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# Desvenlafaxine and Venlafaxine Exert Minimal In Vitro Inhibition of Human Cytochrome P450 and P-Glycoprotein Activities

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**ABSTRACT ~ Objective:** Identification of potential pharmacokinetic drug-drug interactions is an important step in clinical drug development. We assessed and compared the drug-drug interaction potential of desvenlafaxine and venlafaxine, based on their inhibitory potency on human cytochrome P450 (CYP) and P-glycoprotein (P-gp) activities in vitro. **Methods:** Reversible inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, and mechanism-based inhibition of CYP2C9, CYP2C19, CYP2D6, and CYP3A activity by desvenlafaxine and venlafaxine were determined in human liver microsomes. Whether these drugs were substrates for efflux or inhibitors of P-gp were determined in Caco-2 monolayers. **Results:** Desvenlafaxine and venlafaxine showed little or no reversible inhibition of various CYP enzymes (concentration that inhibits 50% [IC<sub>50</sub>] or inhibition constant [K<sub>i</sub>] ~ or >100 μM). In addition, neither drug acted as a mechanism-based inhibitor of CYP2C9, CYP2C19, CYP2D6, or CYP3A as they did not reduce the IC<sub>50</sub> value for any of these enzymes in the presence of preincubations with or without a nicotinamide adenine dinucleotide phosphate-regenerating system. Desvenlafaxine and venlafaxine showed little inhibition of P-gp activity (IC<sub>50</sub> values >250 μM) and did not act as substrates (efflux ratios <2) for efflux in Caco-2 monolayers. **Conclusions:** Considering in vitro and available clinical data, desvenlafaxine and venlafaxine appear to have low potential for pharmacokinetic drug-drug interactions via inhibiting the metabolic clearance of concomitant drugs that are substrates of various CYP enzymes, in particular CYP2D6. In addition, these data suggest that desvenlafaxine and venlafaxine exhibit little potential for pharmacokinetic interactions with concomitant drugs that are substrates or inhibitors of P-gp. *Psychopharmacology Bulletin*. 2009;42(2):47-63.

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## INTRODUCTION

Assessment of pharmacokinetic drug-drug interactions (DDIs) has gained importance in clinical drug development due to the recent trend toward multidrug therapy and increasing awareness that the pharmacodynamics of coadministered drugs can be dramatically altered as a result of changes in their pharmacokinetics during multidrug therapy.<sup>1</sup> The most common mechanism underlying pharmacokinetic DDIs is inhibition of cytochrome P450 (CYP) activities,<sup>2,3</sup> since the first-pass metabolism and metabolic clearance of many drugs are mediated by this enzyme system. Another mechanism that is now recognized as important in DDIs is modulation of P-glycoprotein (P-gp) activity.<sup>4-6</sup> A member of the adenosine triphosphate-binding cassette superfamily of membrane transporter proteins, P-gp is expressed in many tissues including the luminal surface of intestinal epithelia, the renal proximal tubule, the bile canalicular membrane of hepatocytes and the blood-brain barrier. P-gp can play an important role in limiting intestinal drug absorption and brain penetration, and in facilitating renal or biliary excretion of drug substrates.<sup>7-11</sup>

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Selective serotonin reuptake inhibitors (SSRIs) (e.g., paroxetine and sertraline), serotonin- norepinephrine reuptake inhibitors (SNRIs) (e.g., venlafaxine and duloxetine), and bupropion are widely used in the treatment of major depressive disorders. Because many patients require long-term maintenance treatment with these therapies, they are frequently coprescribed with other medications,<sup>12,13</sup> which can increase the risk for DDIs.<sup>14</sup> SSRIs, SNRIs and bupropion exhibit varying degrees of CYP enzyme inhibition.<sup>13,15-20</sup> Clinically relevant DDIs via CYP2D6 inhibition have been reported for some SSRIs,<sup>8,13,21</sup> including paroxetine<sup>22</sup> and sertraline,<sup>23</sup> and SNRIs,<sup>24</sup> as well as for bupropion.<sup>8,13,15,24-27</sup> In addition to being inhibitors of CYP enzymes, some SSRIs and SNRIs have also been shown to be P-gp inhibitors (e.g., sertraline and paroxetine)<sup>28,29</sup> or substrates (e.g., fluoxetine, fluvoxamine, paroxetine, and venlafaxine).<sup>30,31</sup> Although clinically relevant DDIs involving P-gp by SSRIs and SNRIs have not been established, these findings suggest the potential for drug interactions at the level of intestinal drug absorption, brain penetration, and renal or hepatic elimination.

