Identification of Human Cytochrome P450 and Flavin-Containing Monooxygenase Enzymes Involved in the Metabolism of Lorcaserin, a Novel Selective Human 5-Hydroxytryptamine 2C Agonist

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Human P450 and FMO involved in Lorcaserin Metabolism

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Nonstandard Abbreviations:

HLM, human liver microsomes
HRM, human renal microsomes
P450, cytochrome P450
FMO, flavin-containing monooxygenase
LC/MS/MS, liquid chromatography/tandem mass spectrometry
Lorcaserin, a selective serotonin 5-hydroxytryptamine 2C (5-HT2C) receptor agonist, is being developed for weight management. The oxidative metabolism of lorcaserin, mediated by recombinant human cytochrome P450 (P450) and flavin-containing monooxygenase (FMO) enzymes, was examined in vitro to identify the enzymes involved in the generation of its primary oxidative metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin. Human CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP3A4, and FMO1 are major enzymes involved in N-hydroxylorcaserin; CYP2D6 and CYP3A4 in 7-hydroxylorcaserin; CYP1A1, CYP1A2, CYP2D6, and CYP3A4 in 5-hydroxylorcaserin; and CYP3A4 in 1-hydroxylorcaserin formation. In sixteen individual human liver microsomal preparations (HLM), formation of N-hydroxylorcaserin was correlated with CYP2B6, 7-hydroxylorcaserin with CYP2D6, 5-hydroxylorcaserin with CYP1A2 and CYP3A4, and 1-hydroxylorcaserin with CYP3A4 activity at 10.0 µM of lorcaserin. No correlation was observed for N-hydroxylorcaserin with any P450 marker substrate activity at 1.0 µM of lorcaserin. N-Hydroxylorcaserin formation was not inhibited by CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP3A4 inhibitors at the highest concentration tested. Furafylline, quinidine, and ketoconazole, selective inhibitors of CYP1A2, CYP2D6, and CYP3A4, respectively, inhibited 5-hydroxylorcaserin (IC50 = 1.914 µM), 7-hydroxylorcaserin (IC50 = 0.213 µM), and 1-hydroxylorcaserin formation (IC50 = 0.281 µM), respectively. N-Hydroxylorcaserin showed low and high Km components in HLM and 7-hydroxylorcaserin showed lower Km than 5-hydroxylorcaserin and 1-hydroxylorcaserin in HLM. The highest intrinsic clearance was observed for N-hydroxylorcaserin, followed by 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin in HLM. Multiple human P450 and FMO enzymes catalyze formation of four
primary oxidative metabolites of lorcaserin; suggesting lorcaserin has a low probability of drug-drug interactions by concomitant medications.
Introduction

The cytochrome P450 (P450) monooxygenase system is comprised of a superfamily of heme-containing enzymes, expressed in many mammalian tissues with the highest levels found in liver, and capable of catalyzing the metabolism of a wide range of both endogenous and exogenous substrates (Nelson et al., 1996). The mammalian flavin-containing monooxygenases (FMOs), though not catalytically or structurally as diverse as the P450 superfamily, are important phase I enzymes that are responsible for the conversion of lipophilic xenobiotics to more hydrophilic metabolites. The FMO family is a complementary enzyme system to the P450 family and oxidizes at the nitrogen-, sulfur-, selenium-, and phosphorus-centers of xenobiotic compounds (Ziegler, 1991; Sadeque et al., 1992; Hodgson and Goldstein, 2001; Cashman, 2000; Rawden et al., 2000). In general, the oxidative metabolism of xenobiotics is primarily carried out by P450 and to a lesser extent by FMO.

In the adult human liver, members of the CYP3A, CYP2C, and CYP1A subfamilies are predominant P450 enzymes (Shimada et al., 1994; Guengerich, 1995). Among five mammalian FMOs (FMO1, FMO2, FMO3, FMO4, and FMO5), FMO3 is the most abundantly expressed enzyme in adult human liver (Sadeque et al., 1993; Lawton et al., 1994; Phillips et al., 1995) while FMO1 is predominantly expressed in adult human kidney, with some expression in intestinal tissues (Yeung et al., 2000). FMO1 is also a primary constitutive enzyme found in human fetal livers (Dolphin et al., 1996).

Lorcaserin, (1R)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3-benzazepine, is a selective 5-hydroxytryptamine 2C (5-HT2C) receptor agonist and a potential therapeutic agent for weight management (Smith et al., 2008; Smith et al., 2010). For most commonly used drugs,
biotransformation is the major path of elimination and changes in the activity of P450 enzymes is
the major cause of drug-drug interactions. Therefore, it is relevant to assess the relative
contribution of these oxidative enzymes to the overall clearance of lorcaserin and to identify the
P450 and FMO enzymes responsible for lorcaserin metabolism. Human liver microsomes
catalyze the metabolism of lorcaserin to \(N\)-hydroxylorcaserin, 7-hydroxylorcaserin, 5-
hydroxylorcaserin, and 1-hydroxylorcaserin, its four primary oxidative metabolites (Fig. 1). The
objective of this \textit{in vitro} study was to identify the predominant human P450 and FMO enzymes
involved in the metabolism of lorcaserin using human liver and renal microsomes as well as
human recombinant enzymes. For the screening, inhibition, and correlation experiments two
concentrations of lorcaserin were used. Lorcaserin concentration of 1.0 µM was closer to the
clinically relevant plasma concentration (Smith et al., 2010), and 10.0 µM was 10-fold higher of
the initial 1.0 µM concentration.
Methods

Chemicals. Lorcaserin, (1R)-8-Chloro-2,3,4,5-tetrahydro-1-methyl-1H-3-benzazepine hydrochloride hemihydrate, was provided by Cilag AG (Schaffhausen, Switzerland). N-Hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin were synthesized by SAFC Pharma (Manchester, UK). 7-Hydroxylorcaserin was synthesized at Arena Pharmaceuticals, Inc. (San Diego, CA). Dextrorphan-\(d_3\) was purchased from BD Biosciences (Woburn, MA). Other reagents used in this study, which include ethylenediamine tetra-acetic acid (EDTA), magnesium chloride (\(\text{MgCl}_2\)), potassium phosphate monobasic, potassium phosphate dibasic, dextromethorphan, dimethyl sulfoxide (DMSO), tranylcypromine, thioTEPA, quinidine, ketoconazole, 1-aminobenzotriazole (1-ABT), \(N\)-benzylimidazole, methimazole, and \(\beta\)-NADPH, were purchased from Sigma-Aldrich (St. Louis, MO). Furafylline and \(N\)-benzynirvanol were purchased from BD Biosciences (Woburn, MA). High-purity grade acetonitrile and water were purchased from Burdick and Jackson (Muskegon, MI). All reagents were of high-purity grade.

Enzyme Sources. Mixed gender pooled human liver microsomal preparations (HLM) (pooled from 50 donors) and mixed gender pooled human renal microsomal preparations (HRM) (pooled from 8 donors) were purchased from Xenotech, LLC (Lenexa, KS). Individual donor human liver microsomal preparations, membranes from insect cells (BTI-TN-5BI-4) transfected with baculovirus containing cDNA of human P450 enzymes (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6*1, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11), human P450 reductase, human FMO (FMO1, FMO3, and FMO5), and Sf9 (control membranes) were purchased from BD Biosciences (Woburn, MA). CYP2D6*1 is a wild type, which is denoted as CYP2D6 throughout this document.
**Assay Conditions.** The protein concentrations and incubation times used for the metabolic incubations were in the linear range of metabolite production. The metabolic incubations (0.5 mL final volume) were conducted using 1.2 mL polypropylene cluster tubes in a 96-well plate format. All incubation mixtures (described below) were pre-warmed at 37°C for 5 min after which the reactions were initiated by the addition of β-NADPH (1 mM) with gentle mixing and incubated at 37°C in a water bath for 20 min. All incubations were conducted in triplicate unless otherwise stated. A typical incubation mixture for each of the assays is described in sections below with the final reagent concentrations given in parenthesis. All reactions were terminated by the addition of an equal reaction volume (0.5 mL) of ice-cold acetonitrile containing internal standard dextrorphan-\(d_3\). After 10 min of centrifugation at 3,700 rpm (2,572g), 100 µL of supernatant was transferred to another 96-well plate containing 100 µL of 100 mM potassium phosphate buffer with 3 mM MgCl\(_2\) and 1 mM EDTA (pH 7.4) in order to avoid the high amount of organic content in the samples. Samples for all reference standard curves were processed in a similar way as the samples for metabolic incubations, and analyzed by LC-MS/MS.

