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COMPARATIVE METABOLIC CAPABILITIES AND INHIBITORY PROFILES OF CYP2D6.1, CYP2D6.10, AND CYP2D6.17

Hongwu Shen,[1] Minxia M. He, Houfu Liu, Steven A. Wrighton, Li Wang, Bin Guo,[2] and Chuan Li[1]

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China (H.-W.S., H.-F.L., L.W., B.G., C.L.); Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana (M.M.H., S.A.W.)

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Running Title: *In Vitro* Comparison between CYP2D6.1, CYP2D6.10, and CYP2D6.17

Address correspondence to: Dr. Chuan Li, Center for DMPK Research, Shanghai Institute of Materia Medica, SIBS, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China. E-mail: chli@mail.shcnc.ac.cn

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ABBREVIATIONS: CYP, cytochrome P450; LC–MS/MS, liquid chromatography-tandem mass spectrometry.

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ABSTRACT:

Polymorphisms in the cytochrome P450 2D6 (CYP2D6) gene are a major cause of pharmacokinetic variability in human. Although the poor metabolizer phenotype is known to be caused by two null-alleles leading to absence of functional CYP2D6 protein, the large variability among individuals with functional alleles remains largely unexplained. Thus the goal of this study was to examine the intrinsic enzymatic differences that exist among the several active CYP2D6 allelic variants. The relative catalytic activities (enzyme kinetics) of three functionally active human CYP2D6 allelic variants, CYP2D6.1, CYP2D6.10, and CYP2D6.17 were systematically investigated for their ability to metabolize a structurally diverse set of clinically important CYP2D6-metabolized drugs [atomoxetine, bufuralol, codeine, debrisoquine, dextromethorphan, (*S*)-fluoxetine, nortriptyline, and tramadol] and the effects of various CYP2D6-inhibitors [cocaine, (*S*)-fluoxetine, (*S*)-norfluoxetine, imipramine, quinidine, and thioridazine] on these three variants. The most significant difference observed was a consistent but substrate dependent decease in the catalytic efficiencies of cDNA-expressed CYP2D6.10 and CYP2D6.17 as compared to CYP2D6.1, yielding 1.32–27.9% and 7.33–80.4% of the efficiency of CYP2D6.1, respectively. The most important finding from this study is that there are mixed effects on the functionally reduced allelic variants in enzyme-substrate affinity or enzyme-inhibitor affinity, which are lower, higher or comparable to CYP2D6.1. Considering the rather high frequencies of *CYP2D6*10* and *CYP2D6*17* alleles for Asians and African-Americans, respectively, these data provide further insight into ethnic differences in CYP2D6-mediated drug metabolism. However, as with all *in vitro* to *in vivo* extrapolations, caution should be applied to the clinical consequences.

Despite representing only approximately 2–4% of total human liver cytochrome P450 content, CYP2D6 plays a prominent role in the oxidation of xenobiotics. Estimates indicate that CYP2D6 is involved in the metabolism of about 30% of drugs presently on the market, in particular, the metabolism of many of those targeting the central nervous and cardiovascular systems (Gardiner and Begg, 2006). CYP2D6 is a polymorphic member of the cytochrome P450 superfamily (Zanger *et al.*, 2004) where individuals referred to as poor metabolizers carry a gene deletion or null variants of the CYP2D6 gene in which one or more nucleotide substitutions encode for a faulty message or truncated protein and thus are unable to produce active enzyme. There are differences in the proportions of extensive metabolizers to poor metabolizers in various ethnic groups (Sachse *et al.*, 1997; Ji *et al.*, 2002; Gaedigk *et al.*, 2002). For example, the *in vivo* activity of CYP2D6 is absent in 7.7% of Caucasians in contrast to only ~1% of Chinese and Japanese (Shimizu *et al.*, 2003).

People broadly classified as extensive metabolizers also demonstrate a pronounced variation in their metabolic capacity. Despite the very low frequency of CYP2D6 poor metabolizers in Asian populations, these groups actually display lower mean CYP2D6 activity compared to Caucasians with the reference allele *CYP2D6*1*. This reduced activity is represented by a right-shift in the metabolic ratio (parent to metabolite ratio) for several CYP2D6 substrates, including debrisoquine (Kalow *et al.*, 1980), sparteine (Droll *et al.*, 1998), metoprolol (Horai *et al.*, 1989), and dextromethorphan (Tateishi *et al.*, 1999). This lower overall CYP2D6 activity has been attributed to the high frequency in the Asian populations of a reduced activity variant of CYP2D6, i.e., *CYP2D6*10*. This variant possesses $Pro^{34}Ser$ and $Ser^{486}Thr$ amino acid substitutions compared to *CYP2D6*1*

(*http://www.cypalleles.ki.se/cyp2d6.htm*) and is present in approximately 56.2% of Chinese and 38.8% of Japanese (Ji *et al.*, 2002; Shimizu *et al.*, 2003). *CYP2D6*17* is another important variant of CYP2D6 which is highly prevalent (~21%) in African Americans (Gaedigk *et al.*, 2002). The *CYP2D6*17* allele carries three nonsynonymous coding region single nucleotide polymorphisms conferring Thr^{107}Ile, Arg^{296}Cys, and Ser^{486}Thr amino acid substitutions relative to *CYP2D6*1*. Phenotyping studies and *in vitro* data suggest that the metabolism of CYP2D6 substrates may be differentially decreased by *CYP2D6*17* (Masimirembwa *et al.*, 1996; Oscarson *et al.*, 1997; Bapiro *et al.*, 2002). Other functional allelic variants of CYP2D6 include *CYP2D6*2*, *CYP2D6*9*, and *CYP2D6*41*. Based on genotyping, it is possible to identify individuals with poor metabolizer phenotype (i.e., those carrying two null-alleles) with very high certainty. However, it is much more difficult to predict the metabolic capacity of extensive metabolizers who carry one or more altered function alleles.

Expression systems for the cytochrome P450 enzymes have been used to investigate the metabolic capabilities of these enzymes and to determine the metabolic profile of various compounds. A recent study by Fukuda *et al.* (2000) found reduced affinity of CYP2D6.10 for venlafaxine and bufuralol. Others also observed *in vitro* significantly reduced enzymatic activity of CYP2D6.10, CYP2D6.17, and CYP2D6.2 (Ramamoorthy *et al.*, 2001, 2002; Bapiro *et al.*, 2002; Marcucci *et al.*, 2002; Nakamura *et al.*, 2002; Yu *et al.*, 2002; Bogni *et al.*, 2005) compared to that of the reference form CYP2D6.1. These studies suggest that direct extrapolation with other substrates of *CYP2D6*1* to individuals with the reduced activity alleles may not be possible. Therefore, detailed kinetic analyses with CYP2D6.10,

CYP2D6.17, and CYP2D6.1 for other clinically important CYP2D6-metabolized drugs are still required to fully understand potential differences in the metabolic clearance of these agents. Investigation of allele specific enzyme kinetics will not only have implication in ethnic-specific pharmacotherapy, but also may be critical to rational design of *in vitro* metabolism studies in support of drug discovery and development.

The aims of the present studies are to determine the relative catalytic activities (enzyme kinetics) of three functionally active human CYP2D6 allelic variants, CYP2D6.1, CYP2D6.10, and CYP2D6.17 (expressed in Supersomes with NADPH-cytochrome P450 reductase) for their ability to metabolize a structurally diverse set of clinically important drugs and to gain an understanding of the effects of various CYP2D6-inhibitors on these three variants.