Desvenlafaxine (administered clinically as desvenlafaxine succinate) is an SNRI that was recently approved for the treatment of major depressive disorder.<sup>32</sup> Phase 3 clinical trial data demonstrated efficacy of desvenlafaxine compared with placebo at doses ranging from the Food and Drug Administration-approved 50 mg/d dose to 400 mg/d; higher doses within this range were less well tolerated and were not associated with greater efficacy.<sup>32-36</sup>

The objective of this study was to assess and compare the DDI potential of desvenlafaxine and venlafaxine based on their inhibitory potency

on human CYP and P-gp activities in vitro. The reversible inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 activities, and an initial assessment of mechanism-based inhibition of CYP2C9, CYP2C19, CYP2D6, and CYP3A by desvenlafaxine and venlafaxine were determined in human liver microsomes using specific probe substrates. The role of these drugs as inhibitors or substrates of P-gp was also assessed using Caco-2 cell monolayers. These data represent the first report of the effect of the succinate salt of desvenlafaxine on CYP enzyme and P-gp activities assessed in vitro. The potential for clinical DDIs for desvenlafaxine and venlafaxine are discussed based on present in vitro and available in vivo data.

## MATERIALS AND METHODS

Desvenlafaxine succinate monohydrate and venlafaxine hydrochloride were synthesized by Wyeth Research (Pearl River, NY or Princeton, NJ). Resorufin, ethoxyresorufin, and dextrorphan were purchased from Molecular Probes, Inc. (Eugene, OR). S-Mephenytoin, 4'-hydroxymephenytoin, bufuralol hydrochloride, 1'-hydroxybufuralol, 1'-hydroxymidazolam, 6 $\alpha$ -hydroxypaclitaxel, 4'-hydroxydiclofenac, 7-hydroxycoumarin and paclitaxel were purchased from Gentest Corporation (Woburn, MA). [<sup>3</sup>H]-Digoxin (37 Ci/mmol) was purchased from Perkin Elmer (Boston, MA). All cell culture media supplies were purchased from Life Technologies (Gaithersburg, MD). The BioCoat transwell plates for growing the cell cultures and laminin for coating insert membranes were received from Becton-Dickinson (Franklin Lakes, NJ). The Caco-2 subclone (passage #23) was grown in house (from passage #18). High performance liquid chromatography (HPLC)-grade water, methanol, and acetonitrile were obtained from E.M. Science (Gibbstown, NJ). All other chemicals were reagent grade or better and were purchased from Sigma Chemical, Co. (St. Louis, MO) or VWR (West Chester, PA). Liver microsomes consisted of a pool of human liver microsomes from 50 donors (30 males, ages 6 to 77 and 20 females, ages 30 to 78, medical histories available) purchased from XenoTech Corporation (Lot 0310241 or 051022, Kansas City, KS).

### *Determination of IC<sub>50</sub> or K<sub>i</sub> Values for the Reversible Inhibition of CYP Enzymes*

Concentration that inhibits 50% (IC<sub>50</sub>) values were determined for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, and 3A, using a "cocktail" method similar to that described previously,<sup>37</sup> employing isoform-selective probe substrates with substrate concentrations at their approximate K<sub>m</sub> values

TABLE 1

## PRECURSOR IONS, PRODUCT IONS, AND MS CONDITIONS FOR CYP INHIBITION STUDIES

HUMAN CYP ISOZYME	PROBE SUBSTRATE (CONCENTRATION $\mu$ M)	METABOLITE MONITORED	PRECURSOR ION (m/z)	PRODUCT ION (m/z)	COLLISION ENERGY (V)	SOURCE MODE	STUDY
CYP1A2	Ethoxresorufin (1)	Resorufin	214	186	37 (35)	ESI (APCI)	IC <sub>50</sub>
CYP2A6	Coumarin (2.5)	7-hydroxycoumarin	163	107	31	ESI, APCI	IC <sub>50</sub>
CYP2C8	Paclitaxel (10)	6 $\alpha$ -hydroxypaclitaxel	870	286	19 (23)	ESI (APCI)	IC <sub>50</sub>
CYP2C9	Diclofenac (10) (10)	4'-hydroxydiclofenac	312 312	230 (166) 265	45 (77) 21	ESI (APCI) APCI	IC <sub>50</sub> MBI
CYP2C19	S-mephenytoin (40) (15, 30, 60, 120, 300) (60)	4'-hydroxy-S-mephenytoin	235 233 235	150 190 150	27 (25) -20 27	ESI (APCI) (-) ESI APCI	IC <sub>50</sub> K <sub>i</sub> MBI
CYP2D6	Bufuralol (5) (5, 10, 20, 40, 100) (10)	1'-hydroxybufuralol	278 278 278	159 186 or 159 186	35 25 or 35 27	ESI, APCI APCI APCI	IC <sub>50</sub> K <sub>i</sub> MBI
CYP3A	Midazolam (2.5) (2.5) (0.75, 1.5, 3, 6, 15) (2.5)	1'-hydroxymidazolam	342 342 342 342	168 203 203 168	55 39 35 53	ESI, APCI ESI APCI APCI	IC <sub>50</sub> IC <sub>50</sub> K <sub>i</sub> MBI
CYP3A	Testosterone (50) (10, 25, 50, 100, 250)	6 $\beta$ -hydroxytestosterone	305 305	157 (269) 269	35 (23) 21	ESI (APCI) ESI	IC <sub>50</sub> K <sub>i</sub>