**Screening with Recombinant P450 and FMO.** Metabolic activity assays designed to screen human recombinant P450 and FMO enzymes for lorcaserin were performed. Briefly, 50 pmol/mL of P450 (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11) and FMO (FMO1, FMO3, and FMO5) were incubated with 1.0 or 10.0 µM of lorcaserin in 100 mM potassium phosphate buffer containing 3 mM MgCl\(_2\) and 1 mM EDTA (pH 7.4). Parallel control reactions were performed with Sf9 insect cells (cells without P450 expression), under identical conditions.
Incubation with Individual Liver Microsomes. Incubation mixtures contained lorcaserin (1.0 or 10.0 µM), liver microsomal protein (0.25 mg/mL), and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4).

Inhibition of P450 by Chemical Inhibitors in Liver Microsomes. The effect of general and selective chemical inhibitors of P450 enzymes on the metabolism of lorcaserin was investigated using pooled HLM. For general P450 inhibitors, incubation mixtures contained lorcaserin (1.0 or 10.0 µM), 1-ABT (1 mM) or N-benzylimidazole (1 mM), microsomal protein (0.25 mg/mL), and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4). For P450-selective inhibitors, incubation mixtures contained lorcaserin (1.0 or 10.0 µM), individual P450-specific inhibitors, microsomal protein (0.25 mg/mL), and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4). P450-selective inhibitors, furafylline (0.12 - 7.50 µM), tranylcypromine (0.02-1.28 µM), thioTEPA (0.937 - 30 µM), N-benzylvinanol (0.039 - 5.0 µM), quinidine (0.004 - 2.0 µM), and ketoconazole (0.059 - 3.75 µM) were used to investigate the involvement of CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP3A4, respectively. For the control incubations, chemical inhibitors were replaced with appropriate amount of solvent. For CYP1A2, furafylline was pre-incubated for 20 min with human liver microsomal protein in the presence of β-NADPH prior to adding lorcaserin.
Inhibition of FMO and P450 in Renal Microsomes. Additional experiments were designed to evaluate the contribution of FMO and P450 enzymes in human renal microsomes (HRM) for lorcaserin metabolism.

Inhibition of FMO-Mediated Metabolism: To investigate the contribution of FMO enzymes in HRM, three sets of incubations were conducted. Set 1 – Control (without treatment): The incubation mixture consisted of HRM (0.25 mg/mL protein), lorcaserin (1.0 or 10.0 µM), and 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5). Set 2 – Methimazole Treatment: The incubation mixture consisted of HRM (0.25 mg/mL protein), lorcaserin (1.0 or 10.0 µM), general FMO inhibitor methimazole (25 µM), and 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5). Set 3 – Heat Treatment: HRM were pre-heated at 45°C for a total of 3 min in water-bath to inactivate FMO enzymes prior to the incubation. The incubation with pre-heated microsomes was then conducted under identical conditions as described above (Set 1 – Control).

Inhibition of P450-Mediated Metabolism: Similar to FMO, three sets of experiments were conducted to evaluate the contribution of P450 in HRM. The incubations were performed in potassium phosphate buffer at pH 7.4 instead of 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5). Set 1 – Control (without treatment): Incubations were conducted with HRM (0.25 mg/mL protein), lorcaserin (1.0 or 10.0 µM), and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4). Set 2 – 1-ABT Treatment: Incubations were performed under identical conditions as described above (Set 1 – Control) except that 1-ABT (1.0 mM), a general P450 inhibitor, was added to the incubation mixture prior to the addition of lorcaserin. Set 3 – Heat Treatment: Incubations were conducted under the
same conditions as described above (Set 1 – Control) except that the HRM were pre-heated for 3 min at 45°C as described above for FMO.

**Inhibition of FMO in Human Liver Microsomes.** Separate sets of experiments were designed to evaluate the contribution of FMO enzymes in HLM for loracaserin metabolism. *Set 1 – Control (without treatment):* The incubation mixture consisted of HLM (0.25 mg/mL protein), loracaserin (10.0 µM), and 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5).

*Set 2 – Methimazole Treatment:* The incubation mixture consisted of HLM (0.25 mg/mL protein), loracaserin (10.0 µM), general FMO inhibitor methimazole (25 µM), and 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5).

**Enzyme Kinetic Studies.** For the kinetic studies, the incubation mixtures consisted of HLM (0.25 mg/mL protein) or human recombinant P450 (CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) (25 pmol P450/mL) or human recombinant FMO1 (25 pmol FAD/mL); loracaserin (0 - 10 mM) as a substrate; and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA (pH 7.4). After pre-incubation for 5 min in a 37°C water bath, reactions were initiated by the addition of β-NADPH (1 mM) with gentle mixing. The reaction mixtures were then incubated for an additional 20 min for HLM and 10 min for recombinant P450 and FMO enzymes in a 37°C water bath. Samples for all reference standard curves (0.00121 - 2.5 µM) were prepared in a similar way as the samples for metabolic incubations.

The assays for the kinetic studies with HRM (0.25 mg/mL protein) were conducted in similar way as described above for HLM except that 25 mM glycine/100 mM potassium pyrophosphate buffer (pH 8.5) was used.
Sample Analysis by LC-MS/MS. Following sample processing as described above, analyses of lorcaserin metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, were performed by LC-MS/MS. A 10 µL sample was introduced for chromatographic separation using a Mac Mod HALO column (2.7 µm, 3 × 30 mm) (MAC-MOD analytical, Inc. Chadds Ford, PA) at a flow rate of 0.60 mL/min. A binary gradient was applied using water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) as follows: initial 2% mobile phase B for 5 seconds, 2% to 30% mobile phase B over 120 seconds, then 30% to 80% mobile phase B over 30 seconds. Next, the column was washed with 80% mobile phase B for 15 seconds followed by a gradient change from 80% to 2% mobile phase B over 5 seconds. Finally, the column was re-equilibrated with 2% B for 35 seconds. The total analysis time was 3.5 min. Mass spectrometric detection was achieved with an MDS Sciex API-3000 triple quadruple mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with an IonSpray LC/MS interface operated in positive ion mode and using multiple reaction monitoring (MRM). The following Q1/Q3 transitions were monitored: m/z 212.1 to m/z 152.2 for N-hydroxylorcaserin, m/z 212.1 to m/z 160.3 for 7-hydroxylorcaserin, m/z 212.1 to m/z 194.1 for 5-hydroxylorcaserin, m/z 212.1 to m/z 194.2 for 1-hydroxylorcaserin, and m/z 261.2 to m/z 157.2 for dextrorphan-d₃. The MRM transitions for 5-hydroxylorcaserin and 1-hydroxylorcaserin are identical; however, 5-hydroxylorcaserin and 1-hydroxylorcaserin are chromatographically separated. Quantification was performed with regression analysis generated from calibration standards.

Data analysis. Enzyme kinetic parameters $K_m$ and $V_{max}$ were calculated using the Sigma Plot software (Systat Software, Inc., Richmond, CA), which generated a nonlinear least-square
fit to the Michaelis-Menten equation. The intrinsic clearance (CL_int) was calculated as
\[ \text{CL}_{\text{int}} = \frac{V_{\text{max}}}{K_m} \] (Segel, 1976).

The percentage total normalized rates (%TNR) were determined as described by
Rodrigues (1999a). The normalized rate was derived by multiplying the rate of hydroxylation
(nmol/nmol P450/min) of each isoform by the nominal specific content (nmol P450/mg protein)
of the corresponding P450 isoform in native human liver microsomes. The nominal specific
content utilized in these determinations was derived from a pool of liver microsomes \((n = 12)\)
phentyped by BD Biosciences reported by Rodrigues (1999b) for all P450s expect CYP2B6.
Since BD Biosciences data reported by Rodrigues (1999b) indicated that CYP2B6 levels may
have high in this set of population and the high variability of CYP2B6 expression (~39-fold)
reported in the literature (Rodrigues, 1999b; Ogilvie et al., 2008), we used a median average
value of 0.0207 nmol/mg protein derived from a different pool of liver microsomes \((n = 12)\)
phenotyped by BD Biosciences (BD Biosciences catalog 1999-2000). The normalized rate
values obtained were then summed and the %TNRs were determined for each isoform.

