Identification of Human Sulfotransferases Involved in Lorcaserin N-Sulfamate Formation

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ABSTRACT

Lorcaserin [(R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine] hydrochloride hemihydrate, a selective serotonin 5-hydroxytryptamine (5-HT) 5-HT2C receptor agonist, is approved by the U.S. Food and Drug Administration for chronic weight management. Lorcaserin is primarily cleared by metabolism, which involves multiple enzyme systems with various metabolic pathways in humans. The major circulating metabolite is lorcaserin N-sulfamate. Both human liver and renal cytosols catalyze the formation of lorcaserin N-sulfamate, where the liver cytosol showed a higher catalytic efficiency than renal cytosol. Human sulfotransferases (SULTs) SULT1A1, SULT1A2, SULT1E1, and SULT2A1 are involved in the formation of lorcaserin N-sulfamate. The catalytic efficiency of these SULTs for lorcaserin N-sulfamate formation is widely variable, and among the SULT isoforms SULT1A1 was the most efficient. The order of intrinsic clearance for lorcaserin N-sulfamate is SULT1A1 > SULT2A1 > SULT1A2 > SULT1E1. Inhibitory effects of lorcaserin N-sulfamate on major human cytochrome P450 (P450) enzymes were not observed or minimal. Lorcaserin N-sulfamate binds to human plasma protein with high affinity (i.e., >99%). Thus, despite being the major circulating metabolite, the level of free lorcaserin N-sulfamate would be minimal at a lorcaserin therapeutic dose and unlikely be sufficient to cause drug-drug interactions. Considering its formation kinetic parameters, high plasma protein binding affinity, minimal P450 inhibition or induction potential, and stability, the potential for metabolic drug-drug interaction or toxicological effects of lorcaserin N-sulfamate is remote in a normal patient population.

Introduction

Lorcaserin hydrochloride hemihydrate*, a selective serotonin 5-hydroxytryptamine (5-HT) 5-HT2C receptor agonist, is approved by the U.S. Food and Drug Administration for chronic weight management. It is a secondary amine-containing benzazepine [(R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine] (Fig. 1). Lorcaserin is primarily cleared by metabolism. Multiple metabolic pathways such as oxidation, glucuronide conjugation, and sulfo-conjugation have been identified both in vitro and in vivo. Multiple cytochrome P450 (P450) enzymes associated with major metabolic pathways of lorcaserin have been reported; however, the sulfotransferases (SULTs) involved in its sulfo-conjugation have not yet been reported. Lorcaserin N-sulfamate is the major circulating metabolite of lorcaserin.

Sulfo-conjugation is an important metabolic reaction in the detoxification, biosynthesis, and homeostasis of essential endogenous compounds (Coughtrie, 2002; Strott, 2002). SULTs, a supergene family of cytosolic enzymes, catalyze this reaction by transferring a sulfuryl group from a cosubstrate 3’-phosphoadenosine-5’-phosphosulfate (PAPS) to a nucleophilic acceptor molecule (substrate), producing a sulfate conjugate and desulfated 3’-phosphadenosine 5’-phosphosulfate (Schwartz, 2005). In general, sulfate conjugation leads to an increase in hydrophilicity, and thus facilitates the excretion of the conjugated molecule. Therefore, sulfate conjugation is often considered to be an inactivation and detoxification process for xenobiotics and endobiotics (Pacifici and Coughtrie, 2005). Chemically stable sulfo-conjugates, such as N-sulfo-conjugates of aromatic amines, are known to be readily excreted without toxicological consequences (Stillwell et al., 1994; Glatt, 2005). However, sulfo-conjugates of certain chemicals, such as benzylic and allylic alcohols and aromatic hydroxyaldehydes, are short-lived and electrophilic (DeBaun et al., 1970; Miller, 1970, 1994; Glatt, 2005). These may react with DNA and other cellular nucleophiles. Such sulfo-conjugations occur primarily through O-sulfation rather than N-sulfation. While sulfo-conjugates are generally biologically inactive, some can retain their biologic activity—such as cholesterol sulfate, which stimulates the differentiation of skin epidermal cells (Elias et al., 1984), and pregnenolone sulfate, which binds and suppresses the GABA receptor (Corpéchot et al., 1981). Lorcaserin N-sulfamate shows no binding affinity to serotonin 5-HT receptors 5-HT2A, 5-HT2B, and 5-HT2C.

