DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
DMD Fast Forward. Published on January 20, 2012 as doi:10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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

Khawja A. Usmani, Weichao G. Chen, and Abu J.M. Sadeque

Arena Pharmaceuticals, Inc., San Diego, California, USA

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Copyright 2012 by the American Society for Pharmacology and Experimental Therapeutics.

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Running title:

Human P450 and FMO involved in Lorcaserin Metabolism

Corresponding author: Khawja A. Usmani, Arena Pharmaceuticals, Inc., 6166 Nancy Ridge Drive, San Diego, CA 92121, USA; Phone: (858) 453-7200 ext. 1758; Fax: (858) 453-7210; E-mail: kusmani@arenapharm.com

The number of text pages: 28

The number of tables: 4

The number of figures: 10

The number of references: 25

The number of words in the *Abstract*: 249

The number of words in the *Introduction*: 413

The number of words in the *Discussion*: 1,380

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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Lorcaserin, a selective serotonin 5-hydroxytryptamine 2C ($5\text{-}HT_{2C}$) 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 (IC_{50} = 1.914 μM), 7-hydroxylorcaserin (IC_{50} = 0.213 μM), and 1-hydroxylorcaserin formation (IC_{50} = 0.281 μM), respectively. *N*-Hydroxylorcaserin showed low and high K_m components in HLM and 7-hydroxylorcaserin showed lower K_m 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

primary oxidative metabolites of lorcaserin; suggesting lorcaserin has a low probability of drug-drug interactions by concomitant medications.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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, (1*R*)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-3-benzazepine, is a selective 5-hydroxytryptamine 2C (5-HT_{2C}) receptor agonist and a potential therapeutic agent for weight management (Smith et al., 2008; Smith et al., 2010). For most commonly used drugs,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 *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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Methods

Chemicals. Lorcaserin, (1*R*)-8-Chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-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 ($MgCl_2$), potassium phosphate monobasic, potassium phosphate dibasic, dextromethorphan, dimethyl sulfoxide (DMSO), tranylcypromine, thioTEPA, quinidine, ketoconazole, 1-aminobenzotriazole (1-ABT), *N*-benzylimidazole, methimazole, and β-NADPH, were purchased from Sigma-Aldrich (St. Louis, MO). Furafylline and *N*-benzylnirvanol 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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 (2,572*g*), 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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 $MgCl_2$ 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 $MgCl_2$ 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 $MgCl_2$ 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*-benzylnirvanol (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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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^{o}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 $MgCl_2$ 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 lorcaserin metabolism. *Set 1 – Control (without treatment)*: The incubation mixture consisted of HLM (0.25 mg/mL protein), lorcaserin (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), lorcaserin (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); lorcaserin (0 - 10 mM) as a substrate; and 100 mM potassium phosphate buffer containing 3 mM $MgCl_2$ 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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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-d_3. 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

fit to the Michaelis-Menten equation. The intrinsic clearance (CL_{int}) was calculated as $CL_{int} = V_{max}/K_m$ (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) phneotyped 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).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Rate of metabolites formed by various recombinant P450s at 1.0 and 10.0 µM concentrations of lorcaserin 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 lorcaserin concentration, *N*-hydroxylorcaserin 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 lorcaserin concentration, *N*-hydroxylorcaserin 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-hydroxylorcaserin formation at 1.0 and 10.0 µM of lorcaserin 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-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 catalyzed 5-hydroxylorcaserin predominantly (%TNR = 80.1) (Fig. 2C and 2D). CYP3A4 was the primary enzyme for 1-hydroxylorcaserin formation with both 1.0 and 10.0 µM of lorcaserin concentrations.

Human Liver Microsomal Metabolism and Correlation Analysis. *Metabolism by Individual Liver Microsomes:* Lorcaserin metabolism was evaluated in sixteen individual human liver microsomal preparations using 1.0 and 10.0 µM of lorcaserin. As shown in Fig. 3, incubation with 1.0 µM of lorcaserin resulted in the formation of two metabolites, *N*-hydroxylorcaserin and 7-hydroxylorcaserin (Fig. 3A), whereas incubation with 10.0 µM of

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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-

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

hydroxylorcaserin, were formed at the 1.0 μM of lorcaserin concentration. The formation of 7-hydroxylorcaserin showed a moderate correlation with CYP2D6 activity with $r^2 = 0.696$; however, *N*-hydroxylorcaserin 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*-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin formation against the activities of several P450 probe substrates in microsomal preparations. Based on regression analyses, the correlation coefficients (r^2) for *N*-hydroxylorcaserin formation with microsomal P450 activities varied widely. Among them, CYP2B6 showed the highest correlation coefficient ($r^2 = 0.674$) for the formation of *N*-hydroxylorcaserin (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*-hydroxylorcaserin (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-hydroxylorcaserin 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-hydroxylorcaserin 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 6*β*-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 6*β*-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,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Inhibition of Microsomal CYP2C19: Recombinant CYP2C19 catalyzed the formation of *N*-hydroxylorcaserin (Fig. 2). However, *N*-benzylnirvanol, 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 IC_{50} value of 0.213 μM (Fig. 7D) at 10.0 μM of lorcaserin concentration. As expected, using 1.0 μM of lorcaserin concentration, the quinidine exerted much stronger inhibitory effect (~10-fold) on 7-hydroxylorcaserin formation with an IC_{50} value of 0.024 μM (Fig. 7E) compared to 10.0 μM of lorcaserin 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 lorcaserin 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 IC_{50} value of 0.281 μM (Fig. 7F). Consistent with Fig. 2, only recombinant CYP3A4 catalyzed the formation of 1-hydroxylorcasern. 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 lorcaserin metabolites, human renal microsomes (HRM) produced only *N*-hydroxylorcaserin. Human recombinant FMO1, FMO3, and FMO5 were incubated with two

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 [$CL_{int} = (V_{max}/K_m)$] 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_{max}, and CL_{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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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*-

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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 µM 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 µM), whereas at low concentration (1.0 µM), 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 µM). 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 µM 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 µM 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

%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-hydoxylation 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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

(%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 for1-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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgement

The authors are grateful to Michael Ma, Forest Flodin, Salma Sarwary, Jane Angeles and Yong Tang for their bio-analytical support. The authors also thank Wim D'Haeze for his critical reading and formatting the manuscript.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

References

Bjornsson RD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, king SP, Miwa G, Ni L, Kumar G, Mcleod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J, and Wrighton SA (2003) The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* **31:**815-823.

Cashman JR (2000) Human flavin-containing monooxygenase: substrate specificity and role in drug metabolism. *Curr Drug Metab* **2:**181-191.

Chen WG, Xu J, Gwathney W, Prosser W, Morgan M, Cerny MA, Chen C, Sadeque AJM, and Shanahan W (2008) Metabolism, pharmacokinetics, and excretion of lorcaserin, a novel selective serotonin 5HT2C receptor agonist, in healthy male volunteers [abstract]. *Drug Metab Rev* **40 (Suppl 3):**205.

Dolphin, CT, Cullingford, TE, Shephard EA, Smith RL, and Phillips IR (1996) Differential developmental and tissue-specific regulation of expression of the genes encoding three members of the flavin-containing monooxygenase family of man, FMO1, FMO3 and FMO4. *Eur J Biochem* **235:**683-689.

Guengerich FP (1995) Structure, Mechanism and Biochemistry, in *Cytochrome P450* (de Montellano POR ed) pp 473-515, Plenum Press, New York.

Hodgson E and Goldstein JA (2001) Metabolism of toxicants: Phase I reactions and pharmacogenetics, in *Introduction to Biochemical Toxicology*, 3rd ed. (Hodgson E, Smart RC, eds) pp 67-112, John Wiley and Sons Inc., New York.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Krueger SK and Williams DE (2005) Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol Ther* **106:**357-387.

Lawton MP, Cashman JR, Cresteil T, Dolphin CT, Elfarra AA, Hines RN, Hodgson E, Kimura T, Ozols J, Phillips IR, Philpot RM, Poulsen LL, Rettie AE, Shephard EA, Williams DE, and Ziegler DM (1994) A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. *Arch Biochem Biophys* **308:**254-257.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, and Nebert DW (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:**1-42.

Newton DJ, Wang RW, and Lu AYH (1995) Cytochrome P450 inhibitors: evaluation of specificities in the in vitro metabolism of therapeutic agent by human liver microsomes. *Drug Metab Dispos* **23:**154-158.

Ogilvie BW, Usuki E, Yerino P, and Parkinson A (2008) In vitro approaches for studying the inhibition of drug-metabolizing enzymes and identifying the drug-metabolizing enzymes responsible for the metabolism of drug (reaction phenotyping) with emphasis on cytochrome P450, in *Drug-Drug Interactions*, (Rodrigues AD ed) pp 231-358, Informa Healthcare, Yew York.

Phillips IR, Dolphin CT, Clair P, Hadley MR, Hutt AJ, McCombie RR, Smith RL, and Shephard EA (1995) The molecular biology of the flavin-containing monooxygenases of man. *Chem-Biol Interact* **96:**17-32.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Rawden HC, Kokwaro GO, Ward SA, and Edward G (2000) Relative contribution of cytochromes P-450 and flavin-containing monoxygenases to the metabolism of albendazole by human liver microsomes. *Br J Clin Pharmacol* **49:**313-322.

Rodrigues AD (1999a) Applications of heterologous expressed and purified human drug-metabolizing enzymes: an industrial perspective, in *Handbook of Drug Metabolism*, (Wolf TE ed) pp 279-320, Marcel Dekker, New York.

Rodrigues AD (1999b) Integrated cytochrome P450 reaction phenotyping. Attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem Pharmacol* **57:**465-480

Sadeque AJ, Eddy AC, Meier GP, and Rettie AE (1992) Stereoselective sulfoxidation by human flavin-containing monooxygenase: Evidence for catalytic diversity between hepatic, renal, and fetal forms. *Drug Metab Dispos* **20:**832-839.

Sadeque AJ, Thummel KE, and Rettie AE (1993) Purification of macaque liver flavin-containing monooxygenase: a form of the enzyme related immunochemically to an isozyme expressed selectively in adult human liver. *Biochim Biophys Acta* **1162:**127-134.

Segel HI (1976) Biochemical Calculations, 2nd ed. John Wiley & Sons.Ltd., New York.

Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270:**414-423.

Smith BM, Smith JM, Tsai J, Schultz JA, Gilson CA, Estrada SA, Chen RR, Park DM, Prieto EB, Gallardo CS, Sengupta D, Dosa PI, Covel JA, Ren A, Webb RR, Beeley NRA,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Martin M, Morgan M, Espitia S, Saldana HR, Bjenning C, Whelan KT, Grottick AJ, Menzaghi F, and Thomsen WJ (2008) Discovery and structure-activity relationship of (1*R*)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-3-benzazepine (lorcaserin), a selective serotonin 5-HT_{2C} receptor agonist for the treatment of obesity. *J Med Chem* **51:**305-313.

Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, Bays H, Shanahan WR, and The Behavioral Modification and Lorcaserin for Overweight and Obesity Management (BLOOM) Study Group (2010) Multicenter, placebo-controlled trial of lorcaserin for weight management. *N Engl J Med* **363:**245-256.

Venkatakrishnan K, von Moltke LL, Obach RS, and Greenblatt DJ (2003) Drug metabolism and drug interactions: application and clinical value of in vitro models. *Curr Drug Metab* **4:**423-459.

Wrighton SA and Ring BJ (1994) Inhibition of human CYP3A catalyzed 1´-hydroxymidazolam formation by ketoconazole, nifedipine, erythromycin, cimetidine and nizatidine. *Pharmacol Res* **11:**921-924.

Yeung CK, Lang DH, Thummel KE, and Rettie AE (2000) Immunoquantitation of FMO1 in human liver, kidney, and intestine. *Drug Metabol Dispos* **28:**1107-1111.