Correlation coefficients \((r^2)\) were obtained by plotting the formation rate of each
lorcaserin metabolites versus the probe substrate activity for each P450 enzyme using Microsoft
Excel. Percentage of control activity of the enzyme (remaining enzyme activity after the
inhibition) for each concentration of inhibitor was calculated by dividing the enzyme activity in
the presence of inhibitor by the enzyme activity in the absence of inhibitor, and the resulting
ratio is multiplied by 100. IC\(_{50}\) values were calculated by plotting the percent of control activity
versus log [I], inhibitor concentration, using GraphPad Prism (GraphPad Prism Software 4.3, San
Diego, CA). Statistical analysis was performed using Student’s t-test (GraphPad Prism Software
4.3, San Diego, CA).
Results

Metabolism by Recombinant P450 Enzymes. Recombinant human cytochrome P450 enzymes, CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11, were used to evaluate the involvement of P450s in lorcaserin metabolism. Incubations were conducted with two concentrations of lorcaserin, 1.0 and 10.0 μM, for metabolic screening. Formation of four primary metabolites, N-hydroxylorcaserin, 1-hydroxylorcaserin, 5-hydroxylorcaserin, and 7-hydroxylorcaserin, was observed with different rates of formation catalyzed by various P450s tested. However, the N-hydroxylorcaserin metabolite was predominantly formed. As shown in Fig. 2, incubation of 1.0 and 10.0 μM of lorcaserin with human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 catalyzed the formation of N-hydroxylorcaserin with different extent of rates. Recombinant CYP2B6, CYP2C19, and CYP2D6 were the dominant P450 enzymes that catalyzed N-hydroxylorcaserin formation at both 1.0 and 10.0 μM of lorcaserin. At a 1.0 μM of lorcaserin concentration, the formation rate of N-hydroxylorcaserin was dominated by CYP2D6. In contrast, at a 10.0 μM lorcaserin concentration, N-hydroxylorcaserin was predominantly formed by CYP2B6 and exhibited by far the highest turnover rate catalyzed by any P450s tested. This may be due to the difference in kinetic properties of these enzymes (see kinetic determination below). Similarly, CYP2D6 and CYP3A4 are involved in the metabolism of 7-hydroxylorcaserin; CYP1A1, CYP1A2, CYP2D6, and CYP3A4 are involved in the metabolism of 5-hydroxylorcaserin; and CYP3A4 only was involved in 1-hydroxylorcaserin formation (Fig. 2A and B).
Rate of metabolites formed by various recombinant P450s at 1.0 and 10.0 μM concentrations of lorcanerin was normalized with respect to the average expression of individual P450 isoforms in native human liver microsomes (HLM) (Fig. 2C-2D). The normalized results showed that at 1.0 μM of lorcanerin concentration, N-hydroxylorcanerin was predominantly catalyzed by both CYP2B6 and CYP2D6 with %TNR values of 25.9 and 30.0, respectively, whereas CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4 played ancillary role with %TNR 4.54, 14.8, 8.34, 3.14, and 13.3, respectively (Fig. 2C). At 10.0 μM of lorcanerin concentration, N-hydroxylorcanerin was predominantly catalyzed by CYP2B6 with %TNR value of 43.6, whereas CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 played ancillary roles with %TNR of 5.70, 7.04, 13.7, 7.83, 2.91, and 19.3, respectively (Fig. 2D). For CYP2D6 catalyzed 7-hydroxylorcanerin formation at 1.0 and 10.0 μM of lorcanerin concentrations, the %TNR were 38.8 and 52.3, respectively, whereas for CYP3A4 %TNR were 61.2 and 47.7, respectively (Fig. 2C and 2D). Normalized rate for 5-hydroxylorcanerin showed that at 1.0 μM of lorcanerin concentration, it was predominantly catalyzed by CYP1A2 (%TNR = 63.4) and CYP2D6 (%TNR = 36.6), whereas at 10.0 μM of lorcanerin concentration, CYP3A4 catalyzed 5-hydroxylorcanerin predominantly (%TNR = 80.1) (Fig. 2C and 2D). CYP3A4 was the primary enzyme for 1-hydroxylorcanerin formation with both 1.0 and 10.0 μM of lorcanerin concentrations.

**Human Liver Microsomal Metabolism and Correlation Analysis.** *Metabolism by Individual Liver Microsomes:* Lorcanerin metabolism was evaluated in sixteen individual human liver microsomal preparations using 1.0 and 10.0 μM of lorcanerin. As shown in Fig. 3, incubation with 1.0 μM of lorcanerin resulted in the formation of two metabolites, N-hydroxylorcanerin and 7-hydroxylorcanerin (Fig. 3A), whereas incubation with 10.0 μM of
lorcaserin resulted in the formation of two additional metabolites, 5-hydroxylorcaserin and 1-hydroxylorcaserin (Fig. 3B). There was a considerable variability in the rate of formation of these metabolites among the sixteen individual HLM. The formation rate for N-hydroxylorcaserin was significantly higher than that of the other metabolites in individual liver microsomal incubations with 10.0 µM of lorcaserin (Fig. 3B) while for incubations with 1.0 µM of lorcaserin, the trend varied for N-hydroxylorcaserin and 7-hydroxylorcaserin formation (Fig. 3A). Among sixteen individual HLM, a 3.72- and 3.84-fold difference in N-hydroxylorcaserin formation rate was observed with 1.0 and 10.0 µM of lorcaserin, respectively (Fig. 3A and B). Similarly, the formation rate for 7-hydroxylorcaserin varied 16.9- and 9.47-fold with 1.0 and 10.0 µM concentrations of lorcaserin, respectively, among individual HLM (Fig. 3A and B). The formation rate of 5-hydroxylorcaserin and 1-hydroxylorcaserin varied 10.6- and 1.40-fold, respectively, with 10.0 µM of lorcaserin among individual HLM (Fig. 3B). Further examination of the rate of metabolite formation between individual human male and female liver microsomes did not suggest any statistically significant gender-specific variability (p values for N-hydroxylorcaserin 0.88 and 0.11; 7-hydroxylorcaserin 0.94 and 0.76 at 1.0 and 10.0 µM concentrations of lorcaserin, respectively), indicating that the variability in metabolic rate might be due to the variation in the expression level of metabolizing enzyme(s) among the individual donors (Fig. 4A and B). However, irrespective of the gender, all sixteen individual HLM catalyzed lorcaserin metabolism.

Correlation between Lorcaserin Metabolite Formation and P450-Specific Activity: Results from the sixteen individual human liver microsomal incubations at concentrations of 1.0 and 10.0 µM of lorcaserin were used to perform a correlation analysis for lorcaserin metabolite formation versus specific P450 probe substrate activities. Two metabolites, N-hydroxylorcaserin and 7-
hydroxyloracaserin, were formed at the 1.0 μM of lorcaserin concentration. The formation of 7-hydroxyloracaserin showed a moderate correlation with CYP2D6 activity with $r^2 = 0.696$; however, $N$-hydroxyloracaserin did not show any correlation with any of the P450 enzymes at this concentration (Table 1).

