Identification of Human UDP-Glucuronosyltransferases Involved in N-Carbamoyl Glucuronidation of Lorcaserin

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ABSTRACT:
Lorcaserin, a selective serotonin 5-HT$_{2C}$ receptor agonist, is a weight management agent in clinical development. Lorcaserin N-carbamoyl glucuronidation governs the predominant excretory pathway of lorcaserin in humans. Human UDP-glucuronosyltransferases (UGTs) responsible for lorcaserin N-carbamoyl glucuronidation are identified herein. Lorcaserin N-carbamoyl glucurononide formation was characterized by the following approaches: metabolic screening using human tissues (liver, kidney, intestine, and lung) and recombinant enzymes, kinetic analyses, and inhibition studies. Whereas microsomes from all human tissues studied herein were found to be catalytically active for lorcaserin N-carbamoyl glucuronidation, liver microsomes were the most efficient. With recombinant UGT enzymes, lorcaserin N-carbamoyl glucuronidation was predominantly catalyzed by three UGT2Bs (UGT2B7, UGT2B15, and UGT2B17), whereas two UGT1As (UGT1A6 and UGT1A9) played a minor role. UGT2B15 was most efficient, with an apparent $K_{\text{m}}$ value of 51.6 ± 1.9 $\mu$M and $V_{\text{max}}$ value of 237.4 ± 2.8 pmol/mg protein/min. The rank order of catalytic efficiency of human UGT enzymes for lorcaserin N-carbamoyl glucuronidation was UGT2B15 > UGT2B7 > UGT2B17 > UGT1A9 > UGT1A6. Inhibition of lorcaserin N-carbamoyl glucuronidation activities of UGT2B7, UGT2B15, and UGT2B17 in human liver microsomes by mefenamic acid, bisphenol A, and eugenol further substantiated the involvement of these UGT2B isoforms. In conclusion, multiple human UGT enzymes catalyze N-carbamoyl glucuronidation of lorcaserin; therefore, it is unlikely that inhibition of any one of these UGT activities will lead to significant inhibition of the lorcaserin N-carbamoyl glucuronidation pathway. Thus, the potential for drug-drug interaction by concomitant administration of a drug(s) that is metabolized by any of these UGTs is remote.

Introduction

UDP-glucuronosyltransferases (UGTs) compose a superfamily of endoplasmic reticulum membrane-bound enzymes (Mackenzie et al., 1997; Meech and Mackenzie, 2010). These enzymes are expressed in liver and in extrahepatic tissues such as kidney, intestine, skin, breast, uterus, and prostate (Mackenzie et al., 1997; Tukey and Strassburg, 2000, 2001). UGTs glucuronidate many endobiotics and xenobiotics by transferring glucuronic acid from its cofactor uridine 5'-diphosphoglucuronic acid (UDPGA) to lipophilic substrates, thereby transforming them into hydrophilic glucuronides and facilitating their subsequent elimination via the biliary or renal routes. Consequently, glucuronidation is recognized as one of the major metabolic pathways for many drugs (Tukey and Strassburg, 2000; Bock, 2002). The majority of glucuronidation reactions in humans result in O- and N-glucuronides through conjugation of alcohols, carboxylic acids, and amines (Miners and Mackenzie, 1991; Tukey and Strassburg, 2000; Bock, 2002). Although rare, S-glucuronidation (Ethell et al., 2003) and C-glucuronidation (Kerdpin et al., 2006) have also been reported in humans. Another class of glucuronidation reactions, the N-carbamoyl glucuronidation of primary and secondary amines, is being reported more frequently in the literature (Straub et al., 1988; Tremaine et al., 1989; Schaefer, 1992, 2006). In recent years, drugs such as sertraline (Obach et al., 2005), varenicline (Obach et al., 2006), and a novel dipeptidyl peptidase-4 inhibitor (Gunduz et al., 2010) were shown to form N-carbamoyl glucuronide metabolites by UGT enzymes. Herein, N-carbamoyl glucuronidation of lorcaserin by human UGTs is reported.