Ziegler DM (1991) Unique properties of the enzymes of detoxication. *Drug Metabol Disp* **19:**847-852.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

a) Reprint Request

Khawja A. Usmani, Arena Pharmaceuticals, Inc., 6166 Nancy Ridge Drive, San Diego, CA 92121, USA; Phone: (858) 453-7200 ext. 1758; Fax: (858) 453-7210; E-mail: kusmani@arenapharm.com

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

Current affiliation: Weichao G. Chen: DMPK, Vertex Pharmaceuticals, San Diego, CA, 92121.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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-aminbenzotriazole (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 μM lorcaserin); (B) ThioTEPA for CYP2B6 inhibition (10.0 μM lorcaserin); (C) *N*-benzylnirvanol for CYP2C19 inhibition (10.0 μM lorcaserin); (D) quinidine for CYP2D6 inhibition (10.0 μM lorcaserin); (E) quinidine for CYP2D6 inhibition (1.0 μM lorcaserin) ; and (F) ketoconazole for CYP3A4 inhibition (10.0 μM 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 μM 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 μM 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,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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*

P450	Marker Reaction	r^2			
		N-Hydroxy lorcaserin	7-Hydroxy lorcaserin	5-Hydroxy lorcaserin	1-Hydroxy lorcaserin
1A2	Phenacetin *O*-deethylation	0.001	0.000	n.d.[a]	n.d.
2A6	Coumarin 7-hydroxylation	0.193	0.039	n.d.	n.d.
2B6	(S)-Mephenytoin *N*-demethylation	0.007	0.002	n.d.	n.d.
2C8	Paclitaxel 6α-hydroxylation	0.034	0.091	n.d.	n.d.
2C9	Tolbutamide 4'-hydroxylation	0.000	0.003	n.d.	n.d.
2C19	(S)-Mephenytoin 4'-hydroxylation	0.015	0.006	n.d.	n.d.
2D6	Bufuralol 1'-hydroxylation	0.074	0.696	n.d.	n.d.
3A4	Testosterone 6β-hydroxylation	0.000	0.000	n.d.	n.d.
4A11	Lauric acid 12-hydroxylation	0.008	0.000	n.d.	n.d.

[a]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.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

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*

P450	Marker Reaction	r^2			
		N-Hydroxy lorcaserin	7-Hydroxy lorcaserin	5-Hydroxy lorcaserin	1-Hydroxy lorcaserin
1A2	Phenacetin *O*-deethylation	0.007	0.000	0.531	0.122
2A6	Coumarin 7-hydroxylation	0.047	0.020	0.011	0.000
2B6	(S)-Mephenytoin *N*-demethylation	0.674	0.062	0.003	0.088
2C8	Paclitaxel 6α-hydroxylation	0.016	0.000	0.084	0.001
2C9	Tolbutamide 4'-hydroxylation	0.004	0.154	0.040	0.023
2C19	(S)-Mephenytoin 4'-hydroxylation	0.000	0.001	0.014	0.003
2D6	Bufuralol 1'-hydroxylation	0.002	0.819	0.000	0.000
3A4	Testosterone 6β-hydroxylation	0.178	0.000	0.481	0.680*
4A11	Lauric acid 12-hydroxylation	0.055	0.002	0.000	0.000

* Only three out of sixteen individual HLM formed 1-hydroxylorcasrin.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. *Kinetic parameters for lorcaserin metabolite formation in human liver and renal microsomes*

		Kinetic Constant		
Tissue	Metabolites	K_m (μM)	V_{max} (pmol/mg protein/min)	CL_{int} (μL/mg protein/min)
HLM	*N*-hydroxylorcaserin	0.885	6.93	7.83
HLM	*N*-hydroxylorcaserin	590	731	1.24
HLM	7-hydroxylorcaserin	0.991	2.70	2.73
HLM	5-hydroxylorcaserin	483	119	0.245
HLM	1-hydroxylorcaserin	392	35.9	0.0920
HRM[a]	*N*-hydroxylorcaserin	768	2670	3.48

[a] Three other metabolites, 7-, 5-, & 1-hydroxylorcaserin, were not observed in the HRM incubations.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

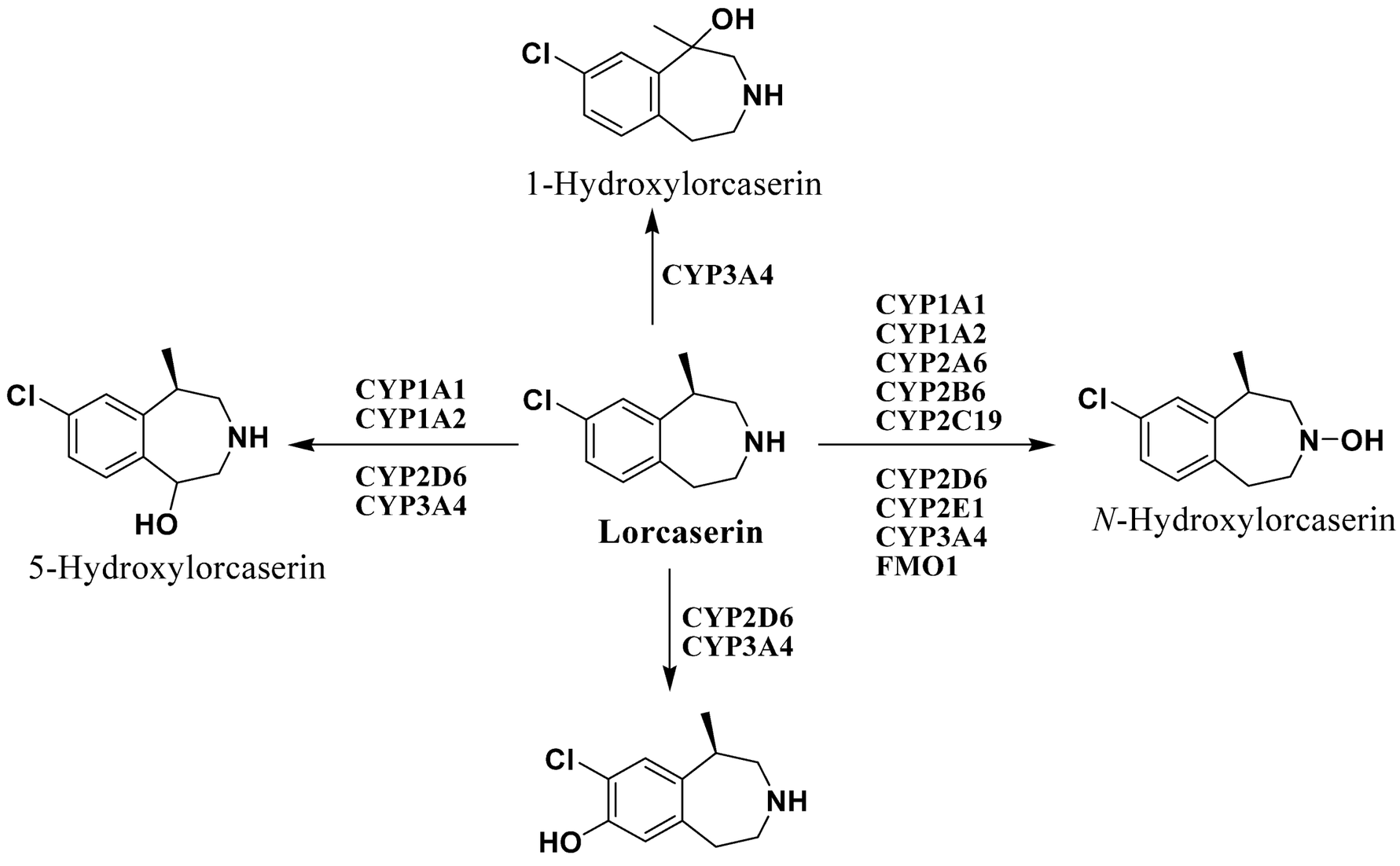
DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Table 4. *Kinetic parameters for lorcaserin metabolites formation in human recombinant P450 and FMO enzymes*

Enzymes	Metabolites	Kinetic Parameters		
		K_m (μM)	V_{max} (nmol/nmol enzyme/min)	CL_{int} (μL/nmol enzyme/min)
CYP1A2	*N*-hydroxylorcaserin	641	9.39	14.7
	5-hydroxylorcaserin	534	3.64	6.80
CYP2A6	*N*-hydroxylorcaserin	785	0.332	0.432
CYP2B6	*N*-hydroxylorcaserin	494	46.9	95.0
CYP2C19	*N*-hydroxylorcaserin	36.9	1.90	51.5
CYP2D6	*N*-hydroxylorcaserin	0.181	0.609	3370
	7-hydroxylorcaserin	0.260	0.110	423
	5-hydroxylorcaserin	0.498	0.0510	102
CYP2E1	*N*-hydroxylorcaserin	768	3.04	3.96
CYP3A4	*N*-hydroxylorcaserin	445	6.38	14.3
	7-hydroxylorcaserin	4170	7.42	1.78
	5-hydroxylorcaserin	191	2.71	14.2
	1-hydroxylorcaserin	213	7.36	34.5
FMO1	*N*-hydroxylorcaserin	816	43.5	53.4