Incubations were conducted with 10.0 μM of lorcaserin to determine the correlation coefficients for $N$-hydroxyloracaserin, 7-hydroxyloracaserin, 5-hydroxyloracaserin, and 1-hydroxyloracaserin formation against the activities of several P450 probe substrates in microsomal preparations. Based on regression analyses, the correlation coefficients ($r^2$) for $N$-hydroxyloracaserin formation with microsomal P450 activities varied widely. Among them, CYP2B6 showed the highest correlation coefficient ($r^2 = 0.674$) for the formation of $N$-hydroxyloracaserin (Table 2 and Fig. 5A). The regression line did not pass through or appear near the origin for any P450 activities, indicating that more than one P450 enzymes are involved in the formation of $N$-hydroxyloracaserin (Fig. 5A). This is in an agreement with the findings that multiple P450 enzymes catalyzed the $N$-hydroxylation pathway (Fig. 2). Regression analysis for 7-hydroxyloracaserin formation versus CYP2D6-mediated bufuralol 1'-hydroxylase activity at 10.0 μM of lorcaserin concentration showed that the regression line passed near the origin with a good correlation coefficient value ($r^2 = 0.819$) (Table 2 and Fig. 5B). This suggests that CYP2D6 was the major contributor of 7-hydroxylation of lorcaserin in HLM. Correlation coefficients were also determined for the formation of 5-hydroxyloracaserin against multiple P450 activities in which CYP1A2-mediated phenacetin $O$-deethylase and CYP3A4-mediated testosterone 6β-hydroxylase activities showed $r^2$ values of 0.531 and 0.481, respectively (Table 2 and Fig. 5C and D). These moderate to weaker $r^2$ values suggested that in addition to CYP1A2 and CYP3A4, other P450 enzyme(s) might be involved in the 5-hydroxylation pathway of...
lorcaserin in HLM. In the case of 1-hydroxylorcaserin, only three out of sixteen individual human liver microsomal preparations that contained the highest activity for testosterone 6β-hydroxylase (CYP3A4) catalyzed the 1-hydroxylation of lorcaserin. Though data with three individual microsomes are very limited for a correlation analysis, however, when tested, an $r^2$ value of 0.680 was observed with testosterone 6β-hydroxylase activity (Table 2).

**Inhibition of P450 by Chemical Inhibitors.** The effect of selective chemical inhibitors for specific cytochrome P450 enzymes on the oxidative metabolism of lorcaserin was examined using pooled HLM. The concentrations of the inhibitors were selected such that the maximum inhibitory effect is observed while maintaining the selectivity towards respective P450 enzymes. As demonstrated above, six hepatic P450 enzymes, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP3A4, exhibited a major contribution in the oxidative metabolism of lorcaserin. Therefore, chemical inhibitors specific to the activity of these P450 enzymes were used in this study. As described above, incubation with 1.0 µM of lorcaserin concentration resulted in the formation of two metabolites, N-hydroxylorcaserin and 7-hydroxylorcaserin, whereas incubation with 10.0 µM of lorcaserin resulted in the formation of two additional metabolites, 5-hydroxylorcaserin and 1-hydroxylorcaserin. Incubations for all P450 inhibition studies were conducted with 1.0 and 10.0 µM of lorcaserin concentrations, however, most of the data shown here is from 10.0 µM of lorcaserin concentration unless otherwise indicated from 1.0 µM.

**Inhibition of P450 Activity by General P450 Inhibitors:** Inhibition studies with two general P450 inhibitors, 1-aminobenzotriazole (1-ABT) and N-benzylimidazole, resulted in significant inhibition (80 - 100%) of the formation of N-hydroxylorcaserin, 7-hydroxylorcaserin,
5-hydroxylorcaserin, and 1-hydroxylorcaserin in HLM, suggesting that these pathways are catalyzed by P450 enzymes in HLM (Fig. 6).

Inhibition of Microsomal CYP1A2: As demonstrated in Fig. 7A, increasing concentrations of furafylline showed an inhibitory effect on 5-hydroxylorcaserin formation with an IC$_{50}$ value of 1.914 µM. This is consistent with Fig. 2 showing that formation of 5-hydroxylorcaserin was catalyzed by multiple recombinant P450s, including CYP1A2. Furafylline showed a moderate effect on 1-hydroxylorcaserin formation without reaching 50% of inhibition; however, its effect on the formation of N-hydroxylorcaserin and 7-hydroxylorcaserin was not significant (Fig. 7A).

Inhibition of Microsomal CYP2A6: The contribution of recombinant CYP2A6 on the metabolism of lorcaserin was minor in that it catalyzed only the formation of N-hydroxylorcaserin with a limited rate of formation (Fig. 2). Addition of varying concentrations of tranylcypromine, a CYP2A6 inhibitor, did not show a substantial inhibitory effect on liver microsomal metabolism of lorcaserin (data not shown).

Inhibition of Microsomal CYP2B6: Recombinant CYP2B6 catalyzed the formation of N-hydroxylorcaserin with a higher rate at 10.0 µM of lorcaserin concentration compared to other recombinant P450 enzymes (Fig. 2). However, CYP2B6 inhibitor thioTEPA showed minimal inhibitory effects on N-hydroxylorcaserin formation at 10.0 µM (Fig. 7B) and 1.0 µM of lorcaserin concentrations (data not shown). This might be due to the fact that the N-hydroxylation pathway is catalyzed by multiple human P450 enzymes (Fig. 2) and that the inhibition of one individual P450 may not affect the overall metabolic pathway of this metabolite in HLM. The inhibitory effect of thioTEPA on the formation of the three other lorcaserin metabolites was also minimal (Fig. 7B).
Inhibition of Microsomal CYP2C19: Recombinant CYP2C19 catalyzed the formation of N-hydroxylorcaserin (Fig. 2). However, N-benzyl nirvanol, a potent CYP2C19 inhibitor, did not inhibit the formation of N-hydroxylorcaserin or other metabolites at relevant concentrations (Fig. 7C).

Inhibition of Microsomal CYP2D6: The inhibitory effect of quinidine, a potent CYP2D6 inhibitor, on the formation of 7-hydroxylorcaserin was prominent, with an IC₅₀ value of 0.213 µM (Fig. 7D) at 10.0 µM of lorcanerin concentration. As expected, using 1.0 µM of lorcanerin concentration, the quinidine exerted much stronger inhibitory effect (~10-fold) on 7-hydroxylorcaserin formation with an IC₅₀ value of 0.024 µM (Fig. 7E) compared to 10.0 µM of lorcanerin concentration (Fig. 7D). These results are in agreement with the finding that recombinant CYP2D6 is the main contributor of 7-hydroxylorcaserin formation (Fig. 2). Nevertheless, quinidine did not inhibit N-hydroxylorcaserin formation at either concentration (1.0 or 10.0 µM) of lorcanerin in HLM (Fig. 7D and E). Formation of 5-hydroxylorcaserin and 1-hydroxylorcaserin were also unaffected by quinidine as well (Fig. 7D).

Inhibition of Microsomal CYP3A4: Ketoconazole, a potent CYP3A4 inhibitor, significantly inhibited the formation of 1-hydroxylorcaserin, with an IC₅₀ value of 0.281 µM (Fig. 7F). Consistent with Fig. 2, only recombinant CYP3A4 catalyzed the formation of 1-hydroxylorcaserin. The inhibitory effect of ketoconazole on the formation of N-hydroxylorcaserin, 7-hydroxylorcaserin, and 5-hydroxylorcaserin was minimal (Fig. 7F).

Metabolism by Human Renal Microsomes and Recombinant FMO Enzymes.
Among the four lorcanerin metabolites, human renal microsomes (HRM) produced only N-hydroxylorcaserin. Human recombinant FMO1, FMO3, and FMO5 were incubated with two
lorcaserin concentrations, 1.0 and 10.0 µM. Only FMO1 contributed to lorcaserin metabolism, through oxidation on the nitrogen-center, resulting in N-hydroxylorcaserin formation (Fig. 8). Neither human renal microsomes nor human recombinant FMO enzymes formed 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin metabolites (data not shown).

**Relative Contribution of FMO and P450 Enzymes in HRM and HLM on Lorcaserin Metabolism.** As depicted in Fig. 9, heating of renal microsomes for 3 min at 45°C prior to the incubation with lorcaserin (glycine buffer, pH 8.4) substantially decreased (> 95%) the formation of N-hydroxylorcaserin. Similarly, addition of methimazole, a competitive inhibitor of FMO enzymes, also significantly inhibited N-hydroxylorcaserin formation (>80%) in HRM (Fig. 9A), suggesting that human FMO (FMO1, which is expressed in human renal tissues) catalyzed the formation of N-hydroxylorcaserin in HRM. When HRM at pH 7.4 in potassium phosphate buffer (P450 assay condition) were treated with 1-ABT, a general inhibitor of P450 enzymes, N-hydroxylorcaserin formation was not inhibited, suggesting that P450 enzymes are not involved in N-hydroxylorcaserin pathway in HRM (Fig. 9B). However, heat treatment of HRM under this condition (pH 7.4) demonstrated >95% inhibition of N-hydroxylorcaserin formation (Fig. 9B). Furthermore, when HLM incubations were treated with methimazole (glycine buffer, pH 8.4, FMO condition), no inhibition of the formation of any of the lorcaserin metabolites was observed, which ruled out a possible contribution of FMO enzymes in these lorcaserin metabolic pathways in HLM (Fig. 10). These findings are in agreement with the demonstration that only recombinant human FMO1 (Fig. 8), and not FMO3 or FMO5, catalyzed the formation of N-hydroxylorcaserin.