Materials and Methods

Chemical and materials. The CYP2D6-substrates, their metabolites and the CYP2D6-inhibitors, (Fig. 1) were obtained from Sigma (St. Louis, MO), Fluka (CH-9471 Buchs, Switzerland), Ultrafine (Manchester, UK), Lilly Research Laboratories (Indianapolis, IN), or the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). *O*-Desmethyl-tramadol was provided by Dr. F.-J. Nan from the National Center for Drug Screening (Shanghai, China). Microsomes prepared from insect cells containing baculovirus-expressing human CYP2D6.1 with NADPH-cytochrome P450 reductase or CYP2D6.10 with NADPH-cytochrome P450 reductase (Supersomes™) were purchased from BD Gentest Corporation (Woburn, MA). CYP2D6.17 Supersomes prepared from human cytochrome P450 reductase and CYP2D6.17-expressing baculovirus infected insect cells were a gift from BD Gentest Corporation. A microsomal preparation from insect cells infected with the baculovirus vector was used as a control for native activity. β-nicotinamide adenine dinucleotide phosphate sodium salt (NADP), glucose-6-phosphate monosodium salt, and glucose-6-phosphate dehydrogenase were purchased from Sigma (St. Louis, MO). HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). Ammonium formate (>99%) was obtained from Aldrich (Milwaukee, WI). Other organic solvents and chemicals used were of analytical grade and were obtained by Shanghai Chemical Reagent Co. (Shanghai, China). High purity HPLC water was made by distilling pre-deionized water twice in house.

Incubation Conditions for Comparison of the Catalytic Activities of CYP2D6 Allelic Variants. Pilot studies were performed with each biotransformation (listed in Table 1) to

ensure that the comparison of metabolic capabilities of the three CYP2D6 allelic variants, i.e., CYP2D6.1, CYP2D6.10, and CYP2D6.17, were determined under linear metabolite formation conditions with respect to time and enzyme-protein concentration. Incubations were performed in duplicate in 96-well plates in a total assay volume of 100 μL with each well containing CYP2D6 Supersomes™ (at 5, 10, and 5 pmol P450/mL for CYP2D6.1, CYP2D6.10, and CYP2D6.17, respectively, except for debrisoquine 4-hydroxylation and (*S*)-fluoxetine *N*-demethylation, for which the concentrations of 20, 30, and 20 pmol P450/mL, respectively, were used), substrate (concentration ranges as indicated), 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system comprising 3.3 mM of magnesium chloride, 3.3 mM of glucose-6-phosphate, 0.5 U/mL of glucose-6-phosphate dehydrogenase, and 1.3 mM of NADP. Before commencement of the reaction by adding the NADPH-generating system, the incubation mixture was preincubated for 3 min at 37°C. The optimal incubation time for the biotransformations was 30 min except for debrisoquine 4-hydroxylation (60 min). For each biotransformation, 6 to 8 substrate concentrations were examined over the following ranges: 0.60 to 96 μM for atomoxetine 4-hydroxylation, 0.625 to 80 μM for bufuralol 1'-hydroxylation, 33 to 8000 μM for codeine *O*-demethylation, 2.74 to 1000 μM for debrisoquine 4-hydroxylation, 0.625 to 320 μM for dextromethorphan *O*-demethylation, 0.10 to 200 μM for fluoxetine *N*-demethylation, 0.625 to 320 μM for nortriptyline 10-hydroxylation, and 1.8 to 4000 μM for tramadol *O*-demethylation. The reactions were terminated by adding 100 μL of acetonitrile (4ºC) and the resulting samples were centrifuged at 3,000 rpm for 10 min to remove protein before 5 μL of the supernatant were applied to LC–MS/MS analysis.

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Inhibition Constant (K_i) Determination for CYP2D6-Inhibitors. The effects of the CYP2D6-inhibitors (Table 2) on the activities of the three CYP2D6 allelic variants mediating atomoxetine 4-hydroxylation and dextromethorphan *O*-demethylation were further measured in terms of inhibition constant (K_i). Both the reactions were performed in 96-well plates and the incubation conditions were similar to those of enzyme kinetic assays as described above. A substrate concentration range from 0.2 to 5-fold of the K_m values for the biotransformation was used for determining the K_i values of the inhibitors affecting atomoxetine 4-hydroxylation or dextromethorphan *O*-demethylation mediated by CYP2D6.1, CYP2D6.10, and CYP2D6.17. The inhibitor concentration ranges for the atomoxetine 4-hydroxylation were 0.20–5, 0.19–15, 0.19–15, 2.5–62.5, 0.08–2, and 0.005–0.5 μM for cocaine, (*S*)-fluoxetine, (*S*)-norfluoxetine, imipramine, thioridazine, and quinidine, respectively, while those for the dextromethorphan *O*-demethylation were 0.8–20, 0.19–15, 0.19–15, 3.2–62.5, 0.025–0.5, and 0.01–0.3 μM, respectively.

Analyses of Metabolites. To determine metabolite formation rates, LC–MS/MS system consisting of a Thermo Finnigan TSQ Quantum triple stage quadrapole mass spectrometer (San Jose, CA) interfaced via an electrospray ionization probe with an Agilent 1100 series liquid chromatograph that included a vacuum degasser, a binary pump, a thermostatted autosampler and a thermostatted column compartment (Waldbronn, German) was utilized. The Agilent Chemstation and Finnigan Xcalibur software packages were used for controlling the LC–MS/MS system, as well as for data acquisition and processing.

LC separations were achieved using a 4-μm Phenomenex Synergi C_{18} column (50 × 2.0 mm, i.d.; Torrance, CA), with a pre-column 0.2-μm filter (Upchurch, Scientific, Oak Harbor,

WA). Prepared samples were separated under one of the following LC binary gradient programs (***I*** and ***II***), with mobile phases of CH_3CN:H_2O (10:490, v:v, containing a mass fraction of 0.02% $HCOONH_4$) for solvent A and CH_3CN:H_2O (450:50, v:v, containing a mass fraction of 0.02% $HCOONH_4$) for solvent B delivered at 0.3 mL/min. Gradient program ***I*** was used for analysis of 4-hydroxyatomoxetine and dextrorphan, which consisted of an initial 0.7-min increase of B from 25 to 95%, followed by an isocratic segment maintaining B at 95% from 0.7 to 2 min. Then B was changed back to 25% at 2.1 minute and maintained until 6 min for the analysis of the next sample. Gradient program ***II*** consisted of an initial 1-min increase of B from 5 to 90%, followed by an isocratic segment maintaining B at 90% from 1 to 2 min. Then B was changed back to 5% at 2.1 minute and maintained until 6 min for column equilibration. Gradient program ***II*** was used for analysis of 1'-hydroxybufuralol, morphine, 4-hydroxydebrisoquine, (*S*)-norfluoxetine, 10-hydroxynortriptyline, and *O*-demethyltramadol.

The mass spectrometer was operated in the positive electrospray ionization and selected reaction monitoring scan modes for all the analytes. The instrumental parameters were tuned to maximize generation of the protonated analyte molecules ($[M+H]^+$) and characteristic fragment ions. The precursor-to-product ion transitions were monitored at *m/z* 272 → 44, 278 → 186, 286 → 165, 192 → 132, 258 → 157, 296 → 134, 280 → 215, and 250 → 58 for 4-hydroxyatomoxetine, 1'-hydroxybufuralol**,** morphine, 4-hydroxydebrisoquine**,** dextrorphan, (*S*)-norfluoxetine, 10-hydroxynortriptyline, and *O*-demethyltramadol, respectively. An online motorized six-port divert valve was used to introduce the LC eluent flow to the mass spectrometer over the period of 2.0–5.5 min for data acquisition, with the other eluent flow

diverted to the waste. Matrix-matched standard curves of the peak area of a given metabolite versus the nominal concentration (nM) were linear, and showed correlation coefficients (r) greater than 0.999.