Lorcaserin is a novel and selective serotonin 5-HT$_{2C}$ receptor agonist in clinical development that has been shown to be an effective agent for weight management in phase III studies (Smith et al., 2010; Fidler et al., 2011). Lorcaserin, (R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (Fig. 1A), is a secondary amine containing benzazepine. As with other benzazepines (Straub et al., 1988; Shaffer et al., 2009), we also observed that lorcaserin undergoes N-carbamoyl glucuronidation in vitro as well as in vivo. In humans, the N-carbamoyl glucuronidation was found to be the major clearance pathway of lorcaserin after oral administration (Chen et al., 2008; Morgan et al., ...
were prepared in incubation buffer (sodium bicarbonate buffer, pH 7.4). As prepared microsomes.

The following human recombinant UGTs expressed in insect cells were high-purity grade. Microsomes from human liver, kidney, intestine, and lung were used to identify the specific UGT isoforms involved in the lorcaserin conjugation. Enzyme kinetic studies were performed with human liver microsomes (liver, kidney, intestine, and lung) and in recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17). Incubation mixtures contained human tissue microsomes (0.25 mg microsomal protein/ml) or recombinant UGT (0.125 mg protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 μg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. The incubation mixtures were kept on ice for 15 min, followed by the addition of lorcaserin (100 μM). All incubations were prewarmed at 37°C for 5 min and initiated by the addition of UDPGA (3 mM), bringing the total reaction volume to 500 μl. After 20 min of incubation at 37°C, the reaction was terminated by adding 500 μl of ice-cold acetonitrile containing the internal standard, lorcaserin sulfamate-6 (25 ng/ml).

Assays with Microsomes from Liver, Kidney, Intestine, and Recombinant UGTs. Incubations for initial screening were carried out to determine the extent of lorcaserin N-carbamoyl glucuronide formation in various human tissue microsomes (liver, kidney, intestine, and lung) and in recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17). Incubation mixtures were prepared by combining human liver microsomes (0.25 mg microsomal protein/ml) or recombinant UGT (0.125 mg protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 μg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. The incubation mixtures were kept on ice for 15 min, followed by the addition of lorcaserin (100 μM). All incubations were prewarmed at 37°C for 5 min and initiated by the addition of UDPGA (3 mM), bringing the total reaction volume to 500 μl. After 20 min incubation at 37°C, the reactions were quenched by adding 500 μl of ice-cold acetonitrile containing the internal standard, lorcaserin sulfamate-6 (25 ng/ml).

Effect of Chemical Inhibitors on Human Liver UGT Activity. High-affinity UGT substrates such as mefenamic acid (Gaganis et al., 2007; Mano et al., 2007a,b), bisphenol A (Hanioka et al., 2008), and eugenol (Turgeon et al., 2003) were used as inhibitors to block UGT2B7-, UGT2B15-, and UGT2B17-catalyzed lorcaserin N-carbamoyl glucuronidation activity in HLMs, respectively. Two concentrations of lorcaserin, 1 μM (clinically relevant) and 100 μM (in the proximity of the K_m value of 128 μM in HLMs), were used during inhibition studies. Two concentrations of mefenamic acid (5 and 25 μM), bisphenol A (5 and 25 μM), and eugenol (20 and 100 μM) were used to inhibit UGT2B7-, UGT2B15-, and UGT2B17-mediated lorcaserin N-carbamoyl glucuronidation in HLMs, respectively. The concentrations of mefenamic acid, bisphenol A, and eugenol, selected to inhibit the reaction, were approximately equal to (low) or five times greater than (high) their respective K_m values, so that the maximum inhibitory effect of these compounds might be observed while maintaining UGT selectivity. In addition, two mixtures of mefenamic acid, bisphenol A, and eugenol together, one at 30 μM (mixture of three low concentrations of three inhibitors) and another at 150 μM (mixture of three high concentrations of three inhibitors), were used to inhibit lorcaserin N-carbamoyl glucuronidation in HLMs.