**Enzyme Kinetic Studies.** Human Liver Microsomes and Recombinant P450 Enzymes: Table 3 represents the kinetic parameters ($K_m$, $V_{max}$, and $CL_{int}$) for the formation of lorcaserin...
metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, by pooled human liver microsomal preparations. There were low $K_m$ (0.885 µM) and high $K_m$ (590.1 µM) components of the enzyme(s) associated with N-hydroxylorcaserin formation in HLM, suggesting that more than one enzyme is involved in N-hydroxylorcaserin formation. This is supported by the finding that multiple recombinant P450 enzymes were shown to catalyze this metabolic pathway (Fig. 2). Comparatively, 7-hydroxylorcaserin had lower $K_m$ values (0.991 µM) than 5-hydroxylorcaserin (483 µM) and 1-hydroxylorcaserin (392 µM). The highest intrinsic clearance rate \( \text{[CL}_{\text{int}} = (V_{\text{max}}/K_m) \) was observed for N-hydroxylorcaserin (7.828 µL/mg protein/min) followed by 7-hydroxylorcaserin (2.73 µL/mg protein/min), 5-hydroxylorcaserin (0.245 µL/mg protein/min), and 1-hydroxylorcaserin (0.092 µL/mg protein/min) (Table 3).

The kinetic parameters ($K_m$, $V_{\text{max}}$, and CL$_{\text{int}}$) obtained with recombinant P450s are presented in Table 4. Data showed that multiple P450s catalyzed the formation of N-hydroxylorcaserin with $K_m$ values ranging from 0.181 - 785 µM, which suggested that the multiple P450 enzymes involved in this pathway have variable affinity and catalytic efficiency towards N-hydroxylation of lorcaserin. These kinetic results are consistent with the results presented in Fig. 2 that CYP2D6 produced more N-hydroxylorcaserin compared to CYP2B6 at a lorcaserin concentration of 1.0 µM, whereas CYP2B6 catalyzed this pathway more efficiently at a lorcaserin concentration of 10.0 µM. In general, formation of the N-hydroxylorcaserin metabolite was higher at 10.0 µM than 1.0 µM of lorcaserin.

Recombinant CYP2D6 catalyzed the 7-hydroxylation of lorcaserin with a low $K_m$ value of 0.260 µM and a high intrinsic clearance of 423 µL/mg protein/min, whereas this pathway was catalyzed by CYP3A4 with a high $K_m$ of 4170 µM and a low intrinsic clearance of 1.779 µL/mg
protein/min. These data suggest that CYP2D6 has higher affinity for the lorcaserin 7-hydroxylation pathway than CYP3A4 (Table 4). Recombinant CYP1A2, CYP2D6, and CYP3A4 are involved in the 5-hydroxylation of lorcaserin, with $K_m$ values of 534, 0.498, and 191 µM, respectively. These results suggest that multiple P450 enzymes are involved in 7-hydroxylation pathway, with CYP2D6 being the highest affinity enzyme (lowest $K_m$, 0.498 µM) (Table 4). Recombinant CYP3A4 catalyzed the 1-hydroxylation of lorcaserin with a $K_m$ value of 213 µM, and no other recombinant P450 enzymes were found to be involved in this metabolic pathway.

**Human Renal Microsomes (HRM) and Recombinant FMO1:** With HRM, only $N$-hydroxylorcaserin formation was observed, with a high $K_m$ value (768 µM) and a low intrinsic clearance of 3.48 µL/mg protein/min (Table 3). A similarly high $K_m$ (816 µM) was also observed with recombinant FMO1, which is expressed in human renal tissues (Table 4). HRM as well as recombinant FMO1 catalyzed the formation of $N$-hydroxylation of lorcaserin, with a higher $K_m$ than HLM and recombinant P450s (Tables 3 and 4). Formation of 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin was not detected over the concentration range used with HRM, suggesting that HRM catalyze the formation of $N$-hydroxylorcaserin only.
Discussion

In vitro drug metabolism studies, such as reaction phenotyping and P450 inhibition, are valuable tools to predict the potential for drug-drug interactions in vivo as well as polymorphic impact on drug disposition (Bjornsson et al., 2003; Venkatakrishnan et al., 2003). The objective of this study was to identify human cytochrome P450 (P450) and flavin-containing monooxygenase (FMO) enzymes responsible for the primary oxidative metabolism of lorcaserin. The following three basic approaches were used for the enzyme identification: 1) use of a set of human recombinant P450 and FMO enzymes, 2) evaluation of lorcaserin metabolism in sixteen individual human liver microsomes (HLM) in order to determine the correlation coefficient for lorcaserin metabolism versus P450 probe substrate activities, and 3) inhibition of lorcaserin metabolism using P450-specific chemical inhibitors. The study was further substantiated by determining the kinetic parameters, $K_m$, $V_{max}$, and $CL_{int}$, of the four primary oxidative metabolites of lorcaserin, using HLM as well as human recombinant P450 and FMO enzymes. The results of this study demonstrated that multiple P450 enzymes catalyzed the metabolism of lorcaserin into the four primary oxidative metabolites, $N$-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin.

It is generally known that when multiple enzymes are involved in the metabolism of a particular metabolite or a metabolic pathway, the correlation studies with a panel of human liver microsomes and individual enzyme-specific inhibitor experiments provide results that are difficult to elucidate. In this situation, human recombinant enzymes are the primary technique to identify the enzymes involved in the metabolism of a drug. Upon screening with human recombinant enzymes, multiple P450 enzymes, CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, were found to be involved in the formation of $N$-
hydroxylorcaserin. It was observed that recombinant CYP2B6, CYP2C19, and CYP2D6 enzymes played a dominant role in the formation of \( N \)-hydroxylorcaserin at both 1.0 and 10.0 \( \mu \text{M} \) of lorcaserin concentrations (Fig. 2A and 2B). CYP2B6 played the predominant role for the formation of \( N \)-hydroxylorcaserin at high concentration of lorcaserin (10.0 \( \mu \text{M} \)), whereas at low concentration (1.0 \( \mu \text{M} \)), the formation rate by CYP2D6 appeared to be higher than by CYP2B6.

However, when compared the normalized rate with respect to average P450 expression in human liver, the contribution by both CYP2B6 (%TNR 25.9) and CYP2D6 (%TNR 30.0) in the formation of \( N \)-hydroxylorcaserin appeared to be very similar at lower lorcaserin concentration (1.0 \( \mu \text{M} \)). Other P450s such as CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4 were also involved in this pathway with their combined %TNR~40.0, which is greater than the individual %TNR of CYP2B6 and CYP2D6. Further, a close examination of Fig. 3A, shows that out of sixteen individual human livers, a few of them (HH18, HH91, HG93, and HH74) formed primarily \( N \)-hydroxylorcaserin but no or minimal formation of 7-hydroxylorcaserin (lower activity/expression of CYP2D6 was suggested by vendors supplied information and our data). We also observed that 7-hydroxylorcaserin formation at 1.0 \( \mu \text{M} \) of lorcaserin concentration was completely inhibited by quinidine, a CYP2D6-selective inhibitor, whereas formation of \( N \)-hydroxylorcaserin remains unaffected by quinidine under identical conditions (Fig. 7E). These lines of evidence clearly suggested that even though CYP2D6 played an important role in \( N \)-hydroxylorcaserin formation at lower lorcaserin concentration, this pathway can be catalyzed without CYP2D6 participation, due to either inhibition or low expression in HLM. At 10.0 \( \mu \text{M} \) of lorcaserin concentration, \( N \)-hydroxylorcaserin was predominantly catalyzed by CYP2B6 (%TNR 43.6), however other P450s (CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) were also involved in this pathway with their combined
%TNR ~56.0. Regression analysis for N-hydroxylorcaserin showed a poor correlation with any particular P450 enzyme activity. While, no single P450-selective inhibitor alone was effective for the inhibition of N-hydroxylorcaserin formation, two general P450 inhibitors, 1-ABT and N-benzylimidazole (both are known to inhibit multiple P450 isoforms), individually inhibited N-hydroxylorcaserin to a greater extent (~ 75 - 95%) in HLM suggesting the participation of multiple P450s in this pathway. Thus, these data clearly suggested that N-hydroxylorcaserin formation is not influenced by any single P450 either at 1.0 or 10.0 µM of lorcaserin concentrations, rather catalyzed by multiple P450 enzymes in human liver microsomes.