Data Analysis. Michaelis constant (K_m) and maximum velocity (V_{max}) values were determined by nonlinear regression analysis utilizing the Michaelis-Menten equation (rate of metabolite-formation as a function of substrate concentration) using GraFit software (Version 5; Erithacus Software Ltd. Surrey, UK). The ratio of V_{max} and K_m was used to calculate *in vitro* intrinsic clearance (CL_{int}). When inhibition assays were performed to determine K_i values, the data were plotted on an Eadie-Hofstee plot for a pilot visual inspection of the inhibition type, and the entire data set was fitted to the appropriate equations by nonlinear regression analysis with simple weighting using the GraFit software.

Results

Enzyme Kinetics of CYP2D6.1, CYP2D6.10, and CYP2D6.17. In the studies presented here, eight CYP2D6-mediated biotransformations, i.e., atomoxetine 4-hydroxylation, bufuralol 1'-hydroxylation, codeine *O*-demethylation, dextromethorphan *O*-demethylation, debrisoquine 4-hydroxylation, (*S*)-fluoxetine *N*-demethylation, nortriptyline 10-hydroxylation, and tramadol *O*-demethylation, were examined. The classic single site Michaelis-Menten equation best fit these biotransformations catalyzed *in vitro* by the three recombinant CYP2D6 variants. Figure 2 contains representative plots of metabolite formation rate versus substrate concentration for CYP2D6-mediated turnover of atomoxetine and dextromethorphan. The parameters such as K_m and V_{max} for different CYP2D6 substrates, as well as the corresponding *in vitro* intrinsic clearance (V_{max}/K_m) values for each variant are presented in Table 1.

Bufuralol 1'-Hydroxylation, Dextromethorphan *O*-Demethylation, and Debrisoquine 4-Hydroxylation (Three Commonly Used CYP2D6 Probe Substrates). Bufuralol and dextromethorphan are two commonly used *in vitro* selective CYP2D6 probe substrates with debrisoquine less commonly used but of historical interest. The estimated kinetic parameters K_m and V_{max} for bufuralol 1'-hydroxylation, dextromethorphan *O*-demethylation, and debrisoquine 4-hydroxylation mediated by CYP2D6.1 were 10.2 μM and 6.29 pmol/min/pmol P450, 2.97 μM and 3.49 pmol/min/pmol P450, and 73.7 μM and 1.32 pmol/min/pmol P450, respectively, which resulted in intrinsic clearance (CL_{int}) values of 0.62, 1.18, and 0.02 μL/min/pmol P450, respectively. Compared to the reference protein (CYP2D6.1), estimates of K_m for bufuralol, dextromethorphan, and debrisoquine were 1.3-,

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3.1-, and 1.3-fold for CYP2D6.10 and 0.92-, 5.8-, and 1.5-fold for CYP2D6.17. In addition, V_{max} values for the reference protein were 21.6-, 6.1-, and 6.6-fold higher than CYP2D6.10 and 4.7-, 1.0-, and 1.1-fold higher than CYP2D6.17. The CL_{int} values of these three substrates catalyzed by CYP2D6.10 were reduced to approximately 5.31–11.8% of that observed with the reference protein and reduced 16.8–64.2% with CYP2D6.17.

Atomoxetine 4-Hydroxylation. Atomoxetine, a potent and selective inhibitor of the presynaptic norepinephrine transporter, is used clinically for the treatment of attention-deficit hyperactivity disorder (ADHD) in children and adults (Newcorn *et al.*, 2005). In humans, atomoxetine undergoes extensive metabolism of 4-hydroxylation by CYP2D6 to form 4-hydroxyatomoxetine, and is also transformed into a minor metabolite, *N*-demethylatomoxetine, mainly by CYP2C19 (Ring *et al.*, 2002; Sauer *et al.*, 2003). The enzyme kinetics of CYP2D6.1, CYP2D6.10, and CYP2D6.17 variants towards atomoxetine 4-hydroxylation were characterized, and the results indicated that CYP2D6.10 and CYP2D6.17 exhibited enzyme efficiency (as CL_{int}), which was 8.58% and 21.9% of CYP2D6.1. CYP2D6.10 exhibited a K_m value for atomoxetine similar to the wild type protein did, but its maximum velocity was 9.2 times lower which resulted in its decreased enzyme efficiency. Whereas, the decreased enzyme efficiency of CYP2D6.17 was mainly attributed to increased K_m (by 3.1-fold) and also to a 1.5-fold decreased V_{max}. For CYP2D6.1, the *in vitro* CL_{int} of atomoxetine was approximately 11-, 6-, or 390-fold higher than that of bufuralol, dextromethorphan or debrisoquine, respectively. Although exhibiting decreased catalytic efficiency relative to CYP2D6.1, as often observed for other CYP2D6-mediated biotransformations, the CL_{int} value for atomoxetine 4-hydroxylation by CYP2D6.10 (0.600

μL/min/pmol P450) or CYP2D6.17 (1.53 μL/min/pmol P450) was still quite high and comparable with those for bufuralol 1'-hydroxylation and dextromethorphan *O*-demethylation by CYP2D6.1.

(*S*)-Fluoxetine *N*-Demethylation. Fluoxetine, a racemic mixture of (*R*)-, (*S*)-fluoxetine, is a selective serotonin reuptake inhibitor (Gram *et al.*, 1994) and indicated for the treatment of depression, associated anxiety, obsessive compulsive disorder, and bulimia, as well as pre-menstrual dysphoric disorder. The major metabolic route of fluoxetine is *N*-demethylation to form norfluoxetine. Identification of the enzyme involved in the formation of norfluoxetine demonstrated that (*S*)-fluoxetine *N*-demethylation correlated only with CYP2D6 catalytic activity, whereas both CYP2D6 and CYP2C9 significantly contribute to the formation of (*R*)-norfluoxetine (Margolis *et al.*, 2000; Ring *et al.*, 2001). Relative to the preceding CYP2D6-substrates in which the K_m values were lower for the reference protein versus the variant, the kinetics of (*S*)-fluoxetine *N*-demethylation to (*S*)-norfluoxetine with CYP2D6.10 was different from CYP2D6.1 yielding K_m values of 0.455 and 0.834 μM for CYP2D6.10 and CYP2D6.1, respectively. The corresponding V_{max} value in CYP2D6.10 was only 4% of that for CYP2D6.1. Accordingly, the CL_{int} value was reduced for CYP2D6.10, demonstrating approximately 7.54% of the efficiency of CYP2D6.1. In contrast, CYP2D6.17 demonstrated both increased K_m with (*S*)-fluoxetine and reduced maximal activity, resulting in a CL_{int} which was 8.17% of CL_{int} for CYP2D6.1.