Abbreviations: APCI, atmospheric pressure chemical ionization; CYP, cytochrome P450; ESI, electrospray ionization; IC<sub>50</sub>, concentration that inhibits 50%; K<sub>i</sub>, inhibition constant; MBI, mechanism-based inhibition; MS, mass spectrometry; V, coefficient of variation.

or below (Table 1). Desvenlafaxine and venlafaxine (0 to 100  $\mu\text{M}$ ) were incubated in triplicate with a human liver microsomal protein concentration of 0.2 mg/mL. The inhibition constant ( $K_i$ ) values for the inhibition of CYP2D6, 2C19, and 3A activity were determined for desvenlafaxine and venlafaxine as judged by their  $\text{IC}_{50}$  values. Desvenlafaxine and venlafaxine drug concentrations were chosen to encompass their  $\text{IC}_{50}$  values. Liver microsomal protein concentrations used ranged from 0.1 to 0.5 mg/mL, dependent upon the CYP enzyme evaluated and probe substrates used to ensure initial rate conditions (Table 1). Control inhibitors, including ketoconazole (1  $\mu\text{M}$  for CYP3A), quinidine (10  $\mu\text{M}$  for CYP2D6), sulfaphenazole (10  $\mu\text{M}$  for CYP2C9), quercetin (20  $\mu\text{M}$  for CYP2C8),  $\alpha$  naphthoflavone (20  $\mu\text{M}$  for CYP1A2) and tranylcypromine (10 and 50  $\mu\text{M}$  for CYP2A6 and CYP2C19, respectively) were incubated separately. Control incubations contained no drug.

Incubations were performed in 96-well square plates containing potassium phosphate buffer (100 mM, pH 7.4), human liver microsomes,  $\text{MgCl}_2$  (10 mM) and a nicotinamide adenine dinucleotide phosphate (NADPH)-regenerating system (3.5 mM glucose-6-phosphate, 0.4 units/mL glucose-6-phosphate dehydrogenase and 1.3 mM  $\text{NADP}^+$ ) at a final volume of 0.5 mL. Sample preparation and incubations were performed on the Tecan Genesis Freedom 200 MAP using Gemini software v4.0 and conducted under conditions shown to be linear with respect to time, protein and substrate concentration. Desvenlafaxine and venlafaxine for  $\text{IC}_{50}$  determination were diluted using a Packard Multiprobe IIEX. Incubations were preheated during preparation of plates, initiated by addition of an NADPH-regenerating system, and plates were shaken at 37°C for 10 to 15 minutes on a Thermomixer R (Eppendorf). Reactions were quenched by the addition of 0.5 mL acetonitrile containing the respective internal standard. The plates were then centrifuged for 10 minutes at 3400 rpm and supernatant transferred to a 96 well plate for concentration under  $\text{N}_2$  prior to analysis by HPLC/tandem mass spectrometry (LC/MS/MS) to determine probe substrate metabolite concentrations using 8-point calibration curves.

#### *Initial Assessment of Mechanism-Based Inhibition of CYP2C9, 2C19, 2D6, and 3A*

Desvenlafaxine and venlafaxine (1, 10, and 100  $\mu\text{M}$ ) were preincubated for 30 minutes at 37°C in potassium phosphate buffer (100 mM, pH 7.4) and  $\text{MgCl}_2$  containing human liver microsomes (0.5 mg/mL) and with or without a NADPH-regenerating system at a final volume of 0.5 mL. Aliquots (100  $\mu\text{L}$ ) of the preincubations were then transferred to separate wells containing potassium phosphate buffer,  $\text{MgCl}_2$ , a

NADPH-regenerating system and a cocktail of isoform-selective probe substrates for CYP2C9, CYP2C19, CYP2D6, and CYP3A4 at a final volume of 0.5 mL. Incubations were performed and samples were analyzed similar to procedures described previously for the IC<sub>50</sub> determination of reversible inhibitions. Separate incubations were performed under identical conditions using known mechanism-based inactivators of CYP2C9 (tienilic acid, 2.5  $\mu$ M), CYP2C19 (ticlopidine, 25  $\mu$ M), CYP2D6 (paroxetine, 10  $\mu$ M) and CYP3A4 (troleandomycin, 10  $\mu$ M).