Among other metabolic transformations reported earlier (Sadeque et al., 2012; Usmani et al., 2012), lorcaserin undergoes sulfo-conjugation through the secondary amine nitrogen, forming an N-sulfamate (Fig. 1). Therefore, the lorcaserin N-sulfo-conjugate does not fit the characteristics of chemically unstable sulfo-conjugates reported previously (Glatt,
2005), and instead is stable and can be readily excreted. Its P450 inhibition, formation kinetic parameters, and plasma protein binding characteristics may help to further rule out potential drug-drug interactions for lorcaserin.

In this paper, we describe the characterization of lorcaserin N-sulfamate through identification of SULTs that catalyze its formation, inhibition, and induction potential on P450 enzymes and its affinity for plasma proteins. In light of these characteristics, we discuss the potential of lorcaserin N-sulfamate for toxicological consequences and metabolic drug-drug interactions.

### Materials and Methods

#### Chemicals and Enzyme Source

Lorcaserin hydrochloride hemihydrate, (R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-benzof[d]azepine hydrochloride hemihydrate, was synthesized by Cilag AG (Schaffhausen, Switzerland). Lorcaserin N-sulfamate, (R)-8-chloro-1-methyl-4,5-dihydro-1H-benzof[d]azepine-3(2H)-sulfonic acid, and lorcaserin N-sulfamate-d₄ (internal standard) were synthesized at Arena Pharmaceuticals, Inc. PAPS tetrathiolum salt, tris-HCl, and trizma-base were purchased from Sigma (St. Louis, MO). High-purity, high-performance liquid chromatography (HPLC)-grade acetonitrile and high-purity HPLC-grade water were purchased from Burdick and Jackson (Muskegon, MI). Ultrapure distilled water was purchased from Invitrogen Corporation (Carlsbad, CA). Formic acid was purchased from EMD Chemicals Inc. (Gibbstown, NJ). Human liver microsomes (pooled mixed gender, Male and female) for three consecutive days. Cells were harvested and microsomes were then centrifuged at 4000 rpm for 20 minutes and then terminated by the addition of 500 μl acetonitrile and mixed thoroughly. Incubations without the addition of PAPS were conducted as the negative control (blank) under identical conditions. Incubations were performed in triplicate.

#### Assay for P450 enzyme induction potential by lorcaserin N-sulfamate.

The metabolic incubations in human liver microsomes with specific P450 probe substrates and lorcaserin N-sulfamate were conducted using a 96-well plate format. The incubation mixture contained 0.25 mg/ml microsomal protein, 0–200 μM lorcaserin N-sulfamate (test compounds as an inhibitor), P450-specific substrates (at respective Kᵥₐ values), 1 mM β-NADPH, and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4) in a final volume of 100 μl. The following P450-specific substrates were added to the incubation mixture at a concentration close to their respective Kᵥₐ values: phenacetin (36 μM), bupropion (100 μM), paclitaxel (5 μM), tolbutamide (150 μM), (S)-mephenytoin (50 μM), dextromethorphan (5 μM), and midazolam (8 μM) for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Parallel control incubations conducted with P450 substrates without the addition of lorcaserin N-sulfamate (as an inhibitor) were used as the positive control. Parallel incubations were also performed without the addition of β-NADPH in the reaction mixture as a blank control (negative control). At the end of the incubation period, the incubations were terminated with the addition of an equal volume of acetonitrile containing the internal standard. The mixtures were then centrifuged at 4000 rpm for 20 minutes and the supernatant was transferred to a clean 96-well microtiter plate. The samples were subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis.