7-Hydroxylation of lorcaserin was catalyzed by both recombinant CYP2D6 and CYP3A4. 7-Hydroxylorcaserin formation showed a good correlation ($r^2 = 0.819$) with microsomal CYP2D6 activity and its formation was strongly inhibited by quinidine ($IC_{50} = 0.213$ and 0.024 µM at 10.0 and 1.0 µM of lorcaserin concentrations, respectively), suggesting that CYP2D6 is the main P450 enzyme for 7-hydroxylorcaserin formation. The rate for 7-hydroxylorcaserin formation by recombinant CYP2D6 and CYP3A4 was further normalized with their average expression level in human liver. For CYP2D6 catalyzed 7-hydroxylorcaserin formation at 1.0 and 10.0 µM of lorcaserin concentrations, the %TNR were 38.8 and 52.3, respectively, whereas for CYP3A4 the %TNR values were 61.2 and 47.7, respectively. This suggests that both CYP2D6 and CYP3A4 showed major contribution for 7-hydroxylorcaserin formation. Thus at a clinically relevant concentration of 1.0 µM of lorcaserin both CYP2D6 and CYP3A4 play important role in the 7-hydroxylation pathway of lorcaserin.

5-Hydroxylorcaserin was formed by recombinant CYP1A1, CYP1A2, CYP2D6, and CYP3A4. Normalized rate for 5-hydroxylorcaserin showed that at 1.0 µM of lorcaserin concentration, it was predominantly catalyzed by CYP1A2 (%TNR = 63.4) and CYP2D6.
(\%TNR = 36.6), whereas at 10.0 µM of lorcaserin concentration, CYP3A4 (\%TNR = 80.1) contributed the most in this pathway (Fig. 2C and D). Although 5-hydroxylorcaserin formation was only inhibited by furafylline (a CYP1A2 inhibitor), and only poorly, no good correlation was observed with any microsomal P450 marker substrate activity for the formation of this metabolite. These results suggested that multiple P450 enzymes catalyze the formation of 5-hydroxylorcaserin. CYP3A4 was the primary enzyme for 1-hydroxylorcaserin formation with both 1.0 and 10.0 µM of lorcaserin concentrations. 1-Hydroxylorcaserin formation was strongly inhibited by ketoconazole, a CYP3A4-selective inhibitor (Wrighton and Ring, 1994; Newton et al., 1995), in liver microsomes, suggesting that CYP3A4 catalyzes this pathway. However, a good correlation analysis was not possible due to the involvement of only a small number (three individuals) of liver microsomes in this pathway.

The human flavin-containing monooxygenase (FMO) are an important enzyme system that catalyzes the oxidation of a variety of nitrogen and sulfur-containing drugs and xenobiotics to more polar metabolites. FMO readily catalyzes the drugs that contain primary, secondary, and tertiary nitrogen centers (Krueger and Williams, 2005). Lorcaserin contains secondary nitrogen, which can be metabolized by FMO. FMO1 is a well expressed isoform in adult human kidney and FMO3 is the major liver isoform (Yeung et al., 2000). Only human recombinant FMO1 enzyme metabolized lorcaserin and catalyzed the formation of  N-hydroxylorcaserin; FMO3 and FMO5 did not have detectable activity towards lorcaserin metabolism. Therefore, both P450 and FMO are involved in the metabolism of lorcaserin on the nitrogen-center, resulting in the formation of the  N-hydroxylorcaserin metabolite. Further assessment of the contribution of P450 and FMO to the formation of  N-hydroxylorcaserin, in human renal microsomes (HRM), demonstrated that heat and methimazole treatments strongly inhibited  N-hydroxylorcaserin
formation, whereas 1-aminobenzotriazole, a general P450 inhibitor, did not. Also, no inhibition was observed with methimazole treatment using HLM, which suggested that human FMO1 (expressed in human kidney), and not FMO3 (expressed in human liver), was involved in the lorcaserin N-hydroxylation pathway. However, kinetic studies with HRM as well as recombinant FMO1 showed lower affinity (higher $K_m$) than HLM and recombinant P450s for the N-hydroxylation pathway, suggesting a minor contribution in human from FMO as compared to P450 enzymes.

A schematic overview of the P450 and FMO enzymes responsible for the various metabolic pathways of lorcaserin is depicted in Fig. 1. It may be mentioned here that based on the human mass balance radioactivity study, lorcaserin and its metabolites were primarily eliminated in human urine (>90% of dose). None of these oxidative metabolic pathways reported here were detected as phase I metabolites, rather were found as derived phase II metabolites in human urine (Chen et al., 2008). In this in vitro study, using various approaches, we have characterized P450 and FMO enzymes associated with lorcaserin metabolism. We demonstrated that multiple human P450 enzymes such as CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 and human FMO1 enzyme are responsible for the oxidative metabolism of lorcaserin. Taken together, these results suggest that lorcaserin is metabolized by multiple enzymes and its metabolism does not depend on any of the single metabolic pathway, thus lorcaserin has a low probability of P450- and FMO-mediated interactions by concomitant medications.
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Authorship contributions

Participated in research design: Usmani, Sadeque, and Chen.

Conducted experiments: Usmani

Performed data analysis: Usmani and Sadeque

Wrote or contributed to the writing of the manuscript: Usmani and Sadeque
References


Footnotes

a) Reprint Request
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b) Part of the studies was presented at the 15th North American ISSX meeting in San Diego, California, October 12-16, 2008.

Legends for figures

Figure 1. Schematic representation of the four primary *in vitro* metabolites of lorcaserin, *N*-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin. Relevant P450 enzymes involved in the formation of each of these lorcaserin metabolites are indicated. In addition to P450 enzymes, *N*-hydroxylorcaserin was also catalyzed by FMO1. Detailed incubation procedures for P450 and FMO enzymes are described under methods.

Figure 2. Rate and percent total normalized rates (%TNR) of formation of lorcaserin metabolites, *N*-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, by human recombinant cytochrome P450 enzymes at (A) 1.0 µM, (B) 10.0 µM, (C) 1.0 µM (% TNR), and (D) 10.0 µM (% TNR) lorcaserin concentrations.

Figure 3. Rate of formation of lorcaserin metabolites, *N*-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, in incubations with microsomes from sixteen individual human livers at (A) 1.0 µM and (B) 10.0 µM lorcaserin concentrations.

Figure 4. Comparison of the rate of formation of lorcaserin metabolites, *N*-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, from eight (A) 1.0 µM lorcaserin - male, (B) 1.0 µM lorcaserin - female, (C) 10.0 µM lorcaserin – male, and (D) 10.0 µM lorcaserin – female individual human liver microsomes.

Figure 5. Correlation analyses of the formation of lorcaserin metabolites, (A) *N*-hydroxylorcaserin versus CYP2B6 activity, (B) 7-hydroxylorcaserin versus CYP2D6 activity, (C) 5-hydroxylorcaserin versus CYP1A2 activity, and (D) 5-hydroxylorcaserin versus CYP3A4 activity, in sixteen individual human liver microsomal preparations.
Figure 6. Inhibition of the formation of lorcaserin metabolites, \(N\)-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, by the general human cytochrome P450-inhibitors, 1-aminobenzotriazole (1-ABT) and \(N\)-benzylimidazole, in human liver microsomal preparations using potassium phosphate buffer (pH 7.4).

Figure 7. Effect of P450-selective chemical inhibitors on the metabolism of lorcaserin in human liver microsomal preparations. Lorcaserin metabolites, \(N\)-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, were monitored in the presence of different concentrations of P450-selective inhibitors; (A) furafylline for CYP1A2 inhibition (10.0 \(\mu\)M lorcaserin); (B) ThioTEPA for CYP2B6 inhibition (10.0 \(\mu\)M lorcaserin); (C) \(N\)-benzylnirvanol for CYP2C19 inhibition (10.0 \(\mu\)M lorcaserin); (D) quinidine for CYP2D6 inhibition (10.0 \(\mu\)M lorcaserin); (E) quinidine for CYP2D6 inhibition (1.0 \(\mu\)M lorcaserin) ; and (F) ketoconazole for CYP3A4 inhibition (10.0 \(\mu\)M lorcaserin) .