### *Culturing of Caco-2 Cells*

Twelve well plates with trans well inserts were used for growing the cells. The insert membranes (0.1 micron, 0.96 cm<sup>2</sup>) were precoated with laminin (5  $\mu$ g/cm<sup>2</sup>, for 1 hour at room temperature). Caco-2 cells (passage #23, subclone VII), normally stored in liquid nitrogen, were quick thawed in a water bath at 37°C and were gently centrifuged (500 rpm for 5 min) using a Sorvall Super T21 centrifuge (Kendro Laboratory Products, Newtown, CT) to discard the freezing medium. Cell pellets were gently resuspended in the growth medium (Dulbecco modified Eagle medium [DMEM], high glucose, with added nonessential amino acids [NEAA] and containing 20% fetal bovine serum [FBS]) and seeded in inserts at density of at least 250,000 cells/cm<sup>2</sup>. Plates were housed in a CO<sub>2</sub>-incubator (VWR Scientific, South Plainfield, NJ) at 37°C and 5% CO<sub>2</sub> atmosphere. Cell medium was changed once every 2 days. The formation and integrity of the cell monolayer was monitored by measuring the transepithelial electrical resistance, using a Millicell ERS electrode (Millipore, Bedford, MA). The formation of monolayer was complete at day 4 to 5 at which point the cultures were switched to differentiation medium (DMEM high glucose, NEAA and containing 5% FBS). Medium was changed every 2 days. Two weeks after starting the cultures on differentiation medium, the cells were washed with plain DMEM medium and were ready for conducting studies.

### *Inhibition of P-glycoprotein Activity*

The bidirectional permeation of [<sup>3</sup>H]-digoxin (5  $\mu$ M) was determined in the presence or absence of desvenlafaxine and venlafaxine (1–250  $\mu$ M, 10  $\mu$ L methanol) or verapamil (100  $\mu$ M). Digoxin (1  $\mu$ Ci/ $\mu$ L ethanol, 5  $\mu$ M) was prepared in incubation medium (DMEM with NEAA, no FBS). Incubations for desvenlafaxine and venlafaxine were conducted on 3 separate days, each using a different set of Caco-2 monolayers, which were performed in triplicate (inserts) for 2 hours at 37°C and 5% CO<sub>2</sub> in a CO<sub>2</sub> incubator. Media from the apical and basolateral compartments were collected and frozen at –80°C until analysis. To an aliquot of medium from the apical (100  $\mu$ L) or basolateral (250 to 300  $\mu$ L) compartment,

Ultima Gold Solution (5 mL) was added and mixtures were counted (10 minutes or until %  $\sigma$  of 2 was reached) to determine digoxin (radioactivity) concentration using a Packard Tri Card Model 3100 TR liquid scintillation counter (Perkin Elmer, Shelton CA).

### *Evaluation as Substrates for P-Glycoprotein*

The bidirectional permeation of desvenlafaxine and venlafaxine was determined at drug concentrations of 5, 25, and 100  $\mu\text{M}$  in the absence or presence of verapamil (100  $\mu\text{M}$ ).  $^3\text{H}$ -Digoxin (5  $\mu\text{M}$ ) was used as a positive control. The maximum concentration of organic solvents in the incubation medium was limited to 2%. Incubations were performed for 2 hours in triplicate (inserts) at 37°C and 5%  $\text{CO}_2$  in a  $\text{CO}_2$  incubator. Media from the apical and basolateral compartments were collected and frozen at -80°C until analyses. The internal standard dextrorphan (50  $\mu\text{L}$ , 200 ng/mL in acetonitrile) was added to thawed samples (10  $\mu\text{L}$ ), followed by the addition of matrix (incubation medium, 90  $\mu\text{L}$ ) and acetonitrile (50  $\mu\text{L}$ ), and the mixtures were vortexed and centrifuged at 10000 g for 8 minutes at 4°C (Allegra 21R Centrifuge, Beckman Coulter, Fullerton, CA) to precipitate protein. The supernatant (200  $\mu\text{L}$ ) was analyzed by LC/MS to determine desvenlafaxine and venlafaxine concentrations using a standard curve (1 to 2500 ng/mL) prepared in incubation medium.

### *Analytical Methods for CYP Inhibition Studies*

#### **Instrumentation**

HPLC analysis was performed on an Agilent Model 1100 HPLC (Agilent Technologies, Palo Alto, CA) with a degasser and column heater and an HTS PAL autosampler (LEAP Technologies, Raleigh, NC). For all  $\text{IC}_{50}$  studies and  $\text{K}_i$  studies for 1'-hydroxybufuralol and 6 $\beta$ -hydroxytestosterone, the column used was a 5  $\mu$  Thermo Aquasil  $\text{C}_{18}$  column, 2.1 mm id  $\times$  50 mm (Bellefonte, PA). For  $\text{K}_i$  studies with 4'-hydroxymephenytoin and 1'-hydroxymidazolam, the column was a 5  $\mu$  Analytical Sales and Service Lancer  $\text{C}_{18}$  column, 2 mm id  $\times$  20 mm (Pompton Lakes, NJ). Mass spectrometric measurements were made on an Applied Biosystems PE Sciex API 3000 or API 4000 triple quadrupole mass spectrometer. Analysis of LC/MS data was performed using Analyst<sup>TM</sup> v1.4.1.