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

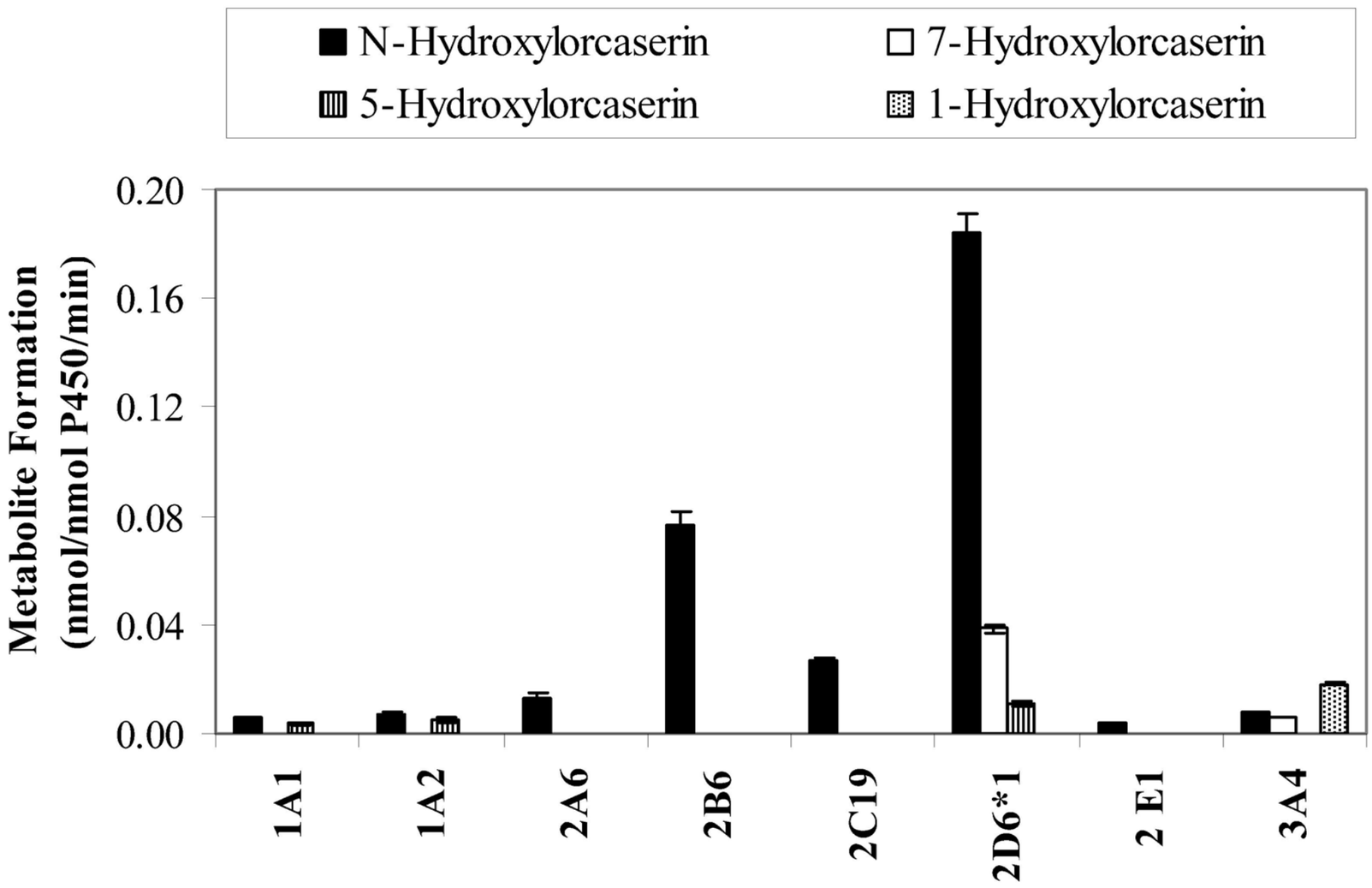
Fig. 1



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

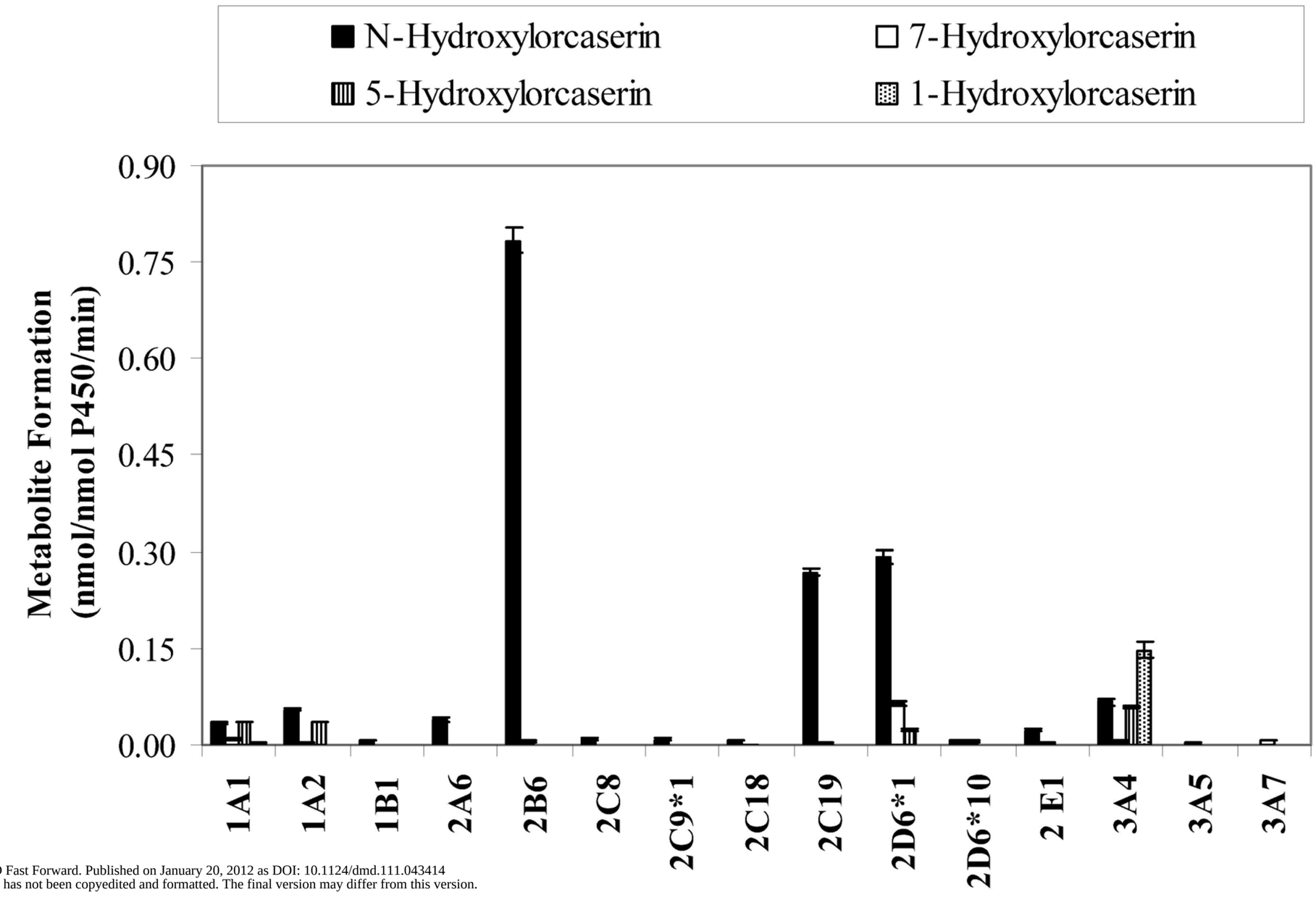
Fig. 2 (A)



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

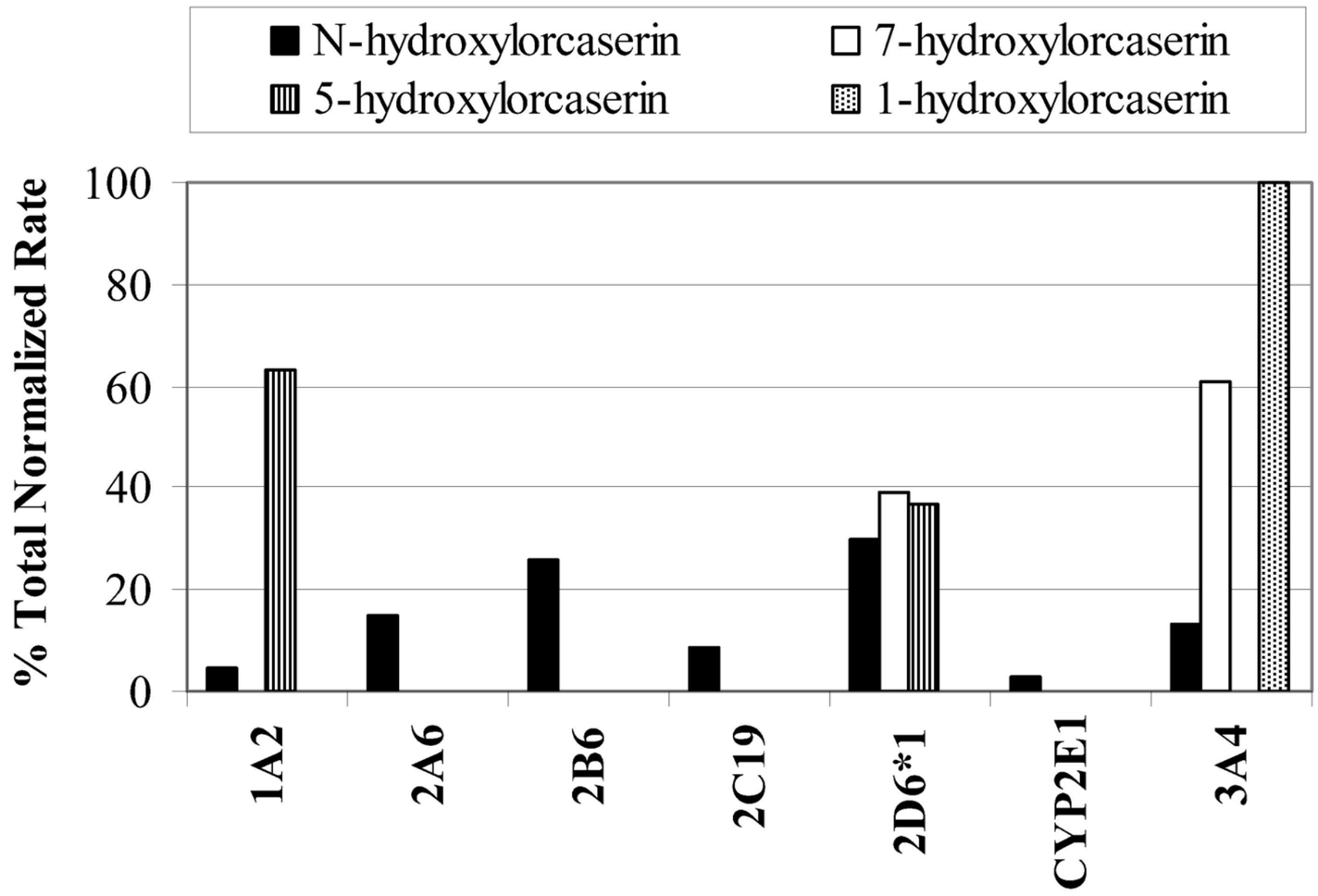
Fig. 2 (B)



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

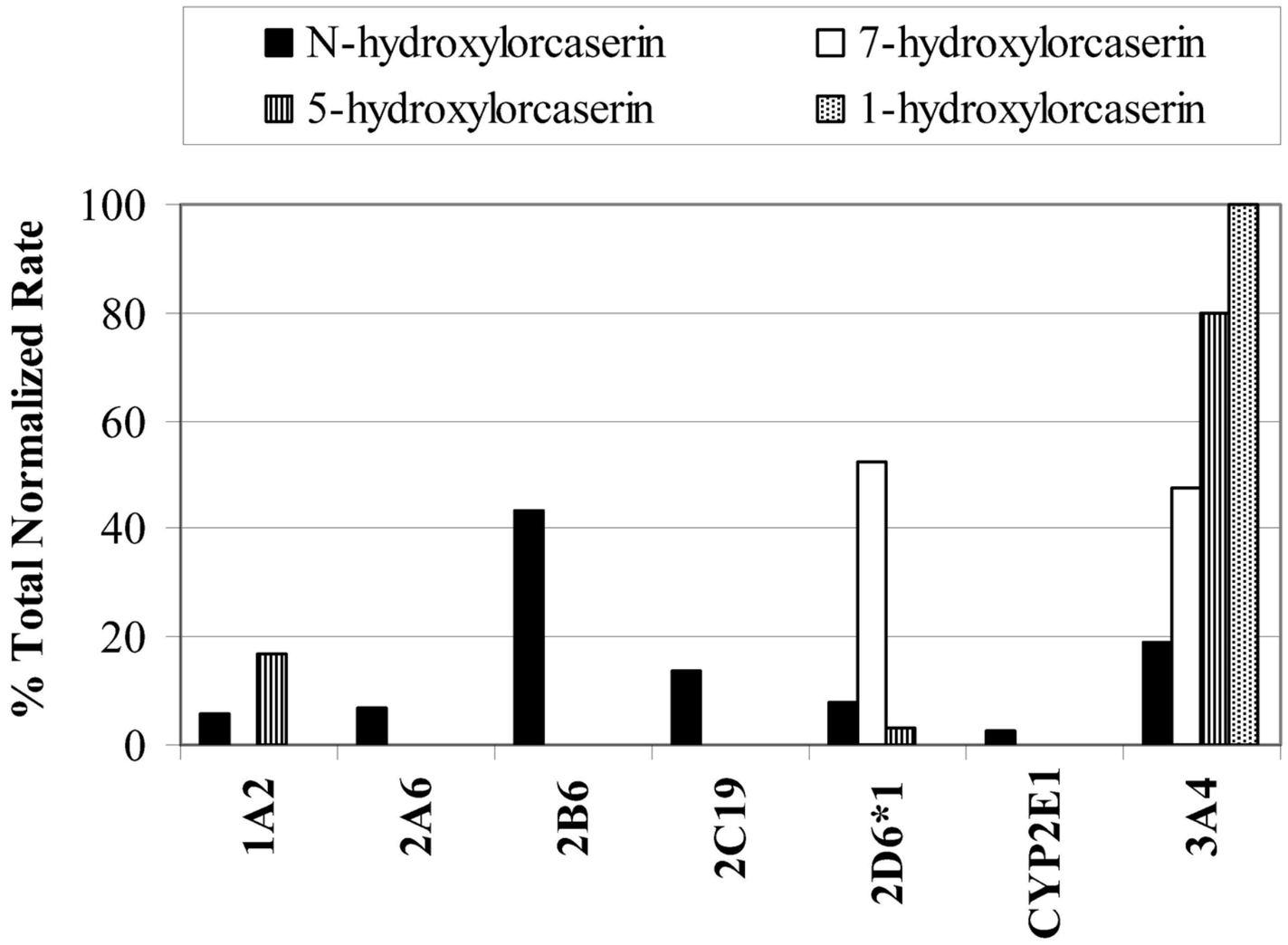
Fig. 2 (C) **(C) 1.0 μM Lorcaserin (%TNR)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 2 (D) **(D) 10.0 μM Lorcaserin (%TNR)**