#### Enzymatic assay sample processing.

After termination of the incubation with an equal volume of acetonitrile, samples were vortexed and centrifuged for 10 minutes at 13,000 rpm in a microcentrifuge. The supernatant was transferred to a clean 96-well microtiter plate and subjected to LC-MS/MS analysis.

#### Assay for P450 enzyme inhibition potential by lorcaserin N-sulfamate.

The metabolic incubations in human liver microsomes with specific P450 probe substrates and lorcaserin N-sulfamate were conducted using a 96-well plate format. The incubation mixture contained 0.25 mg/ml microsomal protein, 0–200 μM lorcaserin N-sulfamate (test compounds as an inhibitor), 1 mM β-NADPH, and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4) in a final volume of 100 μl. The following P450-specific substrates were added to the incubation mixture at a concentration close to their respective Kᵥₐ values: phenacetin (36 μM), bupropion (100 μM), paclitaxel (5 μM), tolbutamide (150 μM), (S)-mephenytoin (50 μM), dextromethorphan (5 μM), and midazolam (8 μM) for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Parallel control incubations conducted with P450 substrates without the addition of lorcaserin N-sulfamate (as an inhibitor) were used as the positive control. Parallel incubations were also performed without the addition of β-NADPH in the reaction mixture as a blank control (negative control). At the end of the incubation period, the incubations were terminated with the addition of an equal volume of acetonitrile containing the internal standard. The mixtures were then centrifuged at 4000 rpm for 20 minutes and the supernatant was transferred to a clean 96-well microtiter plate. The samples were subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis.
After incubation for 24 hours, 200-μl aliquots of the solutions in the donor and receiver chambers for each condition were transferred to 1.2-ml 8-well tissue culture strips. A 200-μl aliquot of the dosing solution was also sampled for analysis. The proteins were precipitated with the addition of three volumes of ice-cold acetonitrile containing internal standard (100 ng/ml lorcaserin sulfamate-d₄) followed by centrifugation at 3000g for 30 minutes. The resulting supernatant (70 μl) was mixed with an equal volume of HPLC-grade water on a 96-well sample plate. The samples were then analyzed by LC-MS/MS. The analyte concentration was determined by comparing with a standard curve of lorcaserin-N-sulfamate generated under identical conditions of protein binding sample preparation.

Samples analysis by LC-MS/MS. Analysis of lorcaserin N-sulfamate was carried out with an Aria LX-2 HPLC system coupled with an MDS Sciex API-4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). A HALO C18, 30 mm and 2.7 μm (Mac-Mod Analytical Inc., Chadds Ford, PA) column was used for separating lorcaserin-N-sulfamate and the internal standard at room temperature. The mobile phase was solvent B (0.1% formic acid in acetonitrile) prepared with authentic lorcaserin (0.001–10 μM) concentration range). The samples for the determination of inhibitory effect of lorcaserin-N-sulfamate was catalyzed by cytosol from human liver, (coma) kidney and by human recombinant SULTs were analyzed according to the method described previously (Usmani et al., 2012).

Enzyme kinetic analysis. The data obtained were analyzed for enzyme kinetic parameters Km and Vmax, using Sigma Plot software (Systat Software Inc., Richmond, CA), which generated a nonlinear least-square fit to the Michaelis-Menten equation. The apparent Km value (Michaelis-Menten constant for substrate affinity) was calculated as the substrate concentration at half of the maximum velocity of the reaction. The intrinsic clearance (CLint) was calculated as CLint = Vmax/Km (Segel, 1976).