Typical incubation mixtures for UGT inhibition assays contained HLM (0.25 mg microsomal protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 μg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. Samples were kept on ice for 15 min, followed by the addition of lorcaserin (1 or 100 μM) and UGT inhibitors (mefenamic acid, bisphenol A, or eugenol, as mentioned above). For the control activity, no inhibitors but appropriate volume of solvents were included in the incubation mixtures. All incubations were prewarmed at 37°C for 5 min, and reactions were then...
initiated by adding UDPGA (3.0 mM), bringing the total reaction volume to 500 μl. After 20 min of incubation at 37°C, the reactions were quenched with the addition of 500 μl of ice-cold acetonitrile containing the internal standard, lorcaserin sulfamate-d₆ (25.0 ng/ml).

**Table 1**

<table>
<thead>
<tr>
<th>UGT Enzymes Source</th>
<th>Kinetic Parameters</th>
<th>Human liver microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kₘ ± S.E.</td>
<td>Vₘₐₓ ± S.E.</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>518.0 ± 63.8</td>
<td>103.3 ± 5.1</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>51.6 ± 1.9</td>
<td>237.4 ± 2.8</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>254.1 ± 13.3</td>
<td>155.3 ± 2.8</td>
</tr>
</tbody>
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**Fig. 2.** Lorcaserin N-carbamoyl glucuronidation activity of various recombinant human UGT enzymes. The enzymatic assay contained 0.125 mg/ml recombinant protein and 100 μM lorcaserin, UDPGA, saccharolactone, and alamethicin in sodium bicarbonate buffer, pH 7.4. Detailed incubations were described under Materials and Methods.
HUMAN UGT INVOLVED IN LORCASERIN N-CARBAMOYL GLUCURONIDATION

FIG. 3. Michaelis-Menten kinetic plot for lorcaserin N-carbamoyl glucuronide formation catalyzed by HLMs. The values are average of triplicate (±S.E.) incubations.

was observed for these UGT isoforms (Fig. 4). The $K_m$ values for UGT1A9, UGT2B7, UGT2B15, and UGT2B17 were 518.0 ± 63.8, 93.8 ± 8.6, 51.6 ± 1.9, and 254.1 ± 13.3 µM, respectively, and the $V_{max}$ values were 103.3 ± 5.1, 186.4 ± 4.4, 237.4 ± 2.8, and 155.3 ± 2.8 pmol/mg protein/min, respectively (Table 1). On the basis of the intrinsic clearance ($CL_{int} = V_{max}/K_m$) values in Table 1, the rank order for the formation of lorcaserin N-carbamoyl glucuronidation was UGT2B15 > UGT2B7 > UGT2B1 > UGT1A9.

Inhibition of UGT Activity in HLMs. As shown in Fig. 5A, a low concentration of mefenamic acid (5 µM), bisphenol A (5 µM), or eugenol (20 µM), added either separately or combined (total inhibitor concentration of 30 µM, designated as combined-low) to the HLM incubation containing 1 µM lorcaserin (substrate), inhibited ≤5% of lorcaserin N-carbamoyl glucuronidation. However, increased concentrations of mefenamic acid (25 µM), bisphenol A (25 µM), and eugenol (100 µM) inhibited 23, 37, and 18% of lorcaserin N-carbamoyl glucuronidation, respectively (Fig. 5A). The addition of a mixture containing the increased concentrations of these inhibitors to the incubation (total inhibitor concentration of 150 µM, designated as combined-high) resulted in 66% inhibition of lorcaserin N-carbamoyl glucuronidation in liver microsomes (Fig. 5A), which is similar to the summed effect (23 + 37 + 18% = 78%) exerted by the individual inhibitors.