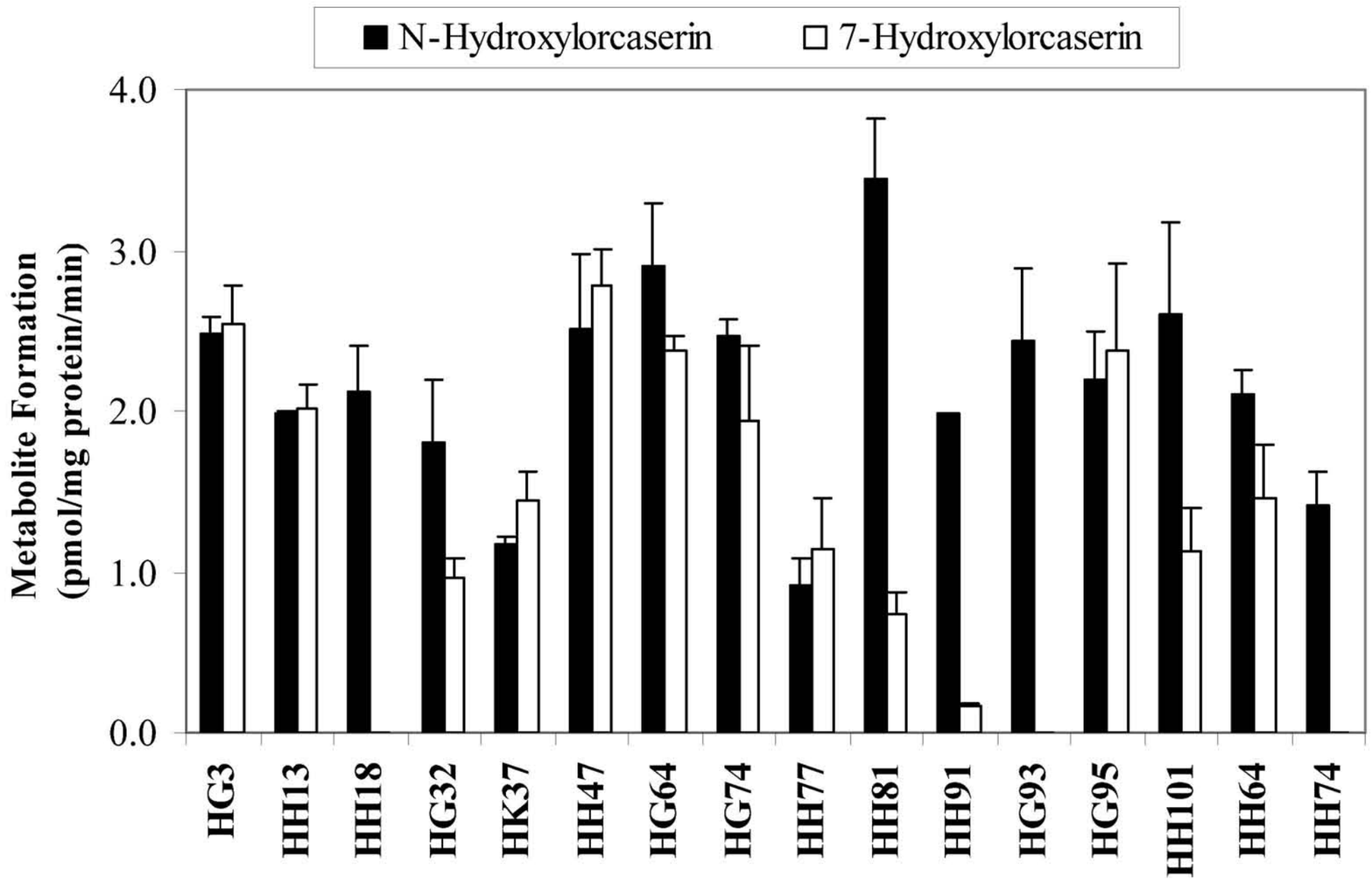


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 3 (A)

(A) 1.0 μM Lorcaserin

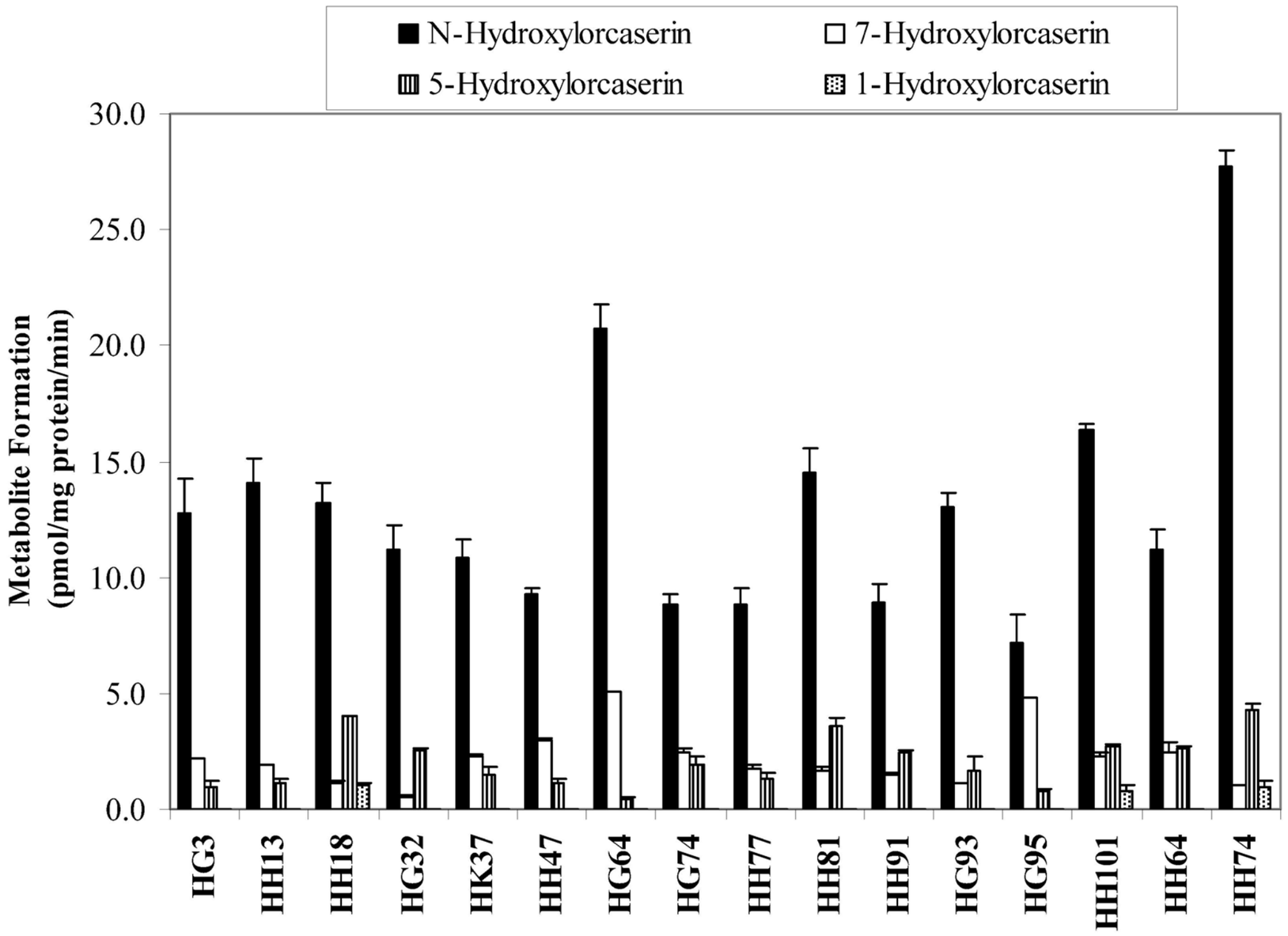


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 3 (B)

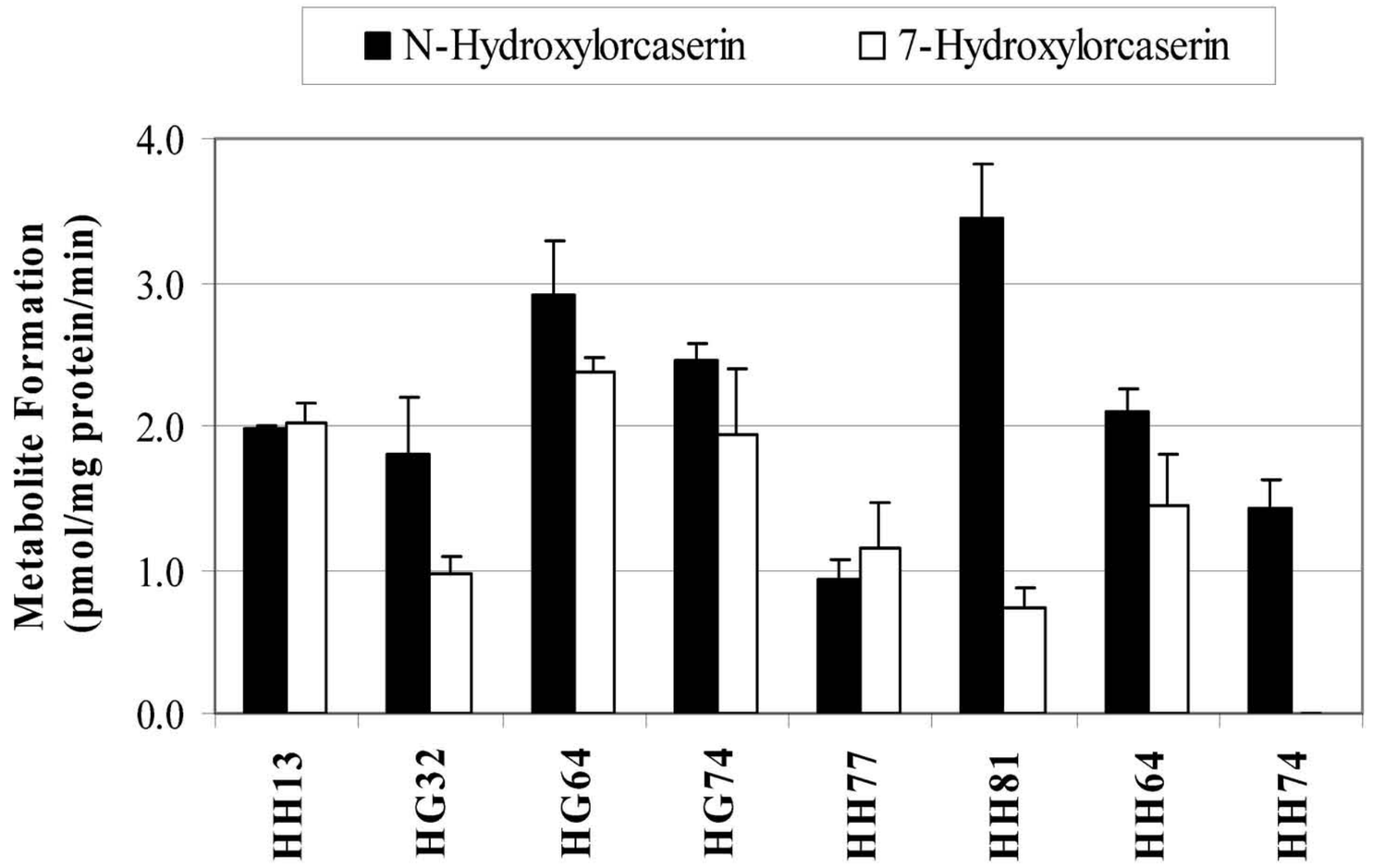
(B) 10.0 μM Lorcaserin



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

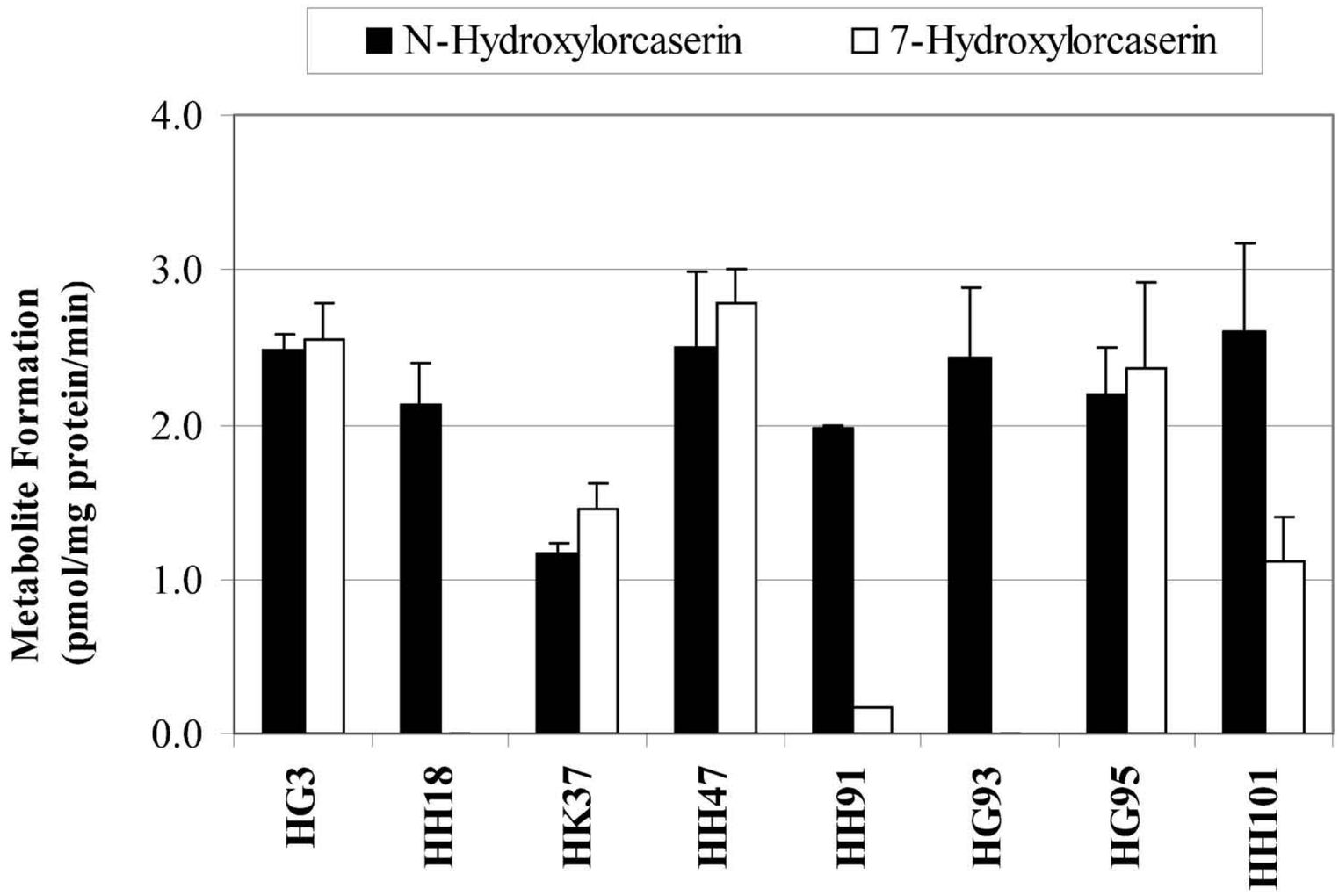
Fig. 4 (A) **(A) 1.0 μM Lorcaserin - Male**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