**Results**

Lorcaserin N-Sulfamate Formation Catalyzed by Liver and Renal Cytosol

Formation of lorcaserin N-sulfamate is catalyzed by human cytosolic SULTs in the presence of PAPS (Fig. 1). In this study, the formation rate of lorcaserin N-sulfamate in vitro in liver and renal cytosol of humans was examined. Incubations were conducted with liver and renal cytosol fractions in the presence of PAPS. The rate constants Km and Vmax for lorcaserin N-sulfamate formation for liver and renal cytosol were calculated from a nonlinear least-square fit to the Michaelis-Menten equation (Figs. 2 and 3). The kinetic parameters Km and Vmax and the intrinsic clearance (CLint = Vmax/Km) are presented in Table 1. The formation of lorcaserin N-sulfamate in human liver cytosol was inhibited by approximately 80% in the presence of 1 μM of 2,6-dichloro-4-nitrophenol (data not shown), a known inhibitor generally used for the inhibition of SULT activity (Seah and Wong, 1994).

Lorcaserin N-Sulfamate Formation Catalyzed by Recombinant SULTs

Initial metabolic screening was performed with five recombinant human SULTs, SULT1A1, SULT1A2, SULT1A3, SULT1E1, and SULT2A1 (Fig. 4). Of these, SULT1A3 did not catalyze lorcaserin N-sulfamate formation and was therefore excluded from further kinetic analyses. The remaining SULTs showed differential activity, with SULT1A1 exhibiting the highest activity and SULT1E1 the lowest activity. SULT1A2 and SULT2A1 showed modest activity. The kinetic parameters are reported in Table 1 and a representative Michaelis-Menten kinetic plot with SULT1A1 is shown in Fig. 5. The order of catalytic efficiency of human SULTs for lorcaserin N-sulfamate formation is as follows: SULT1A1 (CLint = 255 μl/min/mg) > SULT2A1 (CLint = 19.31 μl/min/mg) > SULT1A2 (CLint = 2.86 μl/min/mg) > SULT1E1 (CLint = 1.85 μl/min/mg).

Human Liver P450 Enzyme Inhibition and Induction.

To evaluate the inhibitory effect of lorcaserin-N-sulfamate on major P450 enzymes, the IC50 values for the inhibition of the activity of seven major P450 enzymes was evaluated. The IC50 value for the inhibition of CYP1A2-mediated phenacetin O-deethylase, CYP2B6-mediated bupropion hydroxylase, CYP2C8-mediated paclitaxel 6α-hydroxylase, CYP2C19-mediated (S)-mephenytoin 4’-hydroxylase, CYP2D6-mediated dextromethorphan O-demethylase, and CYP3A4-mediated midazolam 1’-hydroxylase was >100 μM (Fig. 6). However, the IC50 value for CYP2C9-mediated tolbutamide 4’-hydroxylase was 10.3 μM (2840 ng/ml) (Table 2). As shown in Table 2, in the presence of increasing concentrations of human serum albumin in microsomal incubations, the IC50 value for CYP2C9 inhibition was increased such that at a physiologically relevant albumin concentration of 500 μM, the IC50 value observed for CYP2C9 inhibition was >200 μM (Fig. 7). There was no induction observed for major P450 enzymes when

**TABLE 1**

<table>
<thead>
<tr>
<th>SULT Enzyme Source</th>
<th>Km [μM]</th>
<th>Vmax [nmol/mg protein/min]</th>
<th>CLint [ml/min/mg protein]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human liver cytosol</td>
<td>1700</td>
<td>0.0520</td>
<td>0.130</td>
</tr>
<tr>
<td>Human renal cytosol</td>
<td>1470</td>
<td>0.0125</td>
<td>0.00850</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>742.07</td>
<td>189.26</td>
<td>255</td>
</tr>
<tr>
<td>SULT1A2</td>
<td>5419.56</td>
<td>15.52</td>
<td>2.86</td>
</tr>
<tr>
<td>SULT1E1</td>
<td>368.30</td>
<td>0.68</td>
<td>1.85</td>
</tr>
<tr>
<td>SULT2A1</td>
<td>3210.06</td>
<td>61.98</td>
<td>19.31</td>
</tr>
</tbody>
</table>
cultured human hepatocytes were treated with lorcaserin N-sulfamate (up to 20 µM) for 72 hours, suggesting that lorcaserin N-sulfamate is not a human P450 enzyme inducer (data not shown).