Figure 8. Rate of formation of lorcaserin metabolites, \(N\)-hydroxylorcaserin, by human recombinant flavin-containing monooxygenase (FMO1, FMO3, and FMO5) enzymes. The concentration of lorcaserin was 1.0 and 10.0 \(\mu\)M in the incubation mixture. “n.d.” denotes “not detected.”

Figure 9. Inhibitory effect of (A) methimazole, a general FMO inhibitor, and heat on FMO, and (B) 1-ABT, a general P450 inhibitor, and heat on P450 mediated formation of \(N\)-hydroxylorcaserin in human renal microsomal incubations with 1.0 and 10.0 \(\mu\)M of lorcaserin. Glycine buffer (pH 8.5) and potassium phosphate buffer (pH 7.4) were used respectively to support FMO and P450 mediated \(N\)-hydroxylorcaserin formation in human renal microsomal preparations. Both methimazole and heat significantly inhibited \(N\)-hydroxylorcaserin formation,
whereas 1-ABT had no inhibitory effect, consistent with the primacy of FMO1 in N-hydroxylorcaserin formation in renal microsomes.

**Figure 10.** Inhibitory effect of methimazole, a general FMO inhibitor, on the formation of lorcaserin metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, in human liver microsomes was performed using glycine buffer (pH 8.5). There was no or minimal inhibitory effect of methimazole observed on the formation of these metabolites, consistent with the lack of FMO (FMO3) involvement.
# Tables

**Table 1.**  Correlation coefficients ($r^2$) of lorcaserin metabolite formation versus P450 marker activity in sixteen individual human liver microsomes at a lorcaserin concentration of 1.0 µM

<table>
<thead>
<tr>
<th>P450</th>
<th>Marker Reaction</th>
<th>N-Hydroxy lorcaserin</th>
<th>7-Hydroxy lorcaserin</th>
<th>5-Hydroxy lorcaserin</th>
<th>1-Hydroxy lorcaserin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Phenacetin O-deethylation</td>
<td>0.001</td>
<td>0.000</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2A6</td>
<td>Coumarin 7-hydroxylation</td>
<td>0.193</td>
<td>0.039</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2B6</td>
<td>(S)-Mephenytoin N-demethylation</td>
<td>0.007</td>
<td>0.002</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2C8</td>
<td>Paclitaxel 6α-hydroxylation</td>
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<td>0.091</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2C9</td>
<td>Tolbutamide 4'-hydroxylation</td>
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<td>0.003</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2C19</td>
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<td>0.006</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2D6</td>
<td>Bufuralol 1'-hydroxylation</td>
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<td>0.696</td>
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<td>n.d.</td>
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<tr>
<td>3A4</td>
<td>Testosterone 6β-hydroxylation</td>
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<td>0.000</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>4A11</td>
<td>Lauric acid 12-hydroxylation</td>
<td>0.008</td>
<td>0.000</td>
<td>n.d.</td>
<td>n.d.</td>
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*n.d.*: not detected; Formation of 5- and 1-hydroxylorcaserin metabolites were not detected at 1.0 µM of lorcaserin concentration in human liver microsomes.
Table 2. Correlation coefficients ($r^2$) of lorcaserin metabolite formation versus P450 marker activity in sixteen individual human liver microsomes at a lorcaserin concentration of 10.0 µM

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<th>P450</th>
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<td></td>
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<td>1A2</td>
<td>Phenacetin O-deethylation</td>
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<td>0.000</td>
<td>0.531</td>
<td>0.122</td>
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<td>2A6</td>
<td>Coumarin 7-hydroxylation</td>
<td>0.047</td>
<td>0.020</td>
<td>0.011</td>
<td>0.000</td>
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<td>2B6</td>
<td>(S)-Mephenytoin N-demethylation</td>
<td>0.674</td>
<td>0.062</td>
<td>0.003</td>
<td>0.088</td>
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<td>2C8</td>
<td>Paclitaxel 6α-hydroxylation</td>
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<td>0.000</td>
<td>0.084</td>
<td>0.001</td>
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<td>Tolbutamide 4′-hydroxylation</td>
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<td>0.154</td>
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<td>(S)-Mephenytoin 4′-hydroxylation</td>
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<td>0.001</td>
<td>0.014</td>
<td>0.003</td>
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<td>2D6</td>
<td>Bufuralol 1′-hydroxylation</td>
<td>0.002</td>
<td>0.819</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>3A4</td>
<td>Testosterone 6β-hydroxylation</td>
<td>0.178</td>
<td>0.000</td>
<td>0.481</td>
<td>0.680*</td>
</tr>
<tr>
<td>4A11</td>
<td>Lauric acid 12-hydroxylation</td>
<td>0.055</td>
<td>0.002</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Only three out of sixteen individual HLM formed 1-hydroxylorcasrin.
Table 3. Kinetic parameters for lorcaserin metabolite formation in human liver and renal microsomes

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Metabolites</th>
<th>Kinetic Constant</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (pmol/mg protein/min)</td>
<td>$CL_{int}$ (µL/mg protein/min)</td>
</tr>
<tr>
<td>HLM</td>
<td>$N$-hydroxylorcaserin</td>
<td>0.885</td>
<td>6.93</td>
<td>7.83</td>
</tr>
<tr>
<td>HLM</td>
<td>$N$-hydroxylorcaserin</td>
<td>590</td>
<td>731</td>
<td>1.24</td>
</tr>
<tr>
<td>HLM</td>
<td>7-hydroxylorcaserin</td>
<td>0.991</td>
<td>2.70</td>
<td>2.73</td>
</tr>
<tr>
<td>HLM</td>
<td>5-hydroxylorcaserin</td>
<td>483</td>
<td>119</td>
<td>0.245</td>
</tr>
<tr>
<td>HLM</td>
<td>1-hydroxylorcaserin</td>
<td>392</td>
<td>35.9</td>
<td>0.0920</td>
</tr>
<tr>
<td>HRM$^a$</td>
<td>$N$-hydroxylorcaserin</td>
<td>768</td>
<td>2670</td>
<td>3.48</td>
</tr>
</tbody>
</table>

$^a$ Three other metabolites, 7-, 5-, & 1-hydroxylorcaserin, were not observed in the HRM incubations.
Table 4. *Kinetic parameters for lorcaserin metabolites formation in human recombinant P450 and FMO enzymes*

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Metabolites</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/nmol enzyme/min)</th>
<th>CL_int (µL/nmol enzyme/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>N-hydroxylorcaserin</td>
<td>641</td>
<td>9.39</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>5-hydroxylorcaserin</td>
<td>534</td>
<td>3.64</td>
<td>6.80</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>N-hydroxylorcaserin</td>
<td>785</td>
<td>0.332</td>
<td>0.432</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>N-hydroxylorcaserin</td>
<td>494</td>
<td>46.9</td>
<td>95.0</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>N-hydroxylorcaserin</td>
<td>36.9</td>
<td>1.90</td>
<td>51.5</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>N-hydroxylorcaserin</td>
<td>0.181</td>
<td>0.609</td>
<td>3370</td>
</tr>
<tr>
<td></td>
<td>7-hydroxylorcaserin</td>
<td>0.260</td>
<td>0.110</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>5-hydroxylorcaserin</td>
<td>0.498</td>
<td>0.0510</td>
<td>102</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>N-hydroxylorcaserin</td>
<td>768</td>
<td>3.04</td>
<td>3.96</td>
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<tr>
<td>CYP3A4</td>
<td>N-hydroxylorcaserin</td>
<td>445</td>
<td>6.38</td>
<td>14.3</td>
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<td></td>
<td>7-hydroxylorcaserin</td>
<td>4170</td>
<td>7.42</td>
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<td></td>
<td>5-hydroxylorcaserin</td>
<td>191</td>
<td>2.71</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>1-hydroxylorcaserin</td>
<td>213</td>
<td>7.36</td>
<td>34.5</td>
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<tr>
<td>FMO1</td>
<td>N-hydroxylorcaserin</td>
<td>816</td>
<td>43.5</td>
<td>53.4</td>
</tr>
</tbody>
</table>
Fig. 2 (A)