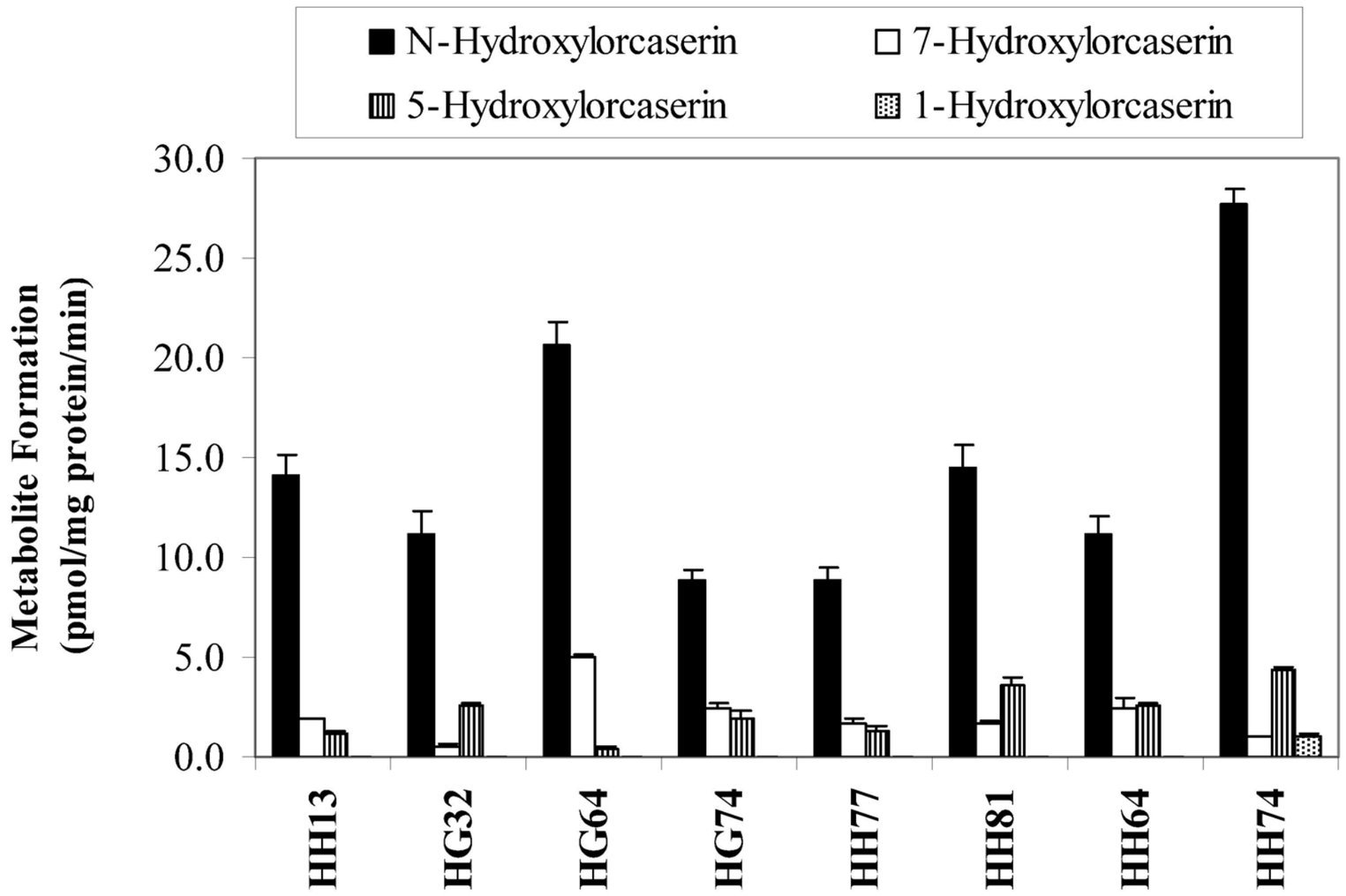
Fig. 4 (B) **(B) 1.0 μM Lorcaserin - Female**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

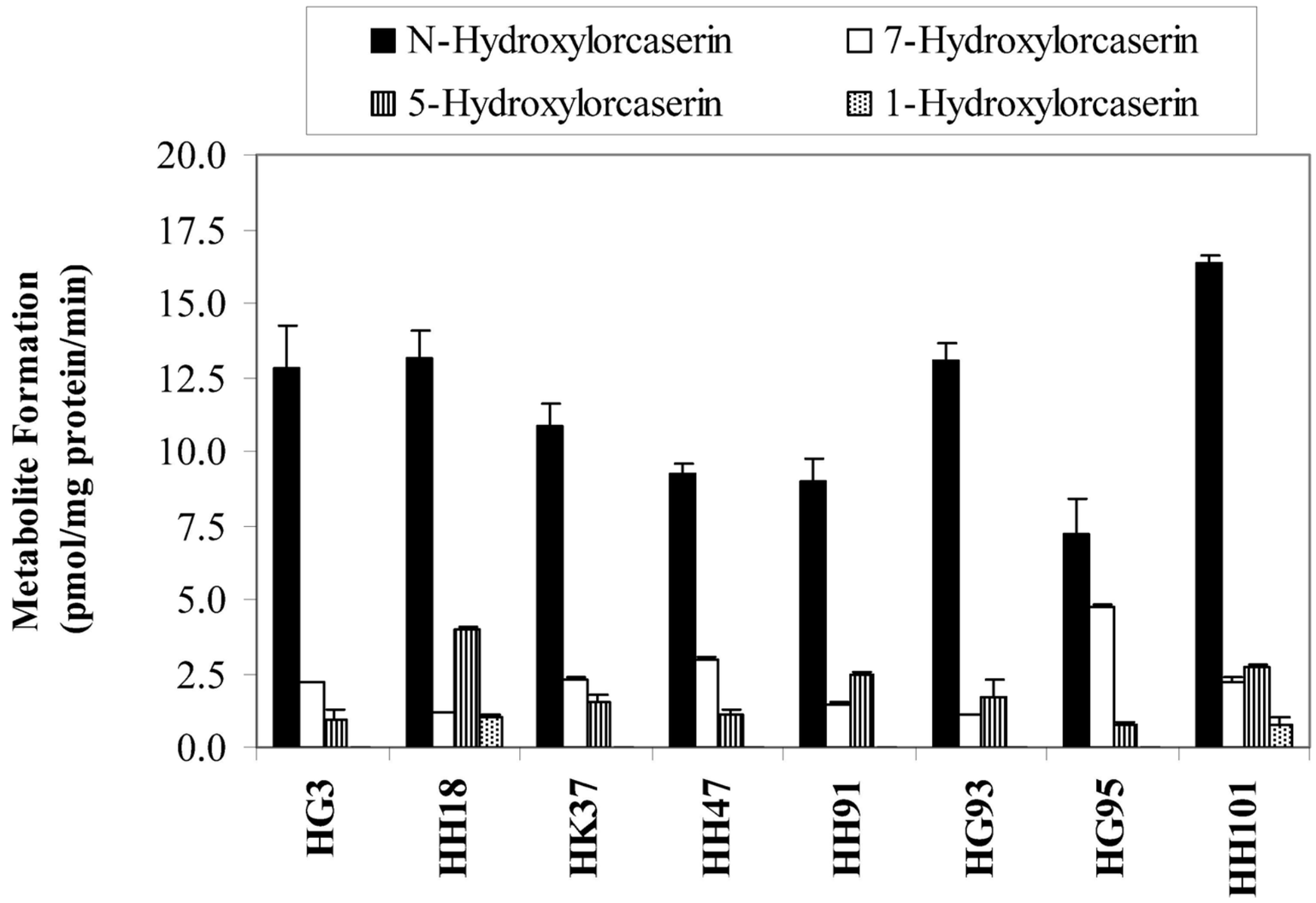
Fig. 4 (C) **(C) 10.0 µM Lorcaserin - Male**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 4 (D) **(D) 10.0 μM Lorcaserin - Female**

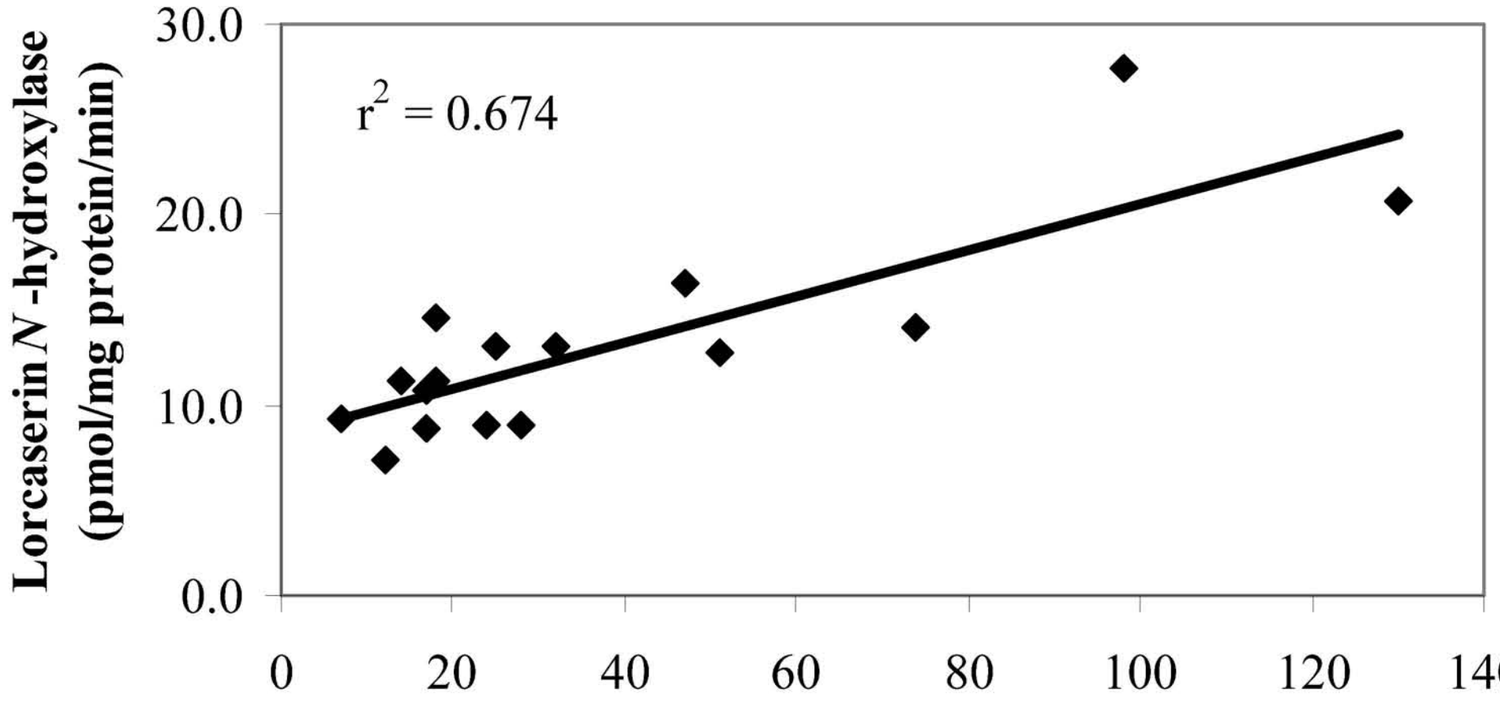


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 5 (A)