Nortriptyline 10-Hydroxylation and Tramadol *O*-Demethylation. Nortriptyline and tramadol were also used as substrates to compare the metabolic capabilities of CYP2D6.1, CYP2D6.10, and CYP2D6.17 allelic variants. Nortriptyline is a tricyclic antidepressant and *in*

vitro studies demonstrated that CYP2D6 was the sole cytochrome P450 mediating the main metabolic pathway, hydroxylation to (*E*)-10-hydroxynortriptyline (Venkatakrishnan *et al.*, 1999). The CYP2D6.1 enzyme was found to have the highest catalytic efficiency for nortriptyline 10-hydroxylation, while the intrinsic clearance values of CYP2D6.10 and CYP2D6.17 were only 1.32% and 7.33% of the CYP2D6.1. The reduced CL_{int} with CYP2D6.10 or CYP2D6.17 was attributed to both an increased K_m (11.8- or 4.5-fold) and a reduced V_{max} (6.4- or 3-fold). Tramadol is an opioid analgesic of the aminocyclohexanol type and its primary metabolites are *O*-desmethyl-tramadol and *N*-desmethyl-tramadol. While the *N*-demethylation is catalyzed by CYP2B6 and CYP3A4, CYP2D6 is primary responsible for *O*-desmethylation (Subrahmanyam *et al.* 2001). In contrast to nortriptyline, both CYP2D6.10 and CYP2D6.17 exhibited similar affinities to CYP2D6.1 for tramadol. A reduced maximum velocity resulted in the CL_{int} values of tramadol catalyzed by CYP2D6.10 or CYP2D6.17, demonstrating 6.9 or 35.7%, of the efficiency of CYP2D6.1, respectively.

Codeine *O*-Demethylation. Although glucuronidation of codeine to codeine-6-glucuronide and subsequent renal excretion is the major route of elimination, the minor biotransformation *O*-demethylation to morphine is mediated by CYP2D6 (only accounting for about 10% of codeine metabolism) which produces its analgesic, antitussive, and anti-diarrheal effects (Mortimer *et al.*, 1990). All the three forms exhibited low but comparable K_m values for codeine, i.e., 1079, 1206, and 1470 μM for CYP2D6.1, CYP2D6.10, and CYP2D6.17, respectively. The decreased enzyme efficiency of CYP2D6.10 relative to CYP2D6.1 (27.9%) was mainly attributed to a 3.2-fold reduced V_{max}. Only a slight decrease in enzyme efficiency was observed for CYP2D6.17, which was about 80.4% of CYP2D6.1.

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Differences Between CYP2D6-Substrates. All the substrates exhibited a reduced *in vitro* intrinsic clearance (CL_{int}) with CYP2D6.10, demonstrating only 1.32–27.9% of the efficiency as compared to CYP2D6.1. The CL_{int} values of CYP2D6.17 towards these substrates were also decreased to 7.33–80.4% of those of CYP2D6.1. The result indicates the estimated CL_{int} values decreased in the order CYP2D6.1 > CYP2D6.17 > CYP2D6.10 (Table 1). The decreases in CL_{int} observed with CYP2D6.10 or CYP2D6.17 relative to the CYP2D6.1 were the results of nonuniform increase in the Michaelis constant (K_m), as well as maximum reaction velocity (V_{max}), across the test CYP2D6-substrates. CYP2D6.10 exhibited only slight K_m changes for atomoxetine, bufuralol, codeine, debrisoquine, (*S*)-fluoxetine, and tramadol when compared to CYP2D6.1, thus the reduced CL_{int} values were mainly attributed to the decreased V_{max}. Meanwhile, both higher K_m values and reduced V_{max} values for dextromethorphan *O*-demethylation and nortriptyline 10-hydroxylation by CYP2D6.10 yielded a lower CL_{int} than CYP2D6.1. The most dramatic difference in CL_{int} between CYP2D6.10 and CYP2D6.1 was observed with nortriptyline as the substrate, whereas the difference was the least with codeine. When compared to CYP2D6.1, CYP2D6.17 appeared to display greater substrate-specific change in K_m than CYP2D6.10. Relative to CYP2D6.1, modest differences in K_m were observed only with the substrates bufuralol, codeine, and tramadol. Meanwhile, atomoxetine, codeine, debrisoquine, and dextromethorphan exhibited comparable or slightly reduced V_{max} values. The enzyme efficiency of CYP2D6.17 towards codeine and debrisoquine were only slightly decreased by approximately 20 and 36%, respectively, as compared to that for CYP2D6.1. Similar to the situation with comparison

between CYP2D6.10 and CYP2D6.1, nortriptyline demonstrated the greatest difference in CL_{int} between CYP2D6.17 and CYP2D6.1 of the substrates investigated.

Interactions of CYP2D6-Inhibitors with CYP2D6.1, CYP2D6.10, and CYP2D6.17. In addition to various CYP2D6-substrates, the interactions between six known CYP2D6-inhibitors of varying drug classes with CYP2D6.1, CYP2D6.10, and CYP2D6.17 were also studied. The inhibitors tested were cocaine, (*S*)-fluoxetine, (*S*)-norfluoxetine, imipramine, quinidine, and thioridazine. Cocaine has been reported to be a competitive inhibitor of CYP2D6-mediated sparteine 2-dehydrogenation (Tyndale *et al.*, 1991). The CYP2D6 substrate fluoxetine and its *N*-demthylated metabolite norfluoxetine are about equal competitive CYP2D6-inhibitors (Stevens and Wrighton, 1993). Although fluoxetine is marketed as a racemic mixture, the (*S*)-fluoxetine and (*S*)-norfluoxetine were found to be more potent inhibitors of CYP2D6 as evidenced by K_i values approximately 5 times lower than the corresponding values for the (*R*)-enantiomers. Like other tricyclic antidepressants, imipramine competitively inhibits CYP2D6-catalyzed dextromethorphan *O*-demethylation, with the estimated K_i value of 28.6 μM (Shin *et al.*, 2002). The anti-arrhythmic quinidine is a well documented potent competitive inhibitor of, but not substrate for, CYP2D6. Quinidine is often used as positive control for *in vitro* and *in vivo* CYP2D6 inhibition studies. Thioridazine has been found to competitively inhibit dextromethorphan *O*-demethylation with the estimated K_i values of 1.4 μM (Shin *et al.*, 1999). To determine the K_i values for these CYP2D6-inibitors, both dextromethorphan *O*-demethylation and atomoxetine 4-hydroxylation were chosen because of their high selectivity and specificity for CYP2D6, and the results are reported in Table 2. In addition, representative Eadie-Hofstee plots for

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quinidine inhibiting dextromethorphan *O*-demethylation and atomoxetine 4-hydroxylation mediated by CYP2D6.1, CYP2D6.10, or CYP2D6.17 are shown in Figure 3.

Inhibition of Dextromethorphan *O*-Demethylase. The inhibition of the three recombinant CYP2D6 allelic variants by the CYP2D6-inhibitors was first measured by using dextromethorphan *O*-demethylase activity. All test inhibitors demonstrated competitive inhibition of CYP2D6.1, and fluoxetine, imipramine, and thioridazine are also competitive substrates. The most potent test inhibitors were quinidine and thioridazine with K_i values of 0.01 and 0.02 μM, respectively. Cocaine, (*S*)-fluoxetine and (*S*)-norfluoxetine were less potent inhibitors with K_i values of approximately 1 μM. Among the tested inhibitors, imipramine exhibited the weakest inhibition with K_i value of 3.3 μM. In addition, all the test inhibitors also demonstrated competitive inhibition of CYP2D6.10 and CYP2D6.17 according to the visual inspection of the Eadie-Hofstee plots. The ratios of K_i values obtained in CYP2D6.10 compared to those in CYP2D6.1 were 0.32 for (*S*)-fluoxetine and 0.41 for (*S*)-norfluoxetine, showing higher affinity of CYP2D6.10 than that of CYP2D6.1; 1.51 for cocaine and 1.11 for imipramine, demonstrating little difference between the two alleles; and 4.64 and 11.8 for quinidine and thioridazine, respectively, exhibiting significantly reduced affinity for CYP2D6.10. CYP2D6.17 displayed greater K_i values (lower affinity) for all the tested inhibitors than CYP2D6.1 with the K_i ratio (2D6.17/2D6.1) varying from 1.4 to 9.2. Similar to the preceding mixed K_m changes for the tested CYP2D6-substrates, CYP2D6.10 exhibited mixed K_i changes for the tested inhibitors when compared to CYP2D6.1. Whereas, CYP2D6.17 displayed greater K_i values for all the test inhibitors than CYP2D6.1 and greater K_m values for most of the test substrates as well.