**Plasma Protein Binding**

Three concentrations, 0.1, 1.0, and 10 µM, of lorcaserin N-sulfamate were used for its plasma protein binding affinity determination. There was <1% of lorcaserin N-sulfamate unbound (<1%fu) to human plasma proteins (>99% bound) at 10 µM, whereas with low concentrations (0.1 and 1 µM) the unbound fraction was below the limit of quantification. The data are presented in Table 3.

**Discussion**

Sulfo-conjugates of xenobiotics and endobiotics are generally biologically inactive and hydrophilic; therefore, this represents a biologically important excretion pathway (Stillwell et al., 1994; Coughtrie, 2002; Strott, 2002; Glatt, 2005). However, the structural features of certain sulfo-conjugated molecules may elicit various biological effects. For example, the O-sulfo-conjugates of aromatic hydroxylamines, benzylic and allylic alcohols are known to be short-lived and electrophilic, and may therefore bind to cellular nucleophiles, including DNA (DeBaun et al., 1970; Glatt, 2005). In addition, some sulfo-conjugates show biological activity, such as cholesterol sulfate, which is known to stimulate differentiation of skin epidermal cells (Elias et al., 1984), and pregnenolone sulfate, which binds and suppresses the GABA receptor (Corpéchot et al., 1981). However, sulfo-conjugation via N-sulfation, such as N-sulfonation of aromatic amines, yields stable sulfo-conjugates that are readily excreted without toxicological consequences (Stillwell et al., 1994; Glatt, 2005). In this paper, we describe the formation and systematic characterization of lorcaserin N-sulfamate, formed by human liver or renal cytosol and recombinant SULTs. The lorcaserin sulfo-conjugate is not the product of O-sulfonation, but rather N-sulfonation, and is known to be chemically stable (Stillwell et al., 1994; Glatt, 2005). Thus, toxicological consequences of lorcaserin N-sulfamate are considered to be unlikely.