### *$\text{IC}_{50}$ Studies for Reversible and Mechanism-Based Inhibition*

Aliquots (10  $\mu\text{L}$ ) of the samples (containing metabolites and internal standard) were injected onto the HPLC using a mobile phase consisting of a linear gradient of acetonitrile containing 0.1% formic acid and

water containing 0.1% formic acid. Specific metabolites of the CYP450 isozymes probe substrates were detected by LC/MS/MS either in the positive electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) mode utilizing a multiple reaction monitoring (MRM) technique. Source temperatures using ESI and APCI modes were 350 and 500°C, respectively, for the reversible studies and 600°C (APCI mode) for the mechanism-based inhibition studies.

### *K<sub>i</sub> Studies*

Aliquots (10 or 20 µL) of the supernatants (containing metabolites and internal standard) were analyzed by LC/MS/MS. For 1'-hydroxybufuralol and 6β-hydroxytestosterone, the mobile phase consisted of a linear gradient of acetonitrile containing 0.1% formic acid and water containing 0.1% formic acid. For 4'-hydroxymephenytoin and 1' hydroxymidazolam, the mobile phase consisted of a linear gradient of acetonitrile and 5 mM ammonium acetate, mixed at a constant flow rate of 0.4 mL/min. 1' hydroxybufuralol and 1' hydroxymidazolam were measured in the APCI mode utilizing an MRM technique. 6β-hydroxytestosterone and 4-hydroxymephenytoin were measured in the positive and negative ESI modes, respectively, utilizing a MRM technique. Source temperatures were 500 to 550°C for 1'-hydroxybufuralol, 350°C 6β-hydroxytestosterone, 300°C 4-hydroxymephenytoin, and 300°C for 1' hydroxymidazolam. An appropriate relevant precursor and daughter was selected for each metabolite (Table 1).

### *Analytical Methods for P-glycoprotein Substrate Studies*

HPLC analysis was performed on an Agilent Model 1100 HPLC (Agilent Technologies, Palo Alto, CA) with an Agilent degasser, an Agilent column heater maintained at 40°C, and an HTS PAL autosampler (LEAP Technologies, Raleigh, NC) maintained at 4°C. Aliquots (50 µL) of the acetonitrile supernatants (containing drugs and the internal standard) were injected onto the HPLC. The column was a Keystone BDS Hypersil C8, 150 mm × 4.6 mm, particle size 5 µm (Bellefonte, PA). The mobile phase consisted of a linear gradient of acetonitrile (A) and 5 mM ammonium acetate (B), mixed in following A/B ratio with a constant flow rate of 1 mL/min: 10/90 (0 to 3.5 min), 95/5 (3.5 to 5.0 min) and 10/90 (5.0 min). All flow from the column, from up to 1 minute of initial flow, was diverted away from the MS.

Drugs were detected by HPLC MS/MS in the positive ESI mode utilizing a MRM technique. Mass spectrometric measurements were made on a Quattro Micro triple quadrupole MS using a Z spray ionization source and with an ionization voltage of 3200 V. Source and desolvation

temperatures were 120 and 220°C, respectively, and cone and desolvation N<sub>2</sub> flows were 100 and 605 L/hr, respectively. An appropriate relevant precursor ion ([M+H]<sup>+</sup>) was selected for each compound using an optimal cone voltage setting. Product ions were then detected following collision-induced dissociation (CID) of the selected precursor ions. Cone and collision cell voltages varied depending upon the compound detected. An appropriate relevant precursor and daughter ion (264.07 for desvenlafaxine and 278.13 for venlafaxine) were selected for each metabolite. Product ions (58.36 for desvenlafaxine and 121.12 for venlafaxine) were then detected following CID of the selected precursor ions. Data analysis was through a Windows NT-based MassLynx™ v4.0.

### *Evaluation of Results and Statistical Analysis*

**CYP Inhibition.** Standard curves were performed at the beginning and end of each run with individual points excluded when back-calculated concentrations deviated from their corresponding nominal values by >20%. In order for a run to be considered acceptable and data reported, no more than 25% of the points could be excluded for any metabolite. Concentrations of all metabolites were determined by extrapolating the response calculated from the peak area ratio to the peak area of internal standard to that of the standard curve. Values for sample wells containing no drugs were averaged and these averages were used as the control values (i.e., 100% enzyme activity). Values for samples containing desvenlafaxine and venlafaxine were expressed as a percentage of this 100% activity value. Calculations were performed with Microsoft Excel 2000. IC<sub>50</sub> values were calculated from plots generated from the inhibitory effect E<sub>max</sub> model (model 103) utilizing WinNonlin Professional, version 4.1. Apparent K<sub>m</sub> values and kinetics of inhibition were determined by nonlinear regression plots (using simple E<sub>max</sub> model [model 101] or sigmoidal E<sub>max</sub> model [model 105] for testosterone, WinNonlin Professional, version 4.1), Lineweaver Burk plots, and Eadie-Hofstee plots. K<sub>i</sub> values were determined by Dixon plots.