Inhibition of Atomoxetine 4-Hydroxylase. To gain a better understanding of the effects of the inhibitors on CYP2D6.1, CYP2D6.10, and CYP2D6.17, the inhibition study was also performed using the probe reaction atomoxetine 4-hydroxylation. All the test CYP2D6-inhibitors demonstrated competitive inhibition of the three recombinant variants. The test inhibitors displayed similar spectra of the effects on the three allelic variants with atomoxetine 4-hydroxylation compared to those with dextromethorphan *O*-demethylation. In terms of 4-hydroxyatomoxetine formation, quinidine and thioridazine also exhibited the most potent inhibition of CYP2D6.1 with K_i values of 0.01 and 0.026 μM, respectively, followed by cocaine, (*S*)-fluoxetine and (*S*)-norfluoxetine with higher K_i values of 0.35–1.04 μM. Imipramine was still the weakest inhibitor with a K_i value approximately 3.04 μM. The K_i ratios for 2D6.10/2D6.1 also demonstrated mixed effect on K_i changes for the inhibitors which could be divided into three groups with (1) (*S*)-norfluoxetine and (*S*)-fluoxetine exhibiting the ratios of 0.57 and 0.73, respectively, (2) cocaine, imipramine, and quinidine showing ratios between 1.23 and 2.3, and (3) thioridazine displaying the greatest ratio of 19. Similar to the results with dextromethorphan, with atomoxetine as the substrate CYP2D6.17 displayed greater K_i values for all the inhibitors compare to CYP2D6.1 with the K_i ratios (2D6.17/2D6.1) varying from 1.12 to 5.6. Except for the two most potent inhibitors quinidine and thioridazine, the differences in K_i values between CYP2D6.10 and CYP2D6.17 appeared to be smaller with atomoxetine 4-hydroxylation than those with dextromethorphan *O*-demethylation. An unexpected observation in the inhibition studies with CYP2D6.17 is that the K_i value for cocaine was 9.29 μM for competitive inhibition of dextromethorphan metabolism, but only 1.60 μM for that of atomoxetine metabolism, the variation in which was

almost 6 fold. The reason behind this repeatable observation (data not shown) remains to be understood.

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Discussion

In the study presented here, the experimental conditions were optimized to focus on measuring initial rates of CYP2D6-substrate biotransformations. Due to lower enzyme efficiencies for CYP2D6.10 and CYP2D6.17 relative to that of CYP2D6.1, sensitive LC–MS/MS methods were developed and utilized for measuring metabolite formation in incubations with the lowest amount of enzyme protein possible and less than 5% of the substrate consumed for all the compounds with all the variants. In addition, efforts were also made to minimize the matrix effects on the LC–MS/MS-based quantification in order to meet validation requirements on precision and accuracy, leading to the generation of reliable experimental data for this study.

Previous communications (Fukuda *et al.*, 2000; Ramamoorthy *et al.*, 2001, 2002; Bapiro *et al.*, 2002; Marcucci *et al.*, 2002; Yu *et al.*, 2002; Nakamura *et al.*, 2002) reporting the comparative catalytic efficiencies of cDNA-expressed CYP2D6.10 or CYP2D6.17, with that of CYP2D6.1 examined the kinetics of common CYP2D6 probe substrate reactions, such as bufuralol 1'-hydroxylation, dextromethorphan *O*-demethylation, and debrisoquine 4-hydroxylation. Both CYP2D6.10 and CYP2D6.17 exhibited decreased catalytic efficiencies for the substrates compared to CYP2D6.1, but the results reported by the different authors were highly variable. For example, in these previous studies bufuralol 1'-hydroxylation mediated by CYP2D6.1, CYP2D6.10, or CYP2D6.17 exhibited K_m values 1.7–25, 12–50, or 17–33 μM, respectively, and the calculated CL_{int} value ranged from 0.04 to 7.88, 0.05 to 0.24, or 0.02 to 0.27 μL/min/pmol P450, respectively. The reason for the discrepancy between the

laboratories might be associated in part with different sources of enzyme used, as well as the different incubation conditions utilized in the studies.

Nakamura *et al.* (2002) reported the thermal instability of CYP2D6.10 at 39ºC and higher temperature. These authors presumed that this instability may be important when abnormal body temperature, e.g. fever, occurs in people by diseases. As to the thermolability of the tested CYP2D6 variants in the present studies, we did not find CYP2D6.10 protein to possess any unusual instability at 37ºC as compared with CYP2D6.1 and CYP2D6.17. The relative enzyme activity of CYP2D6.1, CYP2D6.10, or CYP2D6.17 mediating dextromethorphan *O*-demethylation after preincubation for 30 min was 75.9% ± 3.7%, 78.9% ± 1.7%, or 76.6% ± 6.0%, respectively, while the relative enzyme activity for 60-min preincubation was 56.1% ± 1.7%, 58.5% ± 0.5%, or 55.2% ± 1.8%, respectively. Similar results were obtained using the CYP2D6-mediated reaction atomoxetine 4-hydroxylation. Since the effects of temperature were similar, no correction for enzyme thermolability was made for the comparison of the three CYP2D6 allelic variants with respect to metabolic capabilities and inhibitory profiles.

The cytochrome P450s require P450 reductase as an electron donor to function as monooxygenases. Although the ratio of P450 to the reductase may influence the catalytic activities of CYP2D6, Yamazaki *et al.* (2002) found that bufuralol 1'-hydroxylation activity by CYP2D6 was not enhanced in co-expression systems by the addition of exogenous P450 reductase. The level of P450 reductase in human liver microsomes was found to be subject to considerable variation (14-fold), but this was not reflected in variability in reductase activity (Forrester *et al.*, 1992). In this study, no exogenous NADPH-cytochrome P450 reductase was

supplemented to achieve a constant molar P450/reductase ratio for the three expressed CYP2D6 proteins with co-expressed P450/reductase ratios of 1:0.8, 1:0.4, and 1:1.5 for CYP2D6.1, CYP2D6.10, and CYP2D6.17, respectively. Literature values for the kinetics of dextromethorphan *O*-demethylation resulting from using purified CYP2D6 allelic variants (Yu *et al.*, 2002) provides a valuable comparison for the systems utilized in the current study. Using the purified CYP2D6.1, CYP2D6.10, and CYP2D6.17 with P450 and the reductase in a consistent 1:2 molar ratio, Yu *et al.* (2002) measures V_{max} of 9.0, 2.6, and 8.6 pmol/min/pmol P450 for this reaction, while Table 1 shows a similar order of magnitude in V_{max}, i.e. 3.49, 0.57, and 3.41 pmol/min/pmol P450, respectively.