Prior to the characterization of lorcaserin N-sulfamate formation kinetics, the SULT activity of the cytosolic preparations was verified using para-nitrophenol, a known substrate for SULTs. Typical biphasic kinetics with initial product formation (para-nitrophenol-sulfate) at low para-nitrophenol concentration followed by inhibition of its own product formation at higher concentration was observed (data not shown) as reported previously (Gamage et al., 2003). The kinetic parameters for lorcaserin N-sulfamate formation showed that the $V_{\text{max}}$ and $Cl_{\text{int}}$ values for liver cytosol are about 17- and 15-fold higher, respectively, than that for renal cytosol, suggesting that lorcaserin N-sulfamate is predominantly formed by liver SULTs. An initial screening with recombinant SULTs indicated that SULT1A1, SULT1A2, SULT1E1, and SULT2A1 were involved in lorcaserin N-sulfamate formation, whereas SULT1A3 showed minimal or no activity (Fig. 4). The $Cl_{\text{int}}$ values of SULT1A1
are about 89-, 13-, and 137-fold higher compared with SULT1A2, SULT2A1, and SULT1E1, respectively, suggesting that SULT1A1 is the predominant SULT that catalyzes lorcaserin N-sulfamate formation in humans (Table 1). SULT1A1, SULT2A1, and SULT1E1 account for 53%, 27%, and 6%, respectively, of total SULTs in human liver, whereas in the human kidney these values are 40%, 1%, and 0%, respectively (Riches et al., 2009). Therefore, the SULTs that are involved in lorcaserin N-sulfamate catalysis constituted approximately 86% of the total SULTs expressed in human liver compared with about 41% in kidney, suggesting that the liver is the primary metabolic site for lorcaserin N-sulfamate formation. It is noteworthy that three members of the SULT family (SULT1A1, SULT1A3, and SULT1E1) show very different kinetic behavior for lorcaserin N-sulfamate formation. This may reflect the differential nature of the substrate binding pocket of these isoforms (Gamage et al., 2003). The SULT1A1 binding pocket accepts a wide range of molecules, such as simple planar-substituted phenols (Brix et al., 1999), as well as very large lipophilic substrates (Harris et al., 2000; Li et al., 2001) and polycyclic aromatic compounds (Grimm et al., 2013). Lorcaserin is a secondary amine-containing benzazepine that is more lipophilic due to the aryl chloride portion of the molecule and thereby may be well suited for binding to the hydrophobic pocket of SULT1A1. Although lorcaserin is a nonplanar molecule, the strongly hydrophobic and flexible nature of the active site of SULT1A1 may accommodate lorcaserin as a better substrate compared with other SULTs reported in this study. Lorcaserin has a pK₄ value of 9.53 and the secondary amine in its cyclic aliphatic moiety may become protonated under physiologic reaction pH (7.4). However, lorcaserin appears not to be a substrate for SULT1A3, which may be due to the lack of an aliphatic amine containing an open side chain with at least two carbons, a characteristic of preferred substrates (e.g., dopamine or tyramine) (Brix et al., 1999). SULT1E1 shows high selectivity for endogenous estrogens such as 17β-estradiol as well as for a number of other important drugs including 17α-ethinylestradiol (Forbes-Bamforth and Coughtrie 1994; Zhang et al., 1998). Poor catalytic efficiency of SULT1E1 for lorcaserin N-sulfonation is consistent with previous literature reports wherein fused heterocyclic compounds with secondary amines underwent very negligible sulfonation by SULT1E1; however, such compounds are efficiently catalyzed by SULT1A1 (Cole et al., 2010), which is consistent with the finding that lorcaserin is a better substrate for SULT1A1 than SULT1E1. Despite SULT1A2 being closely related to SULT1A1 in amino acid sequence homology, their substrate affinities are very different. Unlike SULT1A1, simple phenolic compounds are poor substrates for SULT1A2. In the case of SULT1A2, the phenolic ends of two tyrosine moieties, Tyr149 and Tyr240, extend into the substrate binding pocket, which makes it more hydrophilic (thus, compounds such as aromatic hydroxyacids are preferred), possibly through interaction of its hydrophilic aliphatic region (Meinl et al., 2002; Lu et al., 2010). The hydrophobic chloro-benzene moiety of lorcaserin likely makes it a poor substrate for SULT1A2. Similar to SULT1E1, SULT2A1 also showed low affinity for lorcaserin N-sulfamate formation. The active site of SULT2A1 appears to accept more hydrophobic substrates such as steroids, steroid derivates, and bile acids (Comer et al., 1993; Huang et al., 2010). Additionally, specific binding orientation in SULT2A1 is required for specific substrates such as dehydroepiandrosterone and androstosterone (Lu et al., 2008). This may be the reason why lorcaserin is not a high-affinity substrate for SULT2A1.