(A) *N*-Hydroxylorcaserin vs. CYP2B6 Activity

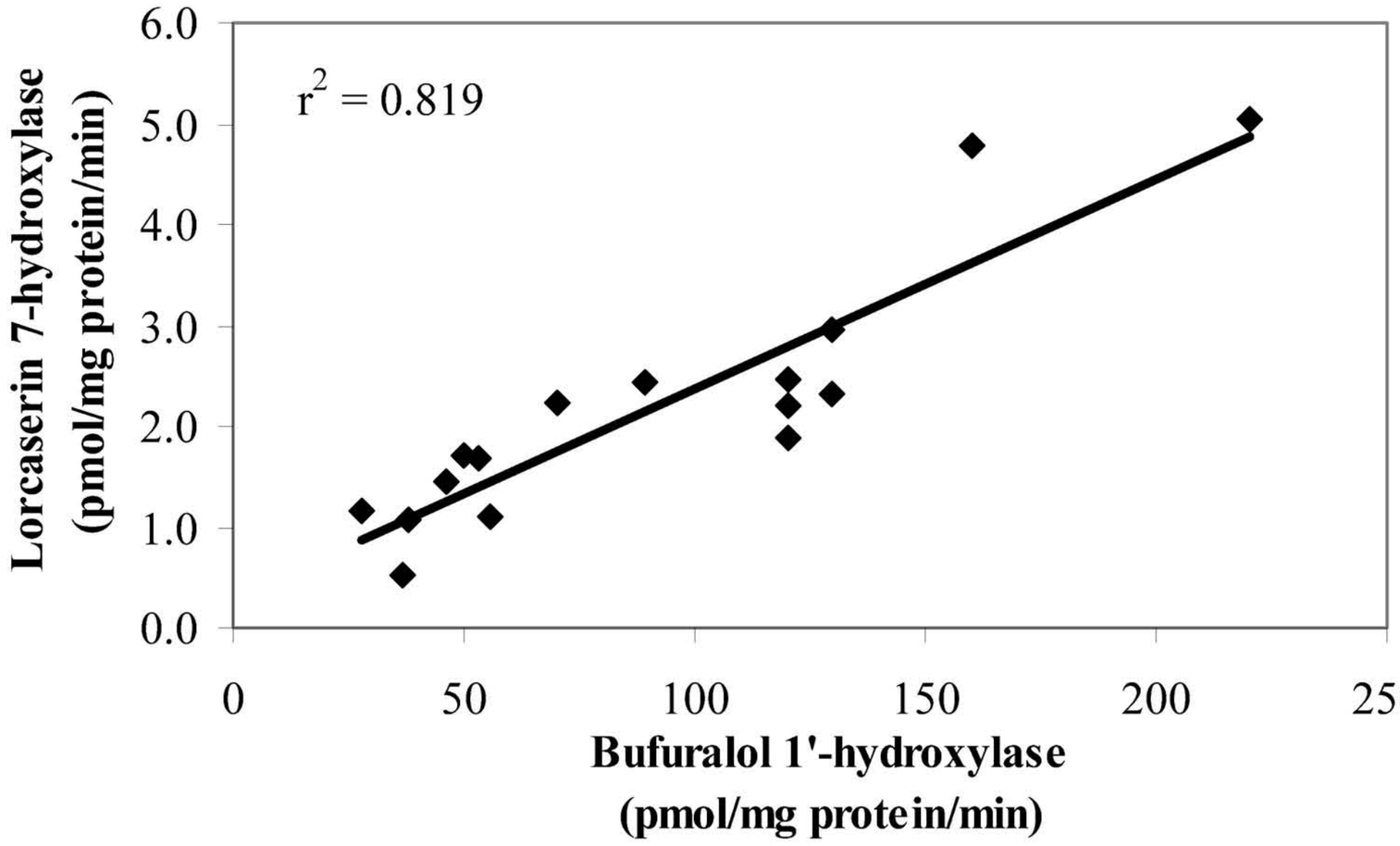


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 5 (B)

(B) 7-Hydroxylorcaserin vs. CYP2D6 Activity

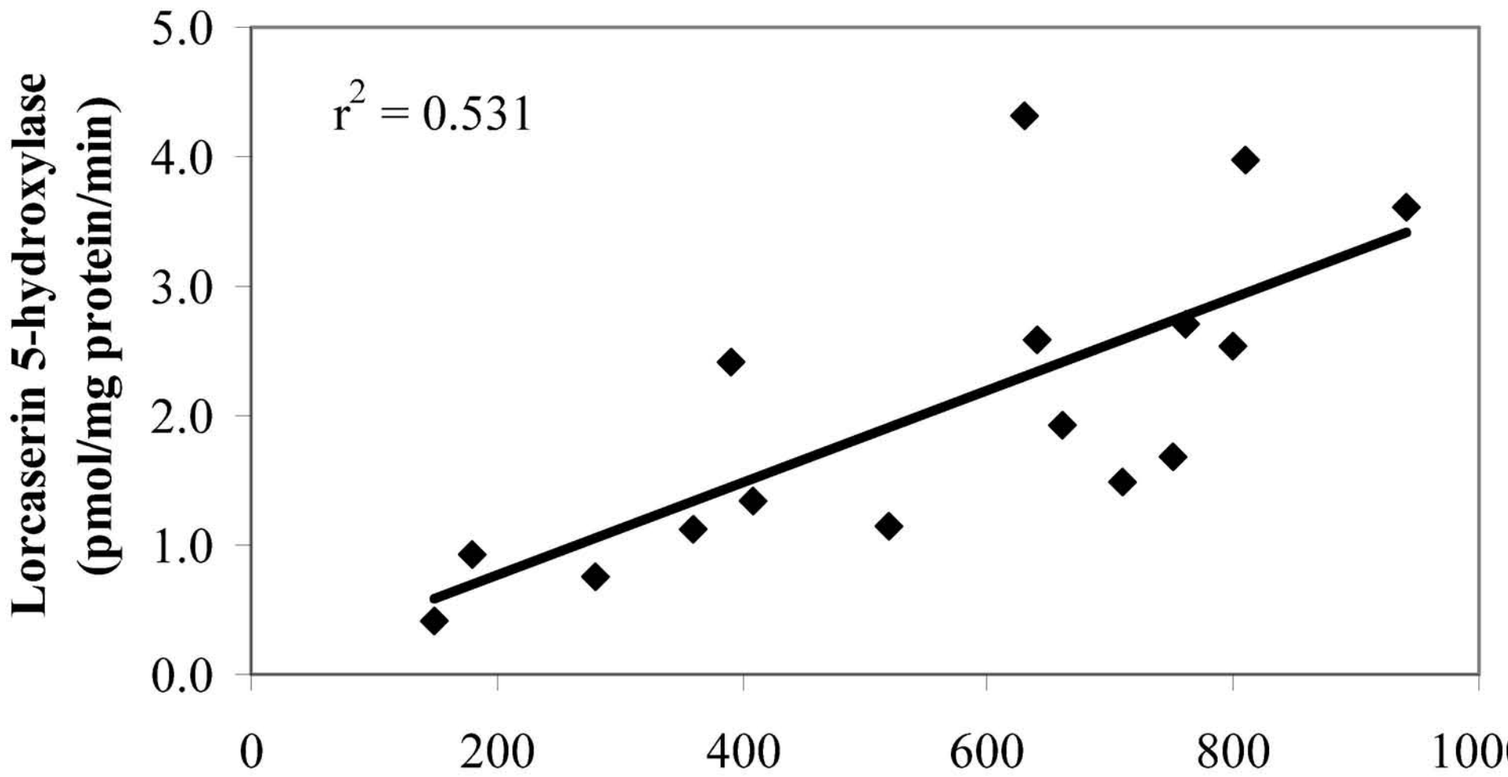


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 5 (C)

(C) 5-Hydroxylorcaserin vs. CYP1A2 Activity

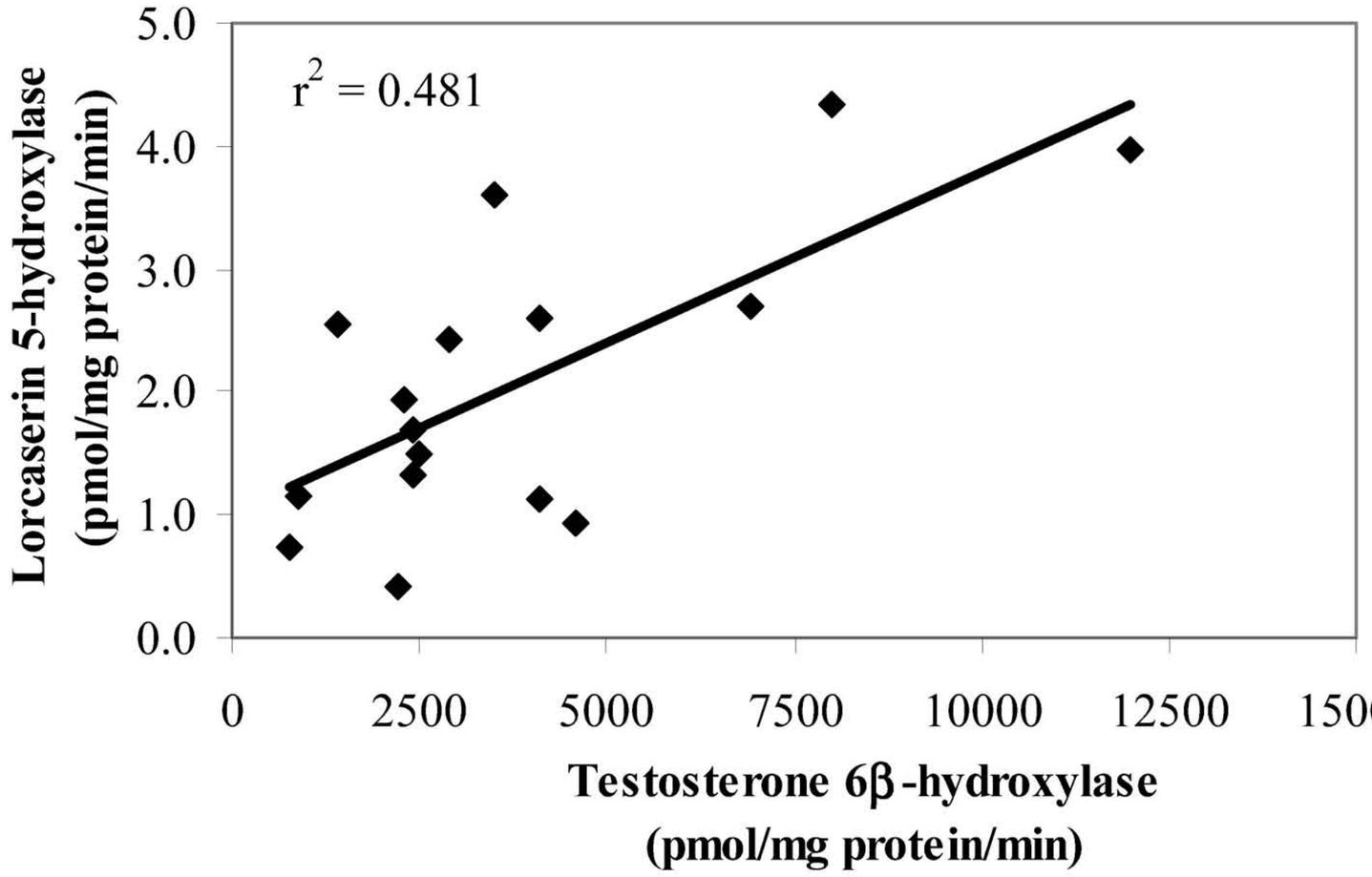


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig 5 (D)