When the lorcaserin (substrate) concentration was increased to 100 µM, mefenamic acid (25.0 µM), bisphenol A (25.0 µM), and eugenol (100 µM) inhibited 15, 30, and 11% of lorcaserin N-carbamoyl glucuronidation, respectively, under identical conditions (Fig. 5B). The addition of a mixture of these three inhibitors (total inhibitor concentration, 150 µM) caused 53% inhibition of lorcaserin N-carbamoyl glucuronidation in liver microsomal incubation (Fig. 5B), which is close to the combined effect (15 + 30 + 11% = 56%) exerted by individual inhibitors under identical substrate conditions (100 µM lorcaserin). No inhibitory effect was observed with low inhibitor concentrations (5 and 20 µM) incubated with 100 µM lorcaserin concentration in HLMs (Fig. 5B).

Discussion

Although N-carbamoyl glucuronidation is considered to be rare, it represents another important metabolic pathway that has increasingly drawn attention from drug metabolism researchers in recent years. Although a number of publications investigated its possible mechanism (Schaefer, 1992, 2006; Shaffer et al., 2005), few studies report the identification of UGT enzymes that catalyze the N-carbamoyl glucuronidation pathway (Obach et al., 2005, 2006; Gunduz et al., 2010). In this study, we identified the human UGT enzymes involved in N-carbamoyl glucuronidation of lorcaserin.

First, we examined the formation of lorcaserin N-carbamoyl glucuronide in microsomes prepared from various human tissues, which include liver, kidney, intestine, and lung. Among these organs, human liver was, by a substantial margin, the most efficient for lorcaserin N-carbamoyl glucuronidation. We also identified that UGT2B7, UGT2B15, and UGT2B17 are the primary enzymes involved in lorcaserin N-carbamoyl glucuronidation. These UGT enzymes are abundantly expressed in liver compared with other tissues (Turgeon et al., 2003; Ohno and Nakajin, 2009). For example, quantitative estimation showed that UGT2B7 is 2-fold and UGT2B15 is 25-fold higher in liver compared with the small intestine (Ohno and Nakajin, 2009). UGT2B15 has the highest intrinsic clearance, followed by UGT2B7 for lorcaserin N-carbamoyl glucuronidation. Thus, human liver tissues have a high pool of catalytic UGT2B enzymes involved in lorcaserin metabolism. Therefore, the high intrinsic clearance for lorcaserin N-carbamoyl glucuronidation by liver microsomes seems to be associated with higher expression of these enzymes in the liver, which is clearly in accordance with these literature reports (Turgeon et al., 2003; Ohno and Nakajin, 2009).

Among extrahepatic tissues, kidney was most efficient at producing the N-carbamoyl glucuronide, followed by intestine and lung. These findings suggest that both hepatic and extrahepatic tissues are involved in lorcaserin N-carbamoyl glucuronidation in humans. Human liver microsomes also showed a much higher turnover rate than any individual recombinant UGT enzyme examined. This finding is similar to the observation previously reported for oxazepam glucuronidation in HLMs compared with recombinant UGTs, where HLMs produced more oxazepam glucuronide than individual recombinant enzymes (Court et al., 2002). Formation of lorcaserin N-carbamoyl glucuronide in the microsomes of lung was the least efficient compared with microsomes from other organs, consistent with observations for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronidation (Ren et al., 2000). Ulrich et al. (1997) reported that lung microsomes from smokers have approximately 2-fold higher glucuronidation activity compared with nonsmokers, suggesting a functional increase in UGT activity in the lung of smokers. UGT2B7 (Ren et al., 2000) and UGT2B17 (Beaulieu et al., 1996; Ohno and Nakajin, 2009) are constitutively expressed in human lung. Several studies have also suggested the induction of UGT2B7 and UGT2B17 in tobacco smokers’ lung (Ren et al., 2000; Carmella et al., 2002; Gallagher et al., 2007). In line with these literature reports, a likely explanation for the 2-fold increased formation of lorcaserin N-carbamoyl glucuronidation in lung microsomes from smokers is the induction of one or more of these UGTs (UGT2B7 and UGT2B17), which are involved in this metabolic pathway of lorcaserin along with UGT2B15.