In the current study kinetic parameters for eight CYP2D6 biotransformations were determined with cDNA-expressed CYP2D6.10 and CYP2D6.17 which consistently exhibited lower catalytic efficiencies as compared to CYP2D6.1. Moreover, the relationships between different CYP2D6 substrates also appear quite variable, suggesting that substrate-dependent intrinsic enzymatic differences exist among the three important active CYP2D6 variants. Therefore, substrate specific and allelic dependent metabolism may affect the dominating metabolic pathway and the relative contributions of the various metabolizing enzymes involved in the clearance of a drug. These findings have important implications for genotype-phenotype prediction for therapeutically useful CYP2D6 substrates. Most *in vitro* studies have focused on the common CYP2D6 probe substrates such as dextromethorphan or bufuralol to simply clarify *in vitro* drug biotransformation data with *in vivo* phenotype data. However, the predicted *in vivo* consequence of a *CYP2D6*17*-containing genotype might be quite different if the practitioner is using the genotype information to predict fluoxetine

clearance (CL_{int} 8% of that observed with CYP2D6.1) versus that of to codeine *O*-demethylation to form morphine (CL_{int} 80% of that observed with CYP2D6.1). A recent study by Bogni *et al.* (2005) showed that CYP2D6.17 was more active than CYP2D6.1 towards haloperidol. In addition, hydroxlylation of riperidone to 9-OH risperidone with *CYP2D6*17* was also found to be more efficient than that with *CYP2D6*1* (Cai *et al.*, 2006). The potential genotype-phenotype problems increase for substrates like haloperidol and riperidone in which the clearance of the agent may be greater with CYP2D6.17 than CYP2D6.1. Among the multitude of clinically used CYP2D6-metabolized drugs, only limited number of the drugs has been investigated. In order to fill this important information gap, it will be necessary to examine *in vitro* all the active CYP2D6 allelic variants with all (new and old) substrates.

Several researchers (Fukuda *et al.*, 2000; Ramamoorthy *et al.*, 2001, 2002; Yu *et al.*, 2002; Hanioka *et al.*, 2006) have reported that CYP2D6.10 exhibits increased K_m for CYP2D6-substrates compared to CYP2D6.1. However, the findings reported here indicate that the difference in K_m between CYP2D6.10 and CYP2D6.1 was mixed and substrate-dependent (Table 1). The $K_{m(CYP2D6.10)}/K_{m(CYP2D6.1)}$ ratio was 0.5 for (*S*)-fluoxetine, 1–2 for atomoxetine, bufuralol, codeine, debrisoquine, and tramadol, and > 3 for dextromethorphan and nortriptyline. The decreased intrinsic clearance of CYP2D6.10 toward the tested CYP2D6-substrates was mainly the result of a substantial decrease in V_{max} by the less efficient variant rather than a different K_m value. Studies on bufuralol 1'-hydroxylation by Zanger *et al.* (2001) and by Nakamura *et al.* (2002) also showed that CYP2D6.10 exhibited small K_m changes compared to CYP2D6.1, with $K_{m(CYP2D6.10)}/K_{m(CYP2D6.1}$ ratios of 1.8 and 1.7,

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respectively. In addition, the current results from the enzyme-inhibition studies using dextromethorphan *O*-demethylase or atomoxetine 4-hydroxylase activity as the CYP2D6 probe (Table 2) indicated mixed differences of enzyme-inhibitor affinity between CYP2D6.10 and CYP2D6.1, i.e., the ratios of $K_{i(CYP2D6.10)}/K_{i(CYP2D6.1)}$ for cocaine, (*S*)-fluoxetine, imipramine, and (*S*)-norfluoxetine were 0.32–1.51, whereas the ratios for quinidine and thioridazine were 2.3 and 19, respectively.

CYP2D6.17 also exhibited mixed effects on K_m as compared to CYP2D6.1, demonstrating a ratio of $K_{m(CYP2D6.17)}/K_{m(CYP2D6.1)}$ 0.7–1.5 for bufuralol, codeine, debrisoquine, and tramadol and > 3 for atomoxetine, dextromethorphan, (*S*)-fluoxetine, and nortriptyline. The results from the current enzyme-inhibition studies indicated that both (*S*)-fluoxetine and (*S*)-norfluoxetine exhibited comparable enzyme-inhibitor affinity for CYP2D6.17 and CYP2D6.1, but the other tested CYP2D6-inhibitors exhibited reduced enzyme-inhibitor affinity for CYP2D6.17 as compared to that for CYP2D6.1. In total, these results indicate that the effects of the variants, CYP2D6*10 and CYP2D6*17 on CL_{int} relative to CYP2D6*1 are substrate dependent affecting K_m, V_{max} or both. Related is the observation that the affinity of inhibitors also varies between the variants and reference allele.

CYP2D6.10 possesses $Pro^{34}Ser$ and $Ser^{486}Thr$ amino acid substitutions, and the effects of these substitutions towards bufuralol, venlafaxine, and *p*-tyramine were elucidated (Fukuda *et al.*, 2000; Niwa *et al.*, 2004). The studies using site-directed mutagenesis suggested that $Ser^{486}Thr$ alone had no significant effects on K_m, while $Pro^{34}Ser$ substitution appear to be relate to the increased K_m for these test substrates. In CYP2D6.17, site direct mutation studies (Oscarson *et al.*, 1997; Bapiro *et al.*, 2002) indicated that both $Thr^{107}Ile$ and $Arg^{296}Cys$

substitutions were required instead of either single amino acid substitution to cause the change in K_m. The results obtained here with the mixed difference in enzyme-substrate affinity, as well as enzyme-inhibitor affinity, between the less functional CYP2D6 allelic variants and CYP2D6.1 may contribute to improvement of *in silico* modeling of CYP2D6.

In summary, although CYP2D6.1 and its allelic variants CYP2D6.10 and CYP2D6.17 only differ by two or three amino acids, the results reported here demonstrated that the allelic variants are associated with substrate-dependent decreases in catalytic properties and enzyme-inhibitor affinities. The notable finding from this study is that the changes with the functionally reduced allelic variants in enzyme-substrate affinity, as well as enzyme-inhibitor affinity, as compared to CYP2D6.1 are mixed, depending on substrates or inhibitors. *CYP2D6*10* and *CYP2D6*17* alleles have been described for Asians and African Americans, respectively, with relatively high frequencies. The results reported here have implications for drug discovery and development; it is advisable to test CYP2D6-metabolized candidates or investigational drugs for allele specific metabolism/interactions. Meanwhile, different metabolism and inhibition by the multiple test compounds with CYP2D6.10 and CYP2D6.17 will have implications on drug dosing, toxicity and the likelihood of drug-drug interactions in those with one or two of the variant enzymes. Ethnic variability in these kinetic effects may also be predicted by these studies, potentially allowing for ethnic-specific pharmacotherapy. As with all *in vitro* to *in vivo* extrapolation, the *in vitro* results should be interpreted cautiously in clinical context, where other drug metabolism pathways may also play a role. It would be interesting to further test in human liver microsomes from individual donors who

are homozygous for the observed difference in the cDNA-expressed CYP2D6.1, CYP2D6.10, and CYP2D6.17.

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Footnotes

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[1] These authors are also affiliated with Graduate School of the Chinese Academy of Sciences.

[2] Current address: Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China.

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Legends for figures

FIG. 1. *Structures of the substrates, their metabolites, and the inhibitors of CYP2D6 examined.*

FIG. 2. *Representative kinetic plots of product formation versus substrate concentration for metabolic turnover of atomoxetine and dextromethorphan in vitro catalyzed by the three recombinant CYP2D6 allelic variants CYP2D6.1 (▲), CYP2D6.10 (●), and CYP2D6.17 (■), respectively. Shown are Michaelis-Menten curves, as well as the transformation of these data to the corresponding Lineweaver-Burk plots, for the formations of dextrorphan from dextromethorphan and 4-hydroxyatomoxetine from atomoxetine.*

FIG. 3. *Representative Eadie-Hofstee plots for quinidine inhibition of CYP2D6-catalyzed dextromethorphan O-demethylation and atomoxetine 4-hydroxylation. The substrate the dextromethorphan concentration ranges were 1.25–40, 2.5–80, and 5.0–160 μM, respectively, while atomoxetine concentration ranges used were 1.0–24, 1.0–24, and 1.5–48 μM for CYP2D6.1, CYP2D6.10 and CYP2D6.17, respectively. The inhibitor concentrations used were 0 (○), 0.005 (□), 0.013 (■), 0.032 (△), 0.08 (▲), 0.2 (◊), and 0.5 μM (♦).*

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TABLE 1

Comparison of enzyme kinetic parameters for CYP2D6.1-, CYP2D6.10-, and CYP2D6.17-mediated metabolite formations

All reactions were run in duplicate. Error estimates are based on the best fit of the average values obtained at each point to the Michaelis-Menten equation using nonlinear regression analysis.

