhibited substrate-dependent inhibition for some inhibitor-substrate pairings.
Plausible mechanisms for P450 substrate-dependent inhibition in vitro include atypical inhibition kinetics, metabolic switching, or the involvement of multiple P450 enzymes. Although a competitive inhibition model reasonably fit all of the observed kinetics, an examination of residuals suggested that an alternate kinetic model may explain the observed inhibition more appropriately. Several inhibitor-substrate pairings exhibited inhibition kinetics that were best fitted using a two-site inhibition model (Galetin et al., 2002). With this model, an inhibitor and substrate may influence the kinetics of each other through an enzyme-substrate-inhibitor complex; this interaction may occur within the active site or through binding at an allosteric site. The active site volume of CYP2D6 has been estimated to be between 540 and 797 Å³, which may allow for simultaneous occupancy by a substrate and inhibitor (Rowland et al., 2006; Porubsky et al., 2008).

Atypical inhibition kinetics, therefore, may contribute to the substrate-dependent inhibition observed in vitro for certain substrate-inhibitor pairs.

Docking studies were performed to determine whether distinct interactions between the substrates or inhibitors and the CYP2D6 active site may contribute to the observed differences in inhibition potency. On the basis of our docking studies, the increased conformational flexibility of bufuralol, desipramine, and metoprolol may afford greater access to a hydrophobic binding pocket (defined by Phe110, Gly244, Leu248, Ile297, and Ala300) (Fig. 2) relative to dextromethorphan. The inhibitors clozapine, debrisoquine, harmine, and Glu216 (Fig. 2). Thus, substrate or inhibitor rigidity and shape may influence observed inhibition profiles.

Another plausible mechanism for substrate-dependent inhibition observed in vitro is the involvement of multiple P450s in substrate metabolism or metabolic switching. The formation of mesoridazine from thoridazine has been estimated to be 50% mediated by CYP2D6 in vitro (Wójcikowski et al., 2006), whereas desipramine, dextromethorphan, and metoprolol exhibit high selectivity for CYP2D6 in vitro ($I_{50}$ of 0.88–1.0) (McGinnity et al., 2008). In concordance with lower in vitro CYP2D6 selectivity, the average fold decrease in inhibition potency of thoridazine compared with that of dextromethorphan was 3-fold. Metoprolol produces two CYP2D6-mediated metabolites, α-hydroxymetoprolol (major) and O-desmethyloxymetoprolol (minor), such that metabolic switching is another possible explanation for the distinctive metoprolol inhibition profile (Belpaire et al., 1998).

The in vivo sensitivity of substrates may also influence selection for a clinical study. Metoprolol exhibited similar sensitivity and dextromethorphan exhibited enhanced sensitivity compared with that of desipramine in vivo. A key characteristic influencing the observed differences in substrate sensitivity and affecting DDI predictions is the $f_{mic}$ value. Metoprolol, with sensitivity similar to that of desipramine ($f_{mic}$ of 0.88), has an $f_{mic}$ of 0.83 (Brown et al., 2005). Dextromethorphan, exhibiting enhanced sensitivity relative to that of desipramine, has an $f_{mic}$ of 0.96 (Nakashima et al., 2005).
2007). Qualitatively, in vivo sensitivity paralleled known \( f_{\text{MCYP2D6}} \) values.

The ability to predict in vivo exposure levels of a given drug (or inhibitor) using modeling and simulation enables the design of DDI studies (Rostami-Hodjegan and Tucker, 2007). The magnitude of in vivo DDIs caused by quinidine was predicted for desipramine, dextromethorphan, and metoprolol and compared with results obtained from the clinical literature (Table 3). All substrates were predicted to exhibit clinically relevant DDIs (AUCI/AUC \( \geq 2 \)) across the dose range examined (50–200 mg). Interchanging substrate parameters in vitro studies should also be considered for use in the clinical DDI potential for a new chemical entity, the substrate used for CYP2D6 in vitro and in vivo, it appears that to routinely establish the DDI based on in vitro and in vivo sensitivities, safety profile, and over-the-counter availability, dextromethorphan may represent the preferred CYP2D6 substrate for in vitro and clinical applications. Finally, because of enantioselective metabolism, making it a less desirable drug that is likely to be coadministered. Bufuralol lacks marketing approval in the United States and may exhibit confounding results.

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