Furthermore, lorcaserin is rapidly and highly absorbed after oral administration in humans. It is metabolized mainly in the liver. On the basis of total radioactivity, >90% of lorcaserin dose is excreted in the urine, and ~33% of dose is accounted for N-carbamoyl glucuronidation (Chen et al., 2008; Morgan et al., 2008). It is conceivable that the sum of activity of each UGT isoform expressed in human liver microsomes involved with lorcaserin N-carbamoyl glucuronidation is equal to the UGT activity of human liver microsomes. Therefore, the contribution of UGT1A9, UGT2B7, UGT2B15, and UGT2B17 to the observed activity in human liver microsomes ($V_{max,UGT}/V_{max, HLM}$) may be estimated as 4.3, 7.8, 10.0, and 6.5%, respectively. It is noteworthy that the sum of these estimated fractions (~29%) is close to the fraction accounted for N-carbamoyl glucuronidation in human
Thus, multiple UGTs are involved in this pathway and the contribution of each individual UGT seemed not to be >10%. Second, it was shown that the UGT2B family, namely UGT2B7, UGT2B15, and UGT2B17, predominantly catalyzed lorcaserin N-carbamoyl glucuronidation and that UGT2B15 was the most efficient UGT, followed by UGT2B7 and UGT2B17. It is noteworthy that UGT2B15 and UGT2B17 share high amino acid sequence identity (>92%), tissue distribution, and specificity for substrates, such as steroids (Hum et al., 1999; Tukey and Strassburg, 2000; Turgeon et al., 2001). In contrast, UGT2B7 shares less amino acid sequence identity (>78%) with UGT2B15 and UGT2B17 and has a much broader substrate specificity, as it is involved in the metabolism of drugs from all major classes, including opioids, cancer drugs, and steroids (Tukey and Strassburg, 2000; Innocenti et al., 2001; Turgeon et al., 2003). Despite their differences in sequence similarity, specificity, and catalytic rate, UGTs show a great deal of substrate redundancy (Miners and Mackenzie, 1991; Tukey and Strassburg, 2000). For example, UGT2B7, UGT2B15, and UGT2B17 all catalyze glucuronidation of testosterone, dihydrotestosterone, and androstane-3α,17β-diol (Turgeon et al., 2001). We observed that lorcaserin N-carbamoyl glucuronidation is also catalyzed by these three steroid-metabolizing enzymes, with comparable rates. Among these three UGTs, UGT2B7 was shown to be the only UGT involved in N-carbamoyl glucuronidation of varenicline (Obach et al., 2006) and was the major UGT for sertraline (Obach et al., 2005); both of these drugs are secondary amines. In contrast, UGT2B7 plays a very minor role in N-carbamoyl glucuronidation of a primary amine, dipeptidyl peptidase-4 inhibitor (Gunduz et al., 2010). We observed, for the first time, that UGT2B15 and UGT2B17, in addition to UGT2B7, are involved in N-carbamoyl glucuronidation of a secondary amine, lorcaserin. These examples suggest that carbamic acids of secondary amines may be a preferred or at least a good substrate for UGT2B7-mediated N-carbamoyl glucuronidation.

Third, because of the substrate overlap among UGTs and the lack of specific chemical inhibitors of UGTs, known high-affinity UGT substrates are often used to inhibit UGT activity toward other substrates, assuming the competitive nature of substrate specificity and binding (Williams et al., 2004). A mixture of three UGT substrates, bisphenol A (UGT2B15), mafenamic acid (UGT2B7), and eugenol (UGT2B17), decreased N-carbamoyl glucuronidation activity by 66 and 53% with 1 and 100 µM of lorcaserin (substrate), respectively, suggesting that at either concentration of substrate, lorcaserin N-car-

![Michaelis-Menten kinetic plots for lorcaserin N-carbamoyl glucuronide formation catalyzed by human recombinant UGTs UGT1A9 (A), UGT2B7 (B), UGT2B15 (C), and UGT2B17 (D).](https://example.com/plot.png)