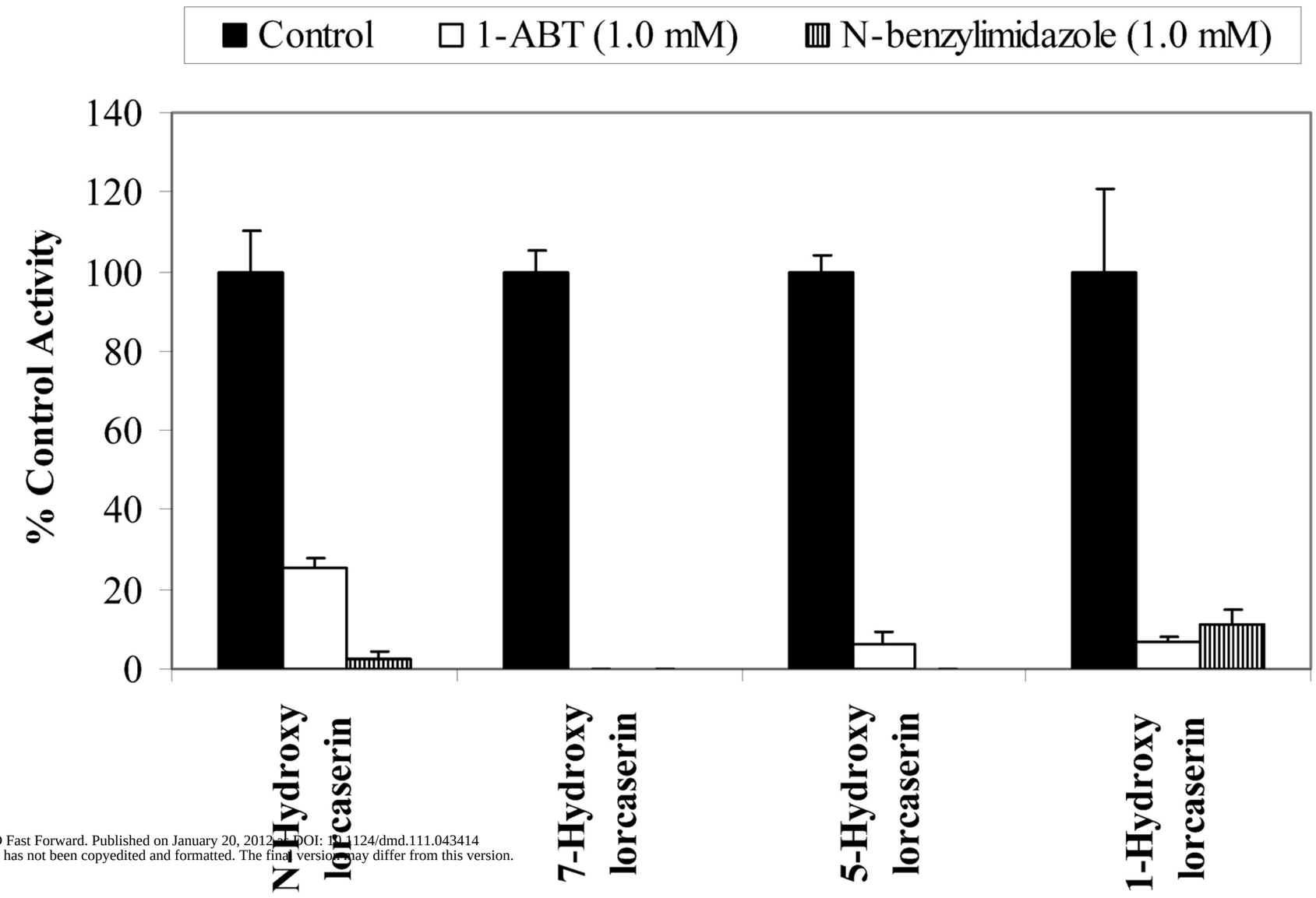
(D) 5-Hydroxylorcaserin vs. CYP3A4 Activity



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

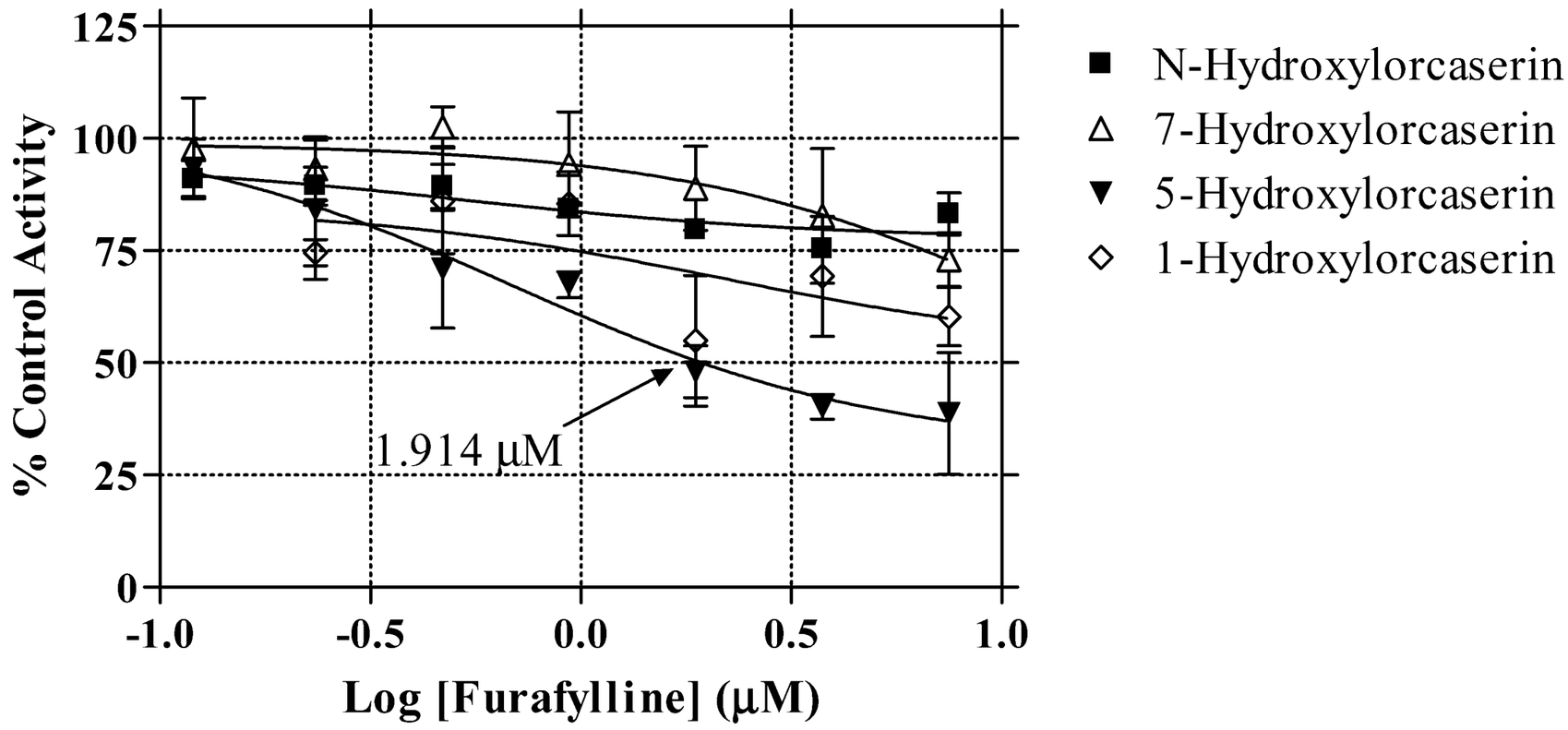
Fig. 6



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

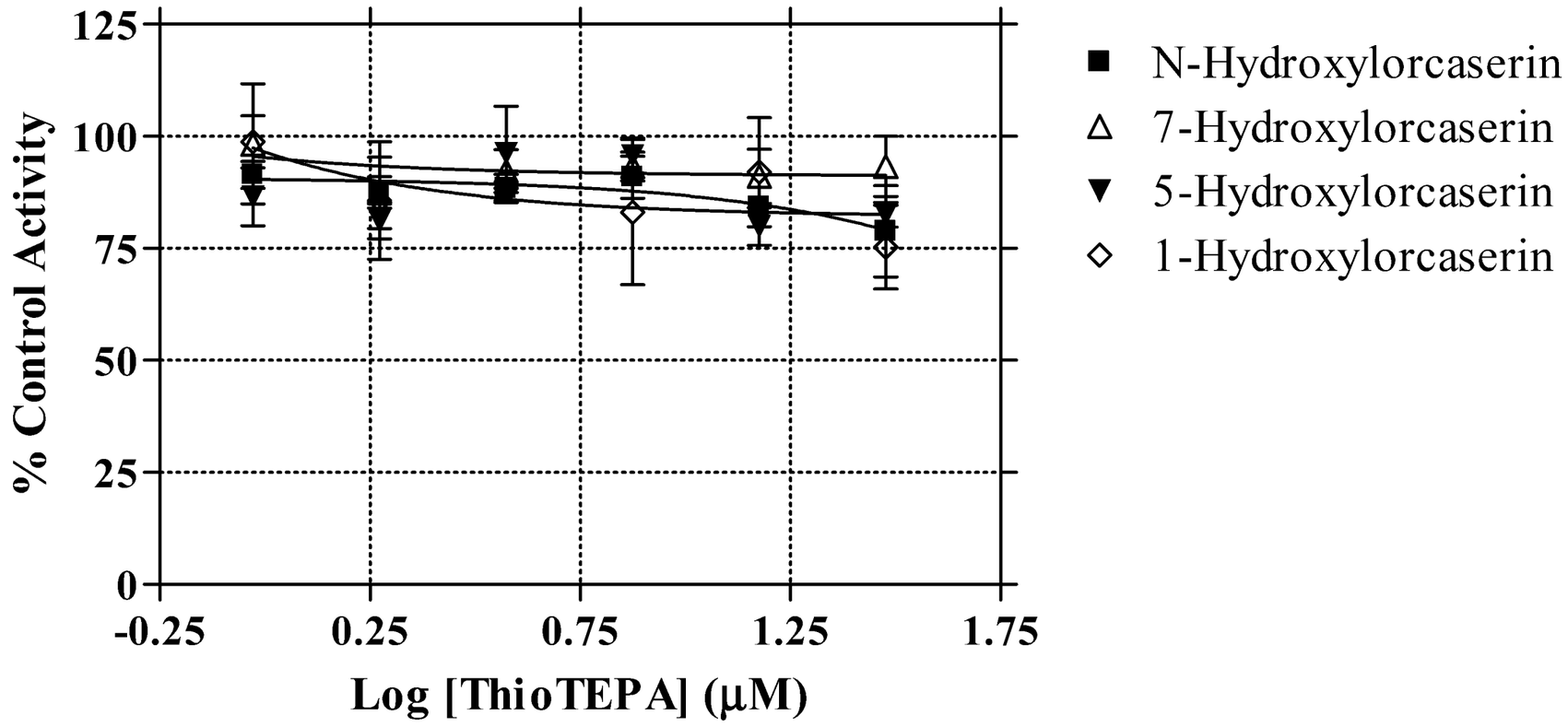
Fig. 7 (A) **(A) CYP1A2 Inhibitor Furafylline (10.0 μM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

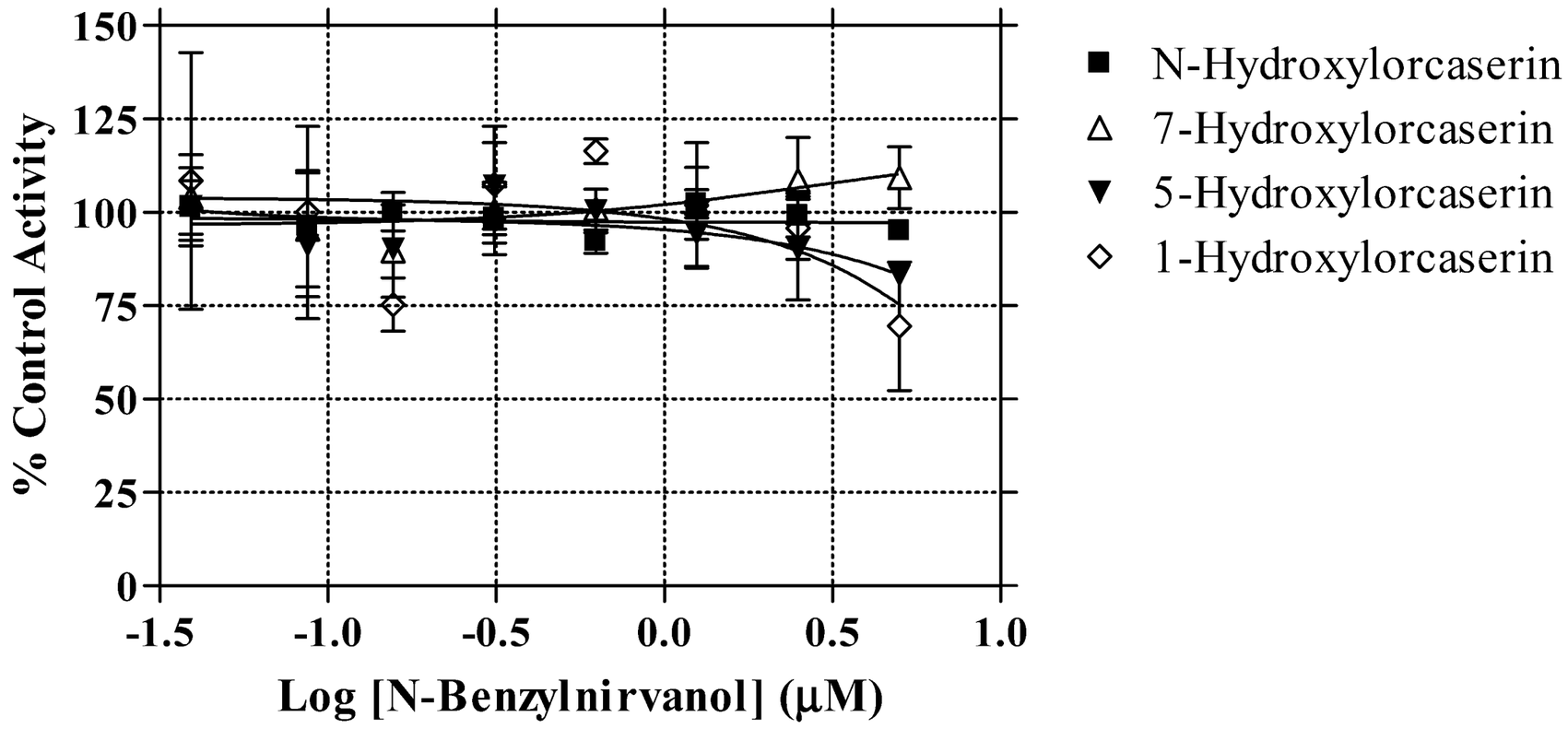
Fig. 7 (B) **(B) CYP2B6 Inhibitor ThioTEPA (10.0 μM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