**P-glycoprotein Efflux or Inhibition.** The transport of digoxin across Caco-2 monolayers was determined by the amount of drug permeated (pmol), the rate of permeation (pmol/sec) and the apparent permeability coefficient (P<sub>app</sub>). P<sub>app</sub> (cm/sec) was calculated by  $P_{app} = (dQ/dt)X1/(A \cdot C_o)$ , where dQ/dt is the rate of drug appearance in the receiver compartment (μmole/sec), C<sub>o</sub> is the initial drug concentration in the donor compartment (μM), and A is the surface area of the monolayer (cm<sup>2</sup>). These values (mean ± SD of n = 3) were calculated in both A→B (apical to basolateral) and B→A (basolateral to apical) directions, and in the absence or presence of desvenlafaxine, venlafaxine,

or verapamil. Efflux was considered to have occurred when the BA/AB ratio was greater than 2. A decrease in the BA/AB ratio in the presence of verapamil would suggest that the observed efflux is at least in part due to P-gp inhibition. Degree of inhibition was calculated using the following relationship: % Inhibition =  $(1 - (i_{B \rightarrow A} - i_{A \rightarrow B} / a_{B \rightarrow A} - a_{A \rightarrow B})) \times 100\%$ , where  $i$  and  $a$  are the amount of digoxin transported in the presence and absence of the putative inhibitor, respectively.<sup>38</sup> IC<sub>50</sub> values were calculated using the inhibitory effect E<sub>max</sub> model (model 103) of WinNonlin Professional, version 4.1.

## RESULTS

### *Reversible Inhibition of CYP Activities*

Desvenlafaxine and venlafaxine showed no or low (IC<sub>50</sub> >100 μM) inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, or CYP3A activities (Table 2). The K<sub>i</sub> values for the inhibition of these CYP enzymes were not determined further.

Desvenlafaxine did not exhibit inhibition of CYP2D6 activity. In contrast, venlafaxine showed moderate inhibition (IC<sub>50</sub> of 69 μM) of CYP2D6 (Table 2). The K<sub>i</sub> values for the inhibition of CYP2D6-mediated bufuralol-1'-hydroxylation were >300 μM for desvenlafaxine compared with 96 μM for venlafaxine (Table 3), with the mode of inhibition determined to be competitive. The K<sub>m</sub> value (20 ± 3.6 μM, mean ± SD, n = 12) for bufuralol-1'-hydroxylation was within the range of values reported previously.<sup>39</sup>

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TABLE 2

ESTIMATED IC<sub>50</sub> VALUES (μM) FOR THE INHIBITION OF CYP ENZYMES IN HUMAN LIVER MICROSOMES

P450	DESVENLAFAXINE	VENLAFAXINE
CYP1A2	130 ± 88	NC
CYP2A6	NC	NC
CYP2C8	NC	NC
CYP2C9	NC	NC
CYP2C19	NC	NC
CYP2D6	NC	69 ± 3
CYP3A <sup>a</sup>	NC	NC
CYP3A <sup>b</sup>	NC	NC

Abbreviation: CYP, cytochrome P450; IC<sub>50</sub>, concentration that inhibits 50%; NC, not calculated due to lack of inhibition at 100 μM; SD, standard deviation.

Values are mean ± SD of 3 separate determinations each performed in triplicate.

<sup>a</sup>Midazolam-1'-hydroxylation.

<sup>b</sup>Testosterone-6β-hydroxylation.

TABLE 3

**K<sub>i</sub> VALUES (μM) AND MODE OF INHIBITION FOR THE INHIBITION OF CYP ENZYMES IN HUMAN LIVER MICROSOMES**

	CYP2D6
Desvenlafaxine	>300
Venlafaxine	96 (competitive)

Abbreviations: CYP, cytochrome P450; K<sub>i</sub>, inhibition constant.

Values are mean of 2 separate determinations each performed in duplicate.

***Mechanism-Based Inhibition of CYP2C9, 2C19, 2D6, and 3A***

Table 4 shows the estimated IC<sub>50</sub> values for the inhibition of selected CYP activities in the presence of preincubations with or without an NADPH-regenerating system. Desvenlafaxine and venlafaxine did not exhibit reduction of IC<sub>50</sub> value for any of the enzymes evaluated, suggesting that these agents were not mechanism-based inhibitors of CYP2C9, CYP2C19, CYP2D6, or CYP3A. The known mechanism-based inhibitors tienilic acid, ticlopidine, paroxetine, and troleandomycin expectedly inhibited CYP2C9, CYP2C19, CYP2D6, and CYP3A activities, respectively, in a NADPH-dependent manner (data not shown).