Biotransformation	K_m (μM)	V_{max} (pmol/min/ pmol P450)	CL_{int} (μL/min/ pmol P450)	CL_{int} ratio (% CYP2D6.1)
CYP2D6.1				
Bufuralol 1'-hydroxylation	10.2 ± 0.6	6.29 ± 0.12	0.617	—
Dextromethorphan *O*-demethylation	2.97 ± 0.39	3.49 ± 0.10	1.18	—
Debrisoquine 4-hydroxylation	73.7 ± 7.0	1.32 ± 0.04	0.0179	—
Atomoxetine 4-hydroxylation	2.73 ± 0.23	19.1 ± 0.4	6.99	—
(*S*)-Fluoxetine *N*-demethylation	0.834 ± 0.111	0.414 ± 0.012	0.496	—
Nortriptyline 10-hydroxylation	4.07 ± 0.36	0.96 ± 0.02	0.236	—
Tramadol *O*-demethylation	286 ± 44	12.9 ± 0.5	0.0451	—
Codeine *O*-demethylation	1079 ± 307	3.68 ± 0.33	0.00341	—
CYP2D6.10				
Bufuralol 1'-hydroxylation	12.9 ± 1.1	0.29 ± 0.01	0.0225	3.65
Dextromethorphan *O*-demethylation	9.14 ± 0.59	0.57 ± 0.01	0.0624	5.31
Debrisoquine 4-hydroxylation	94.5 ± 7.8	0.20 ± 0.01	0.00212	11.8
Atomoxetine 4-hydroxylation	3.45 ± 0.45	2.07 ± 0.07	0.600	8.58
(*S*)-Fluoxetine *N*-demethylation	0.455 ± 0.076	0.017 ± 0.001	0.0374	7.54
Nortriptyline 10-hydroxylation	48.1 ± 8.0	0.15 ± 0.01	0.00312	1.32
Tramadol *O*-demethylation	331 ± 107	1.03 ± 0.09	0.00311	6.90
Codeine *O*-demethylation	1206 ± 239	1.14 ± 0.07	0.00095	27.9
CYP2D6.17				
Bufuralol 1'-hydroxylation	9.47 ± 1.54	1.33 ± 0.07	0.140	22.8
Dextromethorphan *O*-demethylation	17.3 ± 1.0	3.41 ± 0.05	0.197	16.8
Debrisoquine 4-hydroxylation	108 ± 8	1.24 ± 0.03	0.0115	64.2
Atomoxetine 4-hydroxylation	8.33 ± 0.40	12.8 ± 0.18	1.53	21.9
(*S*)-Fluoxetine *N*-demethylation	2.57 ± 0.50	0.104 ± 0.005	0.0405	8.17
Nortriptyline 10-hydroxylation	18.5 ± 4.0	0.32 ± 0.02	0.0173	7.33
Tramadol *O*-demethylation	209 ± 52	3.36 ± 0.21	0.0161	35.7
Codeine *O*-demethylation	1470 ± 253	4.03 ± 0.24	0.00274	80.4

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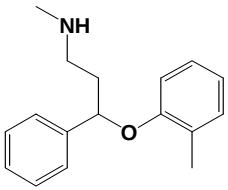
TABLE 2

Inhibition of dextromethorphan O-demethylation and atomoxetine 4-hydroxylation in CYP2D6.1, CYP2D6.10, and CYP2D6.17

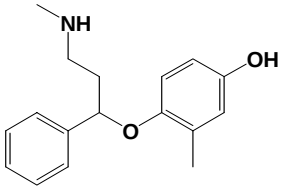
All reactions were run in duplicate. Error estimates are based on the best fit of the average values obtained at each point to the Michaelis-Menten equation using nonlinear regression analysis.

Inhibitor	K_i (μM)			K_i ratio (2D6.10/2D6.1)	K_i ratio (2D6.17/2D6.1)
	CYP2D6.1	CYP2D6.10	CYP2D6.17		
Dextromethorphan O-demethylation					
Cocaine	1.15 ± 0.14	1.74 ± 0.25	9.29 ± 1.13	1.51	8.08
(*S*)-Fluoxetine	1.27 ± 0.12	0.40 ± 0.05	1.73 ± 0.20	0.32	1.36
(*S*)-Norfluoxetine	0.84 ± 0.14	0.34 ± 0.05	1.84 ± 0.31	0.41	2.19
Imipramine	3.34 ± 0.28	3.70 ± 0.43	11.1 ± 0.8	1.11	3.32
Quinidine	0.011 ± 0.001	0.051 ± 0.003	0.079 ± 0.005	4.64	7.18
Thioridazine	0.018 ± 0.003	0.213 ± 0.017	0.166 ± 0.016	11.8	9.22
Atomoxetine 4-hydroxylation					
Cocaine	0.35 ± 0.04	0.43 ± 0.06	1.60 ± 0.21	1.23	4.57
(*S*)-Fluoxetine	0.67 ± 0.07	0.49 ± 0.09	0.82 ± 0.09	0.73	1.22
(*S*)-Norfluoxetine	1.04 ± 0.11	0.59 ± 0.11	1.16 ± 0.13	0.57	1.12
Imipramine	3.04 ± 0.34	4.26 ± 0.43	7.64 ± 0.64	1.40	2.51
Quinidine	0.010 ± 0.001	0.023 ± 0.002	0.056 ± 0.007	2.30	5.60
Thioridazine	0.026 ± 0.002	0.494 ± 0.090	0.103 ± 0.009	19.0	3.96

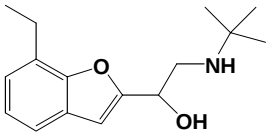
Figure 1



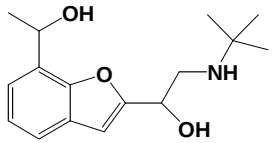
Atomoxetine (S)
$C_{17}H_{21}NO$, MW: 255



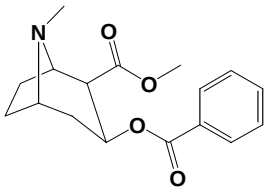
4-Hydroxyatomoxetine (M)
$C_{17}H_{21}NO_2$, MW: 271



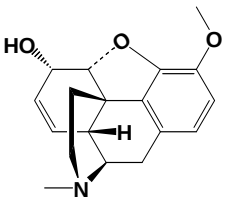
Bufuralol (S)
$C_{16}H_{23}NO_2$, MW: 261



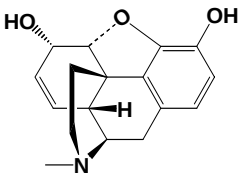
1'-Hydroxybufuralol (M)
$C_{16}H_{23}NO_3$, MW: 277