Details of incubations were described under Materials and Methods.
bamberoyl glucuronidation activity was inhibited, presumably through competition for UGT2B7, UGT2B15, and UGT2B17 activity in HLMs. These findings are consistent with the results obtained from recombinant UGT isoforms that UGT2B7, UGT2B15, and UGT2B17 are the major isozymes that catalyze lorcaserin N-carbamoyl glucuronidation. Some UGT substrates exhibit a high K_m value, thus requiring higher concentration for inhibition when used as a substrate (Williams et al., 2004). For example, up to 500 μM of fenamates, including mefenamic acid, were used to inhibit UGT2B7-catalyzed 4-methylumbelliferone glucuronidation in human kidney cortical microsomes. However, in some instances, a lower concentration of mefenamic acid was sufficient, for example, to inhibit gemfibrozil glucuronidation in human liver microsomes (Mano et al., 2007b). In the case of lorcaserin, a combined mixture of three inhibitors (150 μM) was in the proximity of the K_m value of 128 μM for its N-carbamoyl glucuronidation formation in HLMs. When the three inhibitors were tested individually, the sum of inhibitory effect was 56% (100 μM lorcaserin), which is essentially the same as the 53% inhibition of N-carbamoyl glucuronidation when they were applied combined (150 μM). This observation, together with the K_m values of UGT2B7, UGT2B15, and UGT2B17 for lorcaserin N-carbamoyl glucuronidation, suggests either minimal or absence of nonspecific inhibitory effects (Mano et al., 2007b). A much higher combined concentration of these inhibitors (1200 μM) abolished the lorcaserin N-carbamoyl glucuronidation activity in HLMs (data not shown). However, such a high inhibitor concentration has no clinical relevance and might have an increased nonspecific effect (Williams et al., 2004). It is important to note that the inhibitory effect of individual inhibitors used on lorcaserin N-carbamoyl glucuronidation activity in liver microsomes was in the range of 11 to 37% with either concentration (1 or 100 μM) of lorcaserin, suggesting that a single inhibitor did not sufficiently inhibit lorcaserin N-carbamoyl glucuronidation. This may be due to the wide range of K_m values (Table 1) of the UGTs involved in lorcaserin N-carbamoyl glucuronidation. Considering the differences in kinetic parameters of multiple UGTs involved in the lorcaserin N-carbamoyl glucuronidation pathway, it is unlikely that one substrate (inhibitor) will inhibit all of these UGT enzymes altogether. In a separate study, we also demonstrated that multiple cytochrome P450s and flavin-containing monooxygenase (phase I)-mediated pathways are involved in lorcaserin clearance [see the article by Usmani et al. (2012), this issue]. Thus, inhibition of one pathway may not greatly influence the overall clearance of lorcaserin.

In conclusion, this in vitro study demonstrated that multiple human UGT2B isoforms, such as UGT2B7, UGT2B15, and UGT2B17, pre-dominantly catalyze lorcaserin N-carbamoyl glucuronidation. UGT1A isoforms such as UGT1A6 and UGT1A9 exhibit a minor contribution to this pathway. Considering the fraction of lorcaserin cleared as N-carbamoyl glucuronide, the involvement of multiple UGTs, and contribution from other phase I enzymes in lorcaserin metabolism, it is unlikely that inhibition of any of the UGTs will cause a significant impact on lorcaserin clearance in humans. Thus, drug-drug interaction through inhibition of the N-carbamoyl glucuronidation pathway is unlikely for lorcaserin when taken with concomitant medication.

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

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Fig. 5. Inhibition of lorcaserin N-carbamoyl glucuronidation activity in human liver microsomes by mefenamic acid (5 and 25 μM), bisphenol A (5 and 25 μM), eugenol (20 and 100 μM), and mixtures of these inhibitors. Combined-Low, mixture of mefenamic acid (5 μM), bisphenol A (5 μM), and eugenol (20 μM) (total of 30 μM of three inhibitors); Combined-High, mixture of mefenamic acid (25.0 μM), bisphenol A (25.0 μM), and eugenol (100 μM) (total of 150 μM of three inhibitors). Two concentrations of lorcaserin (substrate), 1 and 100 μM, were used in this experiment.