***Inhibition of P-glycoprotein Activity by Desvenlafaxine and Venlafaxine***

Inhibition of P-gp-mediated digoxin efflux in the presence of increasing concentrations of desvenlafaxine and venlafaxine is summarized in Table 5. Extrapolated IC<sub>50</sub> values were not definable due to minimal inhibition (<20%) at the highest concentration used (i.e., 250 μM). The known P-gp inhibitor, verapamil, had an IC<sub>50</sub> value of 12.2 ± 1.5 μM under the same experiment conditions.

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TABLE 4

**ESTIMATED IC<sub>50</sub> VALUES (μM) FOR THE MECHANISM-BASED INHIBITION OF CYP ENZYMES IN HUMAN LIVER MICROSOMES IN THE PRESENCE OF PREINCUBATION WITH OR WITHOUT AN NADPH REGENERATING SYSTEM**

	NADPH PREINCUBATION	CYP2C9	CYP2C19	CYP2D6	CYP3A
Desvenlafaxine	–	>100	>100	>100	>100
	+	>100	>100	>100	>100
Venlafaxine	–	>100	>100	>100	>100
	+	>100	>100	>100	>100

Abbreviations: CYP, cytochrome P450; IC<sub>50</sub>, concentration that inhibits 50%; NADPH, nicotinamide adenine dinucleotide phosphate.

TABLE 5

**IC<sub>50</sub> VALUES FOR THE INHIBITION OF P-GLYCOPROTEIN-MEDIATED DIGOXIN EFFLUX IN CACO-2 MONOLAYERS**

INHIBITOR	IC <sub>50</sub> (μM) (MEAN ± SD)
Desvenlafaxine	ND
Venlafaxine	ND
Verapamil	12.2 ± 1.5

Abbreviation: IC<sub>50</sub>, concentration that inhibits 50%; ND, not definable due to minimal inhibition (<20%) at the highest concentration used (250 μM); SD, standard deviation. Results are mean ±SD of 3 determinations each conducted on a separate day.

*Desvenlafaxine and Venlafaxine as Substrates for P-glycoprotein*

P<sub>app</sub> and efflux (BA/AB) ratios for desvenlafaxine and venlafaxine are shown in Table 6. Desvenlafaxine and venlafaxine showed efflux ratios of <2, indicating that little P-gp-dependent efflux had taken place in the Caco-2 monolayers. Digoxin showed an efflux ratio of 25 at a drug concentration of 5 μM that was markedly reduced (BA/AB = 1.4) in the presence of verapamil, consistent with digoxin being a P gp substrate.

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**DISCUSSION***CYP-based Drug-Drug Interactions*

Peak plasma concentrations (C<sub>max</sub>) and in vitro K<sub>i</sub> may be used to predict the changes in area under the plasma concentration-versus-time curve (AUC) of a drug in the presence of an inhibitor in vivo.<sup>40</sup> Factors such as microsomal and plasma protein binding, hepatic uptake, and

TABLE 6

**PERMEABILITY IN CACO-2 MONOLAYERS**

COMPOUND	CONC (μM)	P <sub>APP</sub> (AB), × 10 <sup>-6</sup> CM/SEC	P <sub>APP</sub> (BA), × 10 <sup>-6</sup> CM/SEC	BA/AB RATIO
		MEAN ± SD	MEAN ± SD	
Desvenlafaxine	5	9.90 ± 0.41	15.1 ± 1.26	1.5
	25	8.18 ± 0.23	12.4 ± 0.38	1.5
	100	7.27 ± 0.33	9.54 ± 0.59	1.3
Venlafaxine	5	4.62 ± 0.65	6.84 ± 0.50	1.5
	25	4.75 ± 0.53	6.50 ± 0.77	1.4
	100	4.81 ± 0.27	5.39 ± 0.28	1.1
Digoxin	5	0.70 ± 0.01	17.7 ± 0.58	25
Digoxin + Verapamil	5 100	4.58 ± 0.33	6.59 ± 0.19	1.4

Abbreviations: Conc, concentration; P<sub>app</sub>, apparent permeability coefficient; SD, standard deviation. Incubations were performed for 2 hours at 37°C. Results are mean ± SD of n = 3 inserts.

maximum hepatic input concentration, together with the fraction of substrates metabolized by a particular CYP enzyme, may improve the quantitative prediction of DDIs.<sup>41,42</sup> Microsomal binding of the various drugs studied has not been determined in the present study; instead, their apparent  $K_i$  or  $IC_{50}$  values together with available in vivo data are discussed below with respect to the potential for clinical DDIs.