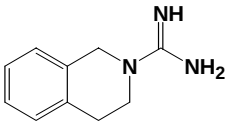
Cocaine (I)
$C_{17}H_{21}NO_4$, MW: 303



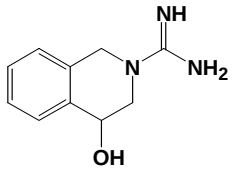
Codeine (S)
$C_{18}H_{21}NO_3$, MW: 299



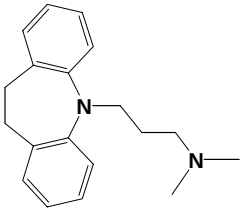
Morphine (M)
$C_{17}H_{19}NO_3$, MW: 285



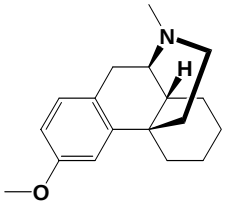
Debrisoquine (S)
$C_{10}H_{13}N_3$, MW: 175



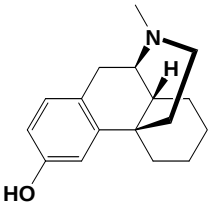
4-Hydroxydebrisoquine (M)
$C_{10}H_{13}N_3O$, MW: 191



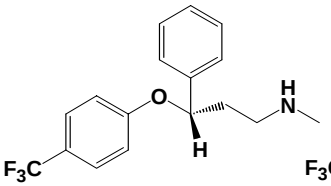
Imipramine (I/S)
$C_{19}H_{24}N_2$, MW: 280



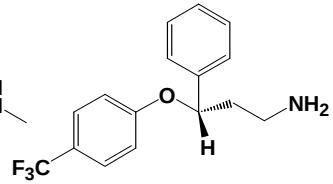
Dextromethorphan (S)
$C_{18}H_{25}NO$, MW: 271



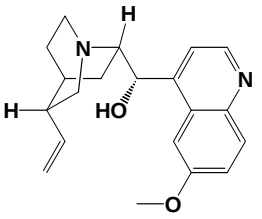
Dextrorphan (M)
$C_{17}H_{23}NO$, MW: 257



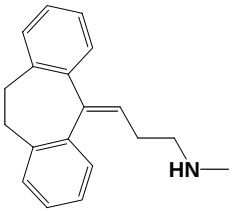
(*S*)-Fluoxetine (S/I)
$C_{17}H_{18}F_3NO$, MW: 309



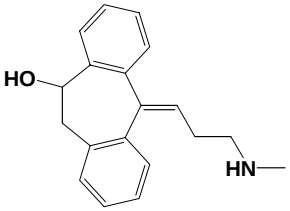
(*S*)-Norfluoxetine (M/I)
$C_{16}H_{16}F_3NO$, MW: 295



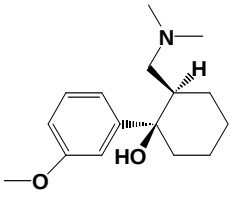
Quinidine (I)
$C_{20}H_{24}N_2O_2$, MW: 324



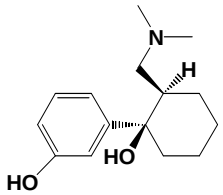
Nortriptyline (S)
$C_{19}H_{21}N$, MW: 263



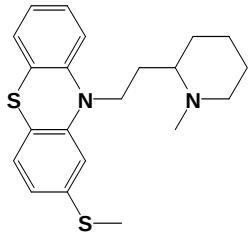
10-Hydroxynortriptyline (M)
$C_{19}H_{21}NO$, MW: 279



Tramadol (S)
$C_{16}H_{25}NO_2$, MW: 263



O-Desmethyl-tramadol (M)
$C_{16}H_{25}NO_2$, MW: 263



Thioridazine (I/S)
$C_{21}H_{26}N_2S_2$, MW: 370

Figure 2

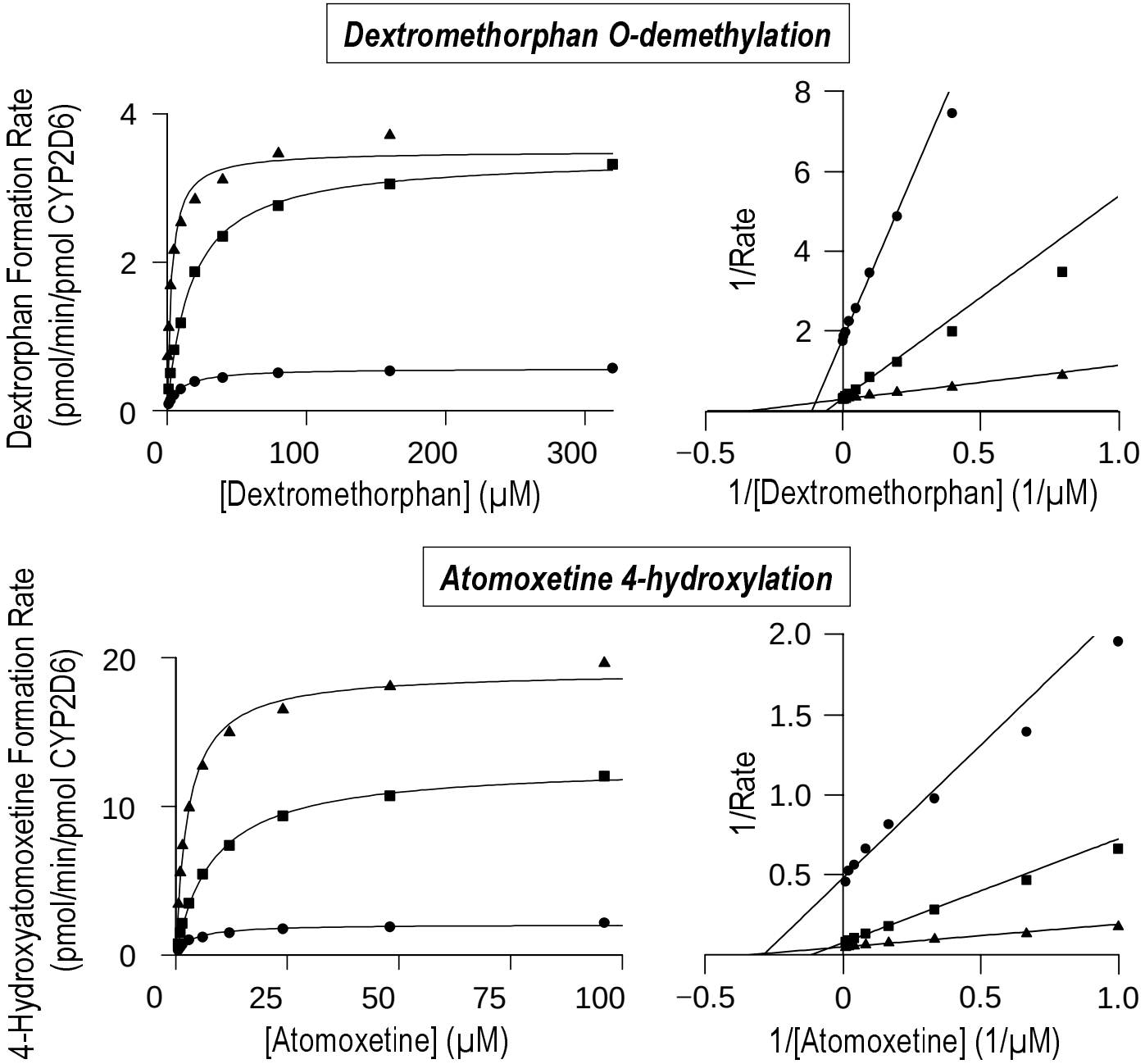


Figure 3