Comparing all of the SULTs used for lorcaserin N-sulfamate formation, SULT1A1 appears to be the most catalytically efficient. Based on the CL_{int} values, the order of catalytic efficiency is SULT1A1 > SULT2A1 > SULT1A2 > SULT1E1. As indicated by the kinetic parameters, lorcaserin does not appear to be a prototypical high-affinity substrate for human SULT enzymes. Thus, drug-drug interactions arising through the SULT-mediated metabolic pathway are not anticipated. Additionally, although lorcaserin N-sulfamate is the major metabolite in circulation, it accounts for only approximately 3% of the total lorcaserin dose in human urine, whereas the major clearance pathways of lorcaserin are oxidation by multiple P450 enzymes (Usmani et al., 2012) and glucuronidation by multiple UDP-glucuronosyltransferase enzymes (Sadeque et al., 2012). The observed pharmacokinetic half-life of lorcaserin N-sulfamate after lorcaserin dosing in humans is much longer than the parent (≈ 40 versus ≈ 11 hours). One likely explanation for the high levels of lorcaserin N-sulfamate in circulation and the prolonged observed half-life is that the very high plasma protein binding affinity (>99%) renders only a small free fraction of the metabolite in circulation available for tissue distribution and elimination. We also observed that lorcaserin N-sulfamate inhibits human liver microsomal tolbutamide hydroxylation with an IC₅₀ value of ≈ 10 μM, a reaction catalyzed by CYP2C9 and catalyzed by human liver microsomal protein.

**TABLE 2**

<table>
<thead>
<tr>
<th>Human Serum Albumin</th>
<th>IC₅₀ (μM)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>10.3 (9.27)</td>
</tr>
<tr>
<td>10</td>
<td>14.6</td>
</tr>
<tr>
<td>100</td>
<td>85.8</td>
</tr>
<tr>
<td>500</td>
<td>&gt; 200</td>
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</table>

This value was calculated based on the free fraction of lorcaserin N-sulfamate determined in human liver microsomal protein.

**TABLE 3**

<table>
<thead>
<tr>
<th>Lorcaserin N-Sulfamate Concentration</th>
<th>Percentage of Plasma Protein Bound (Mean ± S.D., n = 3)</th>
</tr>
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<tbody>
<tr>
<td>1.0</td>
<td>Male: 100% ± 0.00; Female: 100% ± 0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>Male: BLOQ; Female: BLOQ</td>
</tr>
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BLOQ, below the limit of quantification.

The concentration of lorcaserin N-sulfamate in the receiver chamber of the equilibrium dialysis device was below the limit of quantification.
CYP2C9. However, the IC_{50} value becomes >200 μM in the presence of physiologic concentration of human serum albumin (Fig. 7). Additionally, lorcaserin N-sulfamate has no or minimal inhibitory effect on other major P450 enzymes (Fig. 6) and also is not a time-dependent inhibitor of CYP2C9 (data not shown). On the other hand, although the parent drug, lorcaserin, inhibits CYP2D6 activity (approximately 2-fold increase in the area under the curve of dextromethorphan, see the prescribing information for reference, http://www.belviq.com/documents/Belviq_Prescribing_Information.pdf), it is also not a time-dependent inhibitor of CYP2D6. In a separate experiment we found that only 10% of lorcaserin N-sulfamate is bound to microsomal protein, in contrast to >99% bound to human plasma protein. Thus, in an in vivo environment, the availability of free lorcaserin N-sulfamate in circulation would be very negligible at a clinical dose of lorcaserin and is highly unlikely to cause metabolic inhibition of the CYP2C9 pathway. Furthermore, at concentrations up to 20 μM, lorcaserin N-sulfamate did not induce any P450 enzymes in cultured human hepatocytes treated for 72 hours. In comparison, the plasma C_{max} of lorcaserin N-sulfamate exceeds that of lorcaserin [mean C_{max} value = 0.29 μM (56.8 ng/ml), following 10 mg twice daily in humans] by 1- to 5-fold. Taken together, these results suggest that lorcaserin N-sulfamate has a very low potential of affecting P450 metabolic pathways by P450 enzyme inhibition or induction.

In conclusion, lorcaserin N-sulfamate has a very low probability of causing metabolic drug-drug interactions in a normal patient population.

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Authorship Contributions
Participated in research design: Sadeque, Usmani, Palamar, C. Chen, Cerny, W. G. Chen.
Performed data analysis: Palamar, Usmani, C. Chen.
Wrote or contributed to the writing of the manuscript: Sadeque.

References

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