The data in the current study demonstrate that desvenlafaxine exhibits little or no inhibition of the major CYP enzymes, as found in other studies at drug concentrations as high as 100  $\mu\text{M}$ .<sup>16,19,43,44</sup> Furthermore, desvenlafaxine did not show mechanism-based inhibition of the CYP enzymes evaluated. Although previous studies have examined the effect of venlafaxine metabolites (i.e., O-desmethylvenlafaxine and N-desmethylvenlafaxine) on the inhibition of CYP3A,<sup>43</sup> the data herein reflect the first demonstration of the effect of the succinate salt of desvenlafaxine on the reversible and mechanism-based inhibition of various CYP enzymes. Considering its plasma drug concentrations (0.51–1.1  $\mu\text{M}$ ),<sup>45,46</sup> desvenlafaxine may be expected to have minimal potential to inhibit the metabolism of concomitant drugs that are metabolized by these CYP enzymes. In fact, in vivo studies demonstrate that desvenlafaxine produced minor changes in the AUC or  $C_{\text{max}}$  of the CYP 2D6 substrates desipramine and 2-hydroxydesipramine compared with either paroxetine<sup>45</sup> or duloxetine.<sup>46</sup>

Venlafaxine was previously shown to have no or minimal effect (<2 fold) on the dextromethorphan/dextrorphan ratio.<sup>47</sup> That finding appears consistent with low plasma venlafaxine concentrations (0.53 to 0.77  $\mu\text{M}$ )<sup>48,49</sup> relative to the  $K_i$  values reported in this and other studies (=100  $\mu\text{M}$ ).<sup>16,19</sup> Also consistent with the low extent of in vitro inhibition demonstrated in the present study, previous venlafaxine studies showed little inhibition of coadministered drugs metabolized via CYP1A2, CYP2C9, CYP2C19, or CYP3A.<sup>8,15,20</sup> In the current study, venlafaxine did not show mechanism-based inhibition of the CYP enzymes evaluated.

### *P-glycoprotein-based Drug-Drug Interactions*

The inhibitory potency of desvenlafaxine and venlafaxine appeared low under the conditions used in this study, with  $IC_{50}$  values greater than the highest drug concentrations used (250  $\mu\text{M}$ ). The low extent of P-gp inhibition by desvenlafaxine and venlafaxine is in agreement with results reported previously ( $IC_{50}$  values not definable) using L-MDR1 cells and the fluorometric substrate calcein-acetoxymethyl ester.<sup>28</sup> The use of in vitro inhibition data to extrapolate into in vivo DDI has not been well established for P-gp, in part because improved understanding of the quantitative contribution of the transport proteins and the occurrence of

multiple simultaneous processes is needed to accurately predict in vivo effects from in vitro assays.<sup>40</sup> The minimal in vitro P-gp inhibition ( $IC_{50}$  values  $>250 \mu M$ ) observed suggests that these drugs may be expected to have a minimal or no effect on the pharmacokinetics of concomitant drugs that are P-gp substrates.

Desvenlafaxine and venlafaxine (efflux ratios = 1.6) were not considered substrates for efflux in this study, despite the earlier finding that venlafaxine stimulates multidrug resistance (MDR) and MDR-associated protein gene expression in Caco-2 cells.<sup>50</sup> Results from the present report for desvenlafaxine and venlafaxine are apparently in contrast with findings by Uhr and colleagues, who showed that the brain uptake of desvenlafaxine and venlafaxine was enhanced in MDR1a/b knockout mice<sup>31</sup> and that polymorphisms in the drug transporter gene may predict desvenlafaxine and venlafaxine treatment response in depression.<sup>51</sup> Likewise, the antidepressant doxepin was concluded to be a P-gp substrate due to higher cerebrum drug concentrations in MDR1ab knockout mice,<sup>31</sup> but was not considered a substrate for P-gp in Madin-Darby canine kidney cells expressing human P-gp.<sup>30</sup> Since species differences in P-gp activity have been reported pertaining to their respective drug resistance profiles and sensitivity to modulators, caution should be exercised when extrapolating data from knockout animal models to human situations.<sup>52</sup>

In conclusion, this is the first study in which desvenlafaxine (as the succinate salt) and venlafaxine were evaluated within a single study with respect to their inhibitory effects on CYP and P-gp activities in vitro. Due to their limited or lack of in vitro inhibition of various CYP enzymes and considering their therapeutic plasma drug concentrations, desvenlafaxine and venlafaxine appear to have a low potential to inhibit the metabolic disposition of concomitant drugs that are substrates for the major CYP enzymes assessed, in particular for CYP2D6. The in vitro observations are consistent with results of clinical studies that demonstrated minimal effect of desvenlafaxine or venlafaxine on the pharmacokinetics of concomitant medications that are metabolized by the various CYP enzymes. Due to their low in vitro inhibition of P-gp activity, and the demonstration of minimal or no efflux in the Caco-2 system, desvenlafaxine and venlafaxine would be expected to exhibit little or no pharmacokinetic interactions with concomitant drugs that are substrates or inhibitors of P-gp. ❀

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