(A) 1.0 μM Lorcanerin

- N-Hydroxylorcanerin
- 7-Hydroxylorcanerin
- 5-Hydroxylorcanerin
- 1-Hydroxylorcanerin

Metabolite Formation (nmol/nmol P450/min)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Metabolite Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td></td>
</tr>
<tr>
<td>1A2</td>
<td></td>
</tr>
<tr>
<td>2A6</td>
<td></td>
</tr>
<tr>
<td>2B6</td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td></td>
</tr>
<tr>
<td>2D6*1</td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2 (B)

(B) 10.0 μM Loracaserin

- N-Hydroxyloracaserin
- 7-Hydroxyloracaserin
- 5-Hydroxyloracaserin
- 1-Hydroxyloracaserin

Metabolite Formation (nmol/nmol P450/min)

1A1  1A2  1B1  2A6  2B6  2C8  2C9*-1  2C18  2C19  2D6*-1  2D6*-10  2E1  3A4  3A5  3A7
Fig. 2 (C)  

(C) 1.0 μM Lorcaserin (%TNR)

- N-hydroxylorcaserin
- 7-hydroxylorcaserin
- 5-hydroxylorcaserin
- 1-hydroxylorcaserin

% Total Normalized Rate

1A2  2A6  2B6  2C19  2D6*1  CYP2E1  3A4
Fig. 2 (D)  

(D) 10.0 μM Lorcaserin (%TNR)

- N-hydroxylorcaserin
- 7-hydroxylorcaserin
- 5-hydroxylorcaserin
- 1-hydroxylorcaserin

% Total Normalized Rate

1A2  | 2A6  | 2B6  | 2C19 | 2D6+1 | CYP2E1 | 3A4

0   | 20   | 50   | 10   | 10    | 0      | 0
Fig. 3 (A) 1.0 μM Lorcaserin

(A) 1.0 μM Lorcaserin

- N-Hydroxylorcaserin
- 7-Hydroxylorcaserin

Metabolite Formation (pmol/mg protein/min)

Samples: HG3, HH13, HH18, HG32, HK37, HH47, HG64, HG74, HH77, HH81, HH91, HG93, HG95, HH101, HH64, HH74
Fig. 3 (B)

(B) 10.0 μM Lorcaserin

- N-Hydroxylorcaserin
- 7-Hydroxylorcaserin
- 5-Hydroxylorcaserin
- 1-Hydroxylorcaserin

Metabolite Formation (μmol/mg protein/min)

HG3, HH13, HH18, HG32, HK37, HH47, HG64, HG74, HH77, HH81, HH91, HG93, HG95, HH101, HH64, HH74
Fig. 4 (A) (A) 1.0 μM Lorcaserin - Male

Metabolite Formation (pmol/mg protein/min)

- N-Hydroxylorcaserin
- 7-Hydroxylorcaserin

HH13  HG32  HG64  HG74  HH77  HH81  HH64  HH74
Fig. 4 (B)  

(B) 1.0 μM Lorcanerin - Female

![Graph showing metabolite formation (pmol/mg protein/min) for different samples](image-url)
**Fig. 4 (C)**

**C) 10.0 μM Lorcaserin - Male**

![Bar chart showing metabolite formation (pmol/mg protein/min) for different samples.](chart.png)

- **N-Hydroxylorcaserin**
- **7-Hydroxylorcaserin**
- **5-Hydroxylorcaserin**
- **1-Hydroxylorcaserin**

**Samples:** HH13, HG32, HG64, HG74, HH77, HH81, HH64, HH74
Fig. 4 (D)  

(D) 10.0 μM Lorcaserin - Female

[Graph showing metabolite formation (pmol/mg protein/min) for different samples over time, with error bars indicating variability.]
Fig. 5 (A)  

(A) N-Hydroxyloracserin vs. CYP2B6 Activity

\[ r^2 = 0.674 \]

![Graph showing the relationship between Loracserin N-hydroxylase (pmol/mg protein/min) and (S)-Mephenytoin N-demethylase (pmol/mg protein/min). The graph includes data points and a line of best fit.]
Fig. 5 (B)  

(B) 7-Hydroxylorcanerin vs. CYP2D6 Activity

\[ r^2 = 0.819 \]
Fig. 5 (C)  

(C) 5-Hydroxylorcaserin vs. CYP1A2 Activity

![Graph showing the relationship between 5-hydroxylorcaserin and CYP1A2 activity. The graph includes a trend line and data points. The correlation coefficient $r^2 = 0.531$.](image-url)
Fig 5 (D)  5-Hydroxylorcaserin vs. CYP3A4 Activity

$\text{Lorcaserin 5-hydroxylase (pmol/mg protein/min)}$

$\text{Testosterone 6\beta-hydroxylase (pmol/mg protein/min)}$

$r^2 = 0.481$
Fig. 6

![Bar graph showing % Control Activity for different hydroxylated furosemide derivatives](image-url)
Fig. 7 (A)  (A) CYP1A2 Inhibitor Furafylline (10.0 μM Lorcanerin)

- N-Hydroxylorcanerin
- 7-Hydroxylorcanerin
- 5-Hydroxylorcanerin
- 1-Hydroxylorcanerin

% Control Activity

Log [Furafylline] (μM)

1.914 μM
Fig. 7 (B)  (B) CYP2B6 Inhibitor ThioTEPA (10.0 μM Lorcanin)

![Graph showing the inhibition of CYP2B6 by ThioTEPA at 10.0 μM Lorcanin. The graph plots % Control Activity against Log [ThioTEPA] (μM). Different symbols represent different metabolites: □ N-Hydroxylorcanin, △ 7-Hydroxylorcanin, ▼ 5-Hydroxylorcanin, and ◇ 1-Hydroxylorcanin.]
Fig. 7 (C)  

(C) CYP2C19 Inhibitor N-benzylnirvanol (10.0 μM Lorcanerin)

- N-Hydroxylorcanerin
- 7-Hydroxylorcanerin
- 5-Hydroxylorcanerin
- 1-Hydroxylorcanerin

% Control Activity vs. Log [N-Benzylnirvanol] (μM)
Fig. 7 (D)  

(D) CYP2D6 Inhibitor Quinidine (10.0 μM Lorcaserin)

- □ N-Hydroxylorcaserin
- △ 7-Hydroxylorcaserin
- ▼ 5-Hydroxylorcaserin
- ◇ 1-Hydroxylorcaserin

% Control Activity

Log [Quinidine] (μM)

0.213 μM

0 25 50 75 100 125

-2.5 -2.0 -1.5 -1.0 -0.5 0 0.5
Fig. 7 (E)  (E) CYP2D6 Inhibitor Quinidine (1.0 μM Lorcaserin)

N-Hydroxylorcaserin

7-Hydroxylorcaserin

% Control Activity

Log [Quinidine] (μM)
Fig. 7 (F)  (F) CYP3A4 Inhibitor Ketoconazole (10.0 μM Lorcanerin)

- N-Hydroxylorcanerin
- 7-Hydroxylorcanerin
- 5-Hydroxylorcanerin
- 1-Hydroxylorcanerin

% Control Activity vs Log [Ketoconazole] (μM)

0.281 μM
Fig. 8

![Graph showing metabolite formation for N-Hydroxylcaserin at different concentrations](image-url)
Fig. 9 (A)  
(A) Glycine buffer pH 8.5 (FMO)

- Control
- Methimazole (25.0 μM)
- Heat 3 min (45°C)

% Control Activity

Lorcaserin (1.0 μM)  Lorcaserin (10.0 μM)
Fig. 9 (B)  (B) Phosphate buffer pH 7.4 (P450)

![Graph showing % Control Activity vs. Lorcaserin concentrations]

- **Control**
- **1-ABT (1.0 mM)**
- **Heat 3 min (45°C)**

**Lorcaserin (1.0 μM)**

**Lorcaserin (10.0 μM)**
Fig. 10

![Bar graph showing control and Methimazole (25.0 μM) effects on loracrin activity](image-url)

- **N-Hydroxy loracrin**
- **7-Hydroxy loracrin**
- **5-Hydroxy loracrin**
- **1-Hydroxy loracrin**

**X-axis:** Different hydroxylation products of loracrin

**Y-axis:** % Control Activity