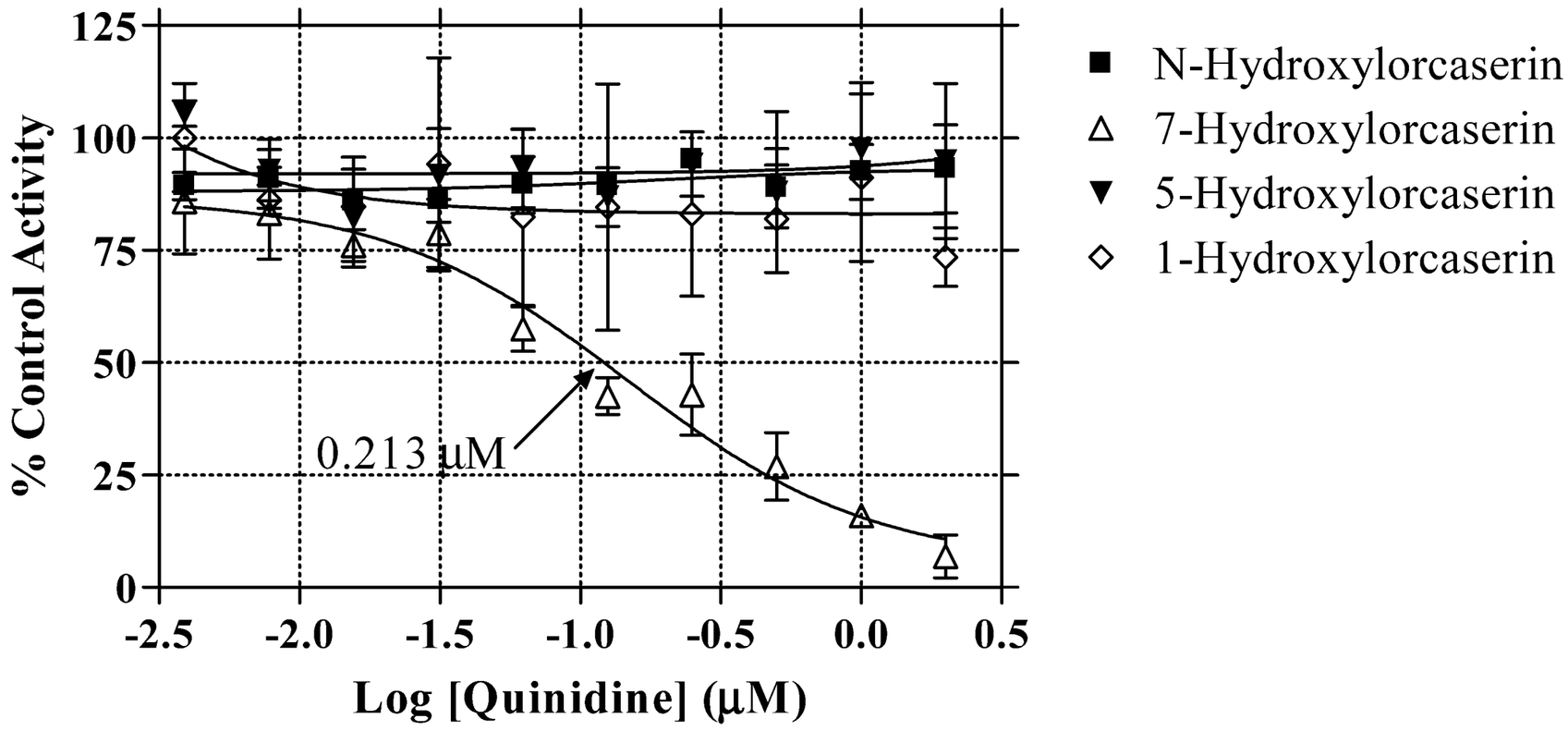
Fig. 7 (C) **(C) CYP2C19 Inhibitor *N*-benzylnirvanol (10.0 µM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

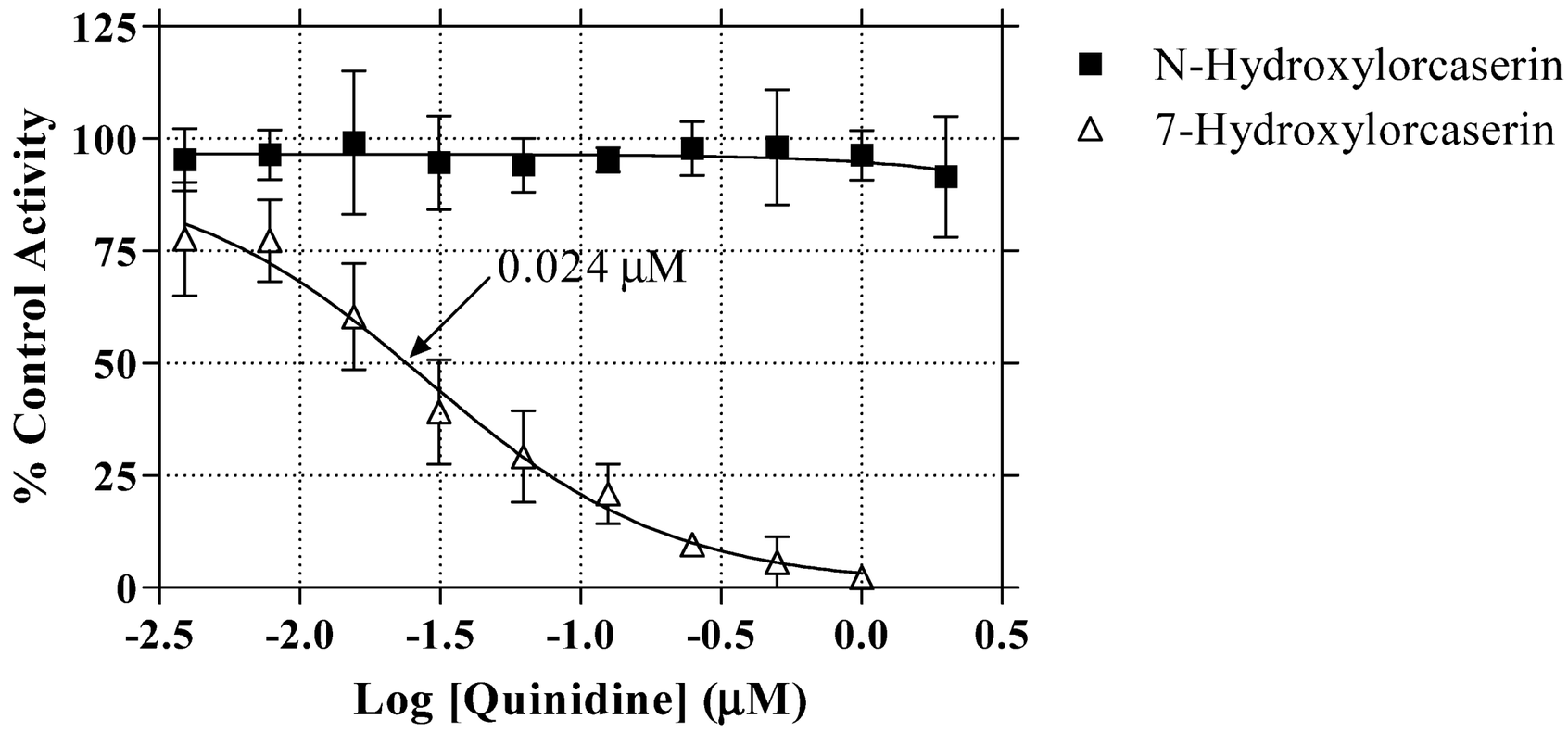
Fig. 7 (D) **(D) CYP2D6 Inhibitor Quinidine (10.0 µM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

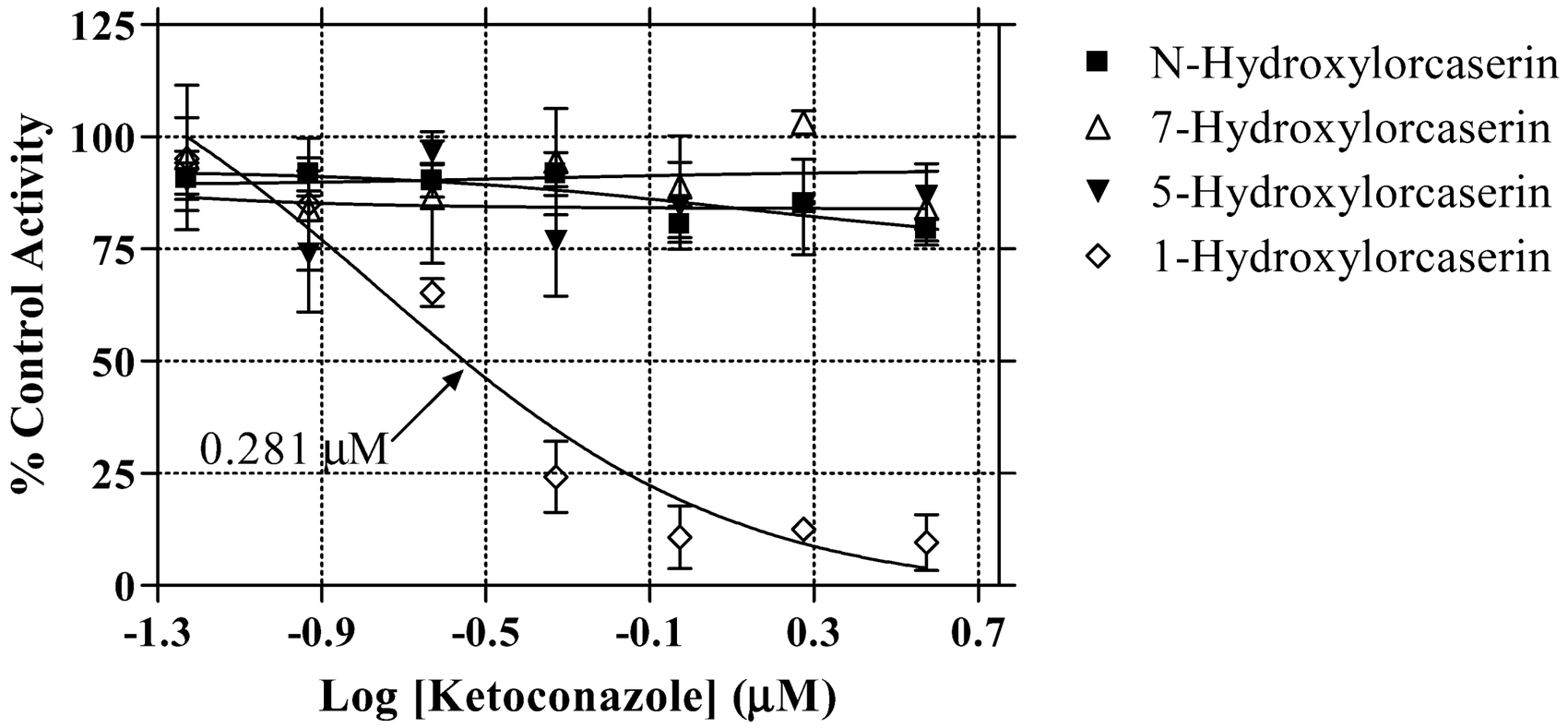
Fig. 7 (E) **(E) CYP2D6 Inhibitor Quinidine (1.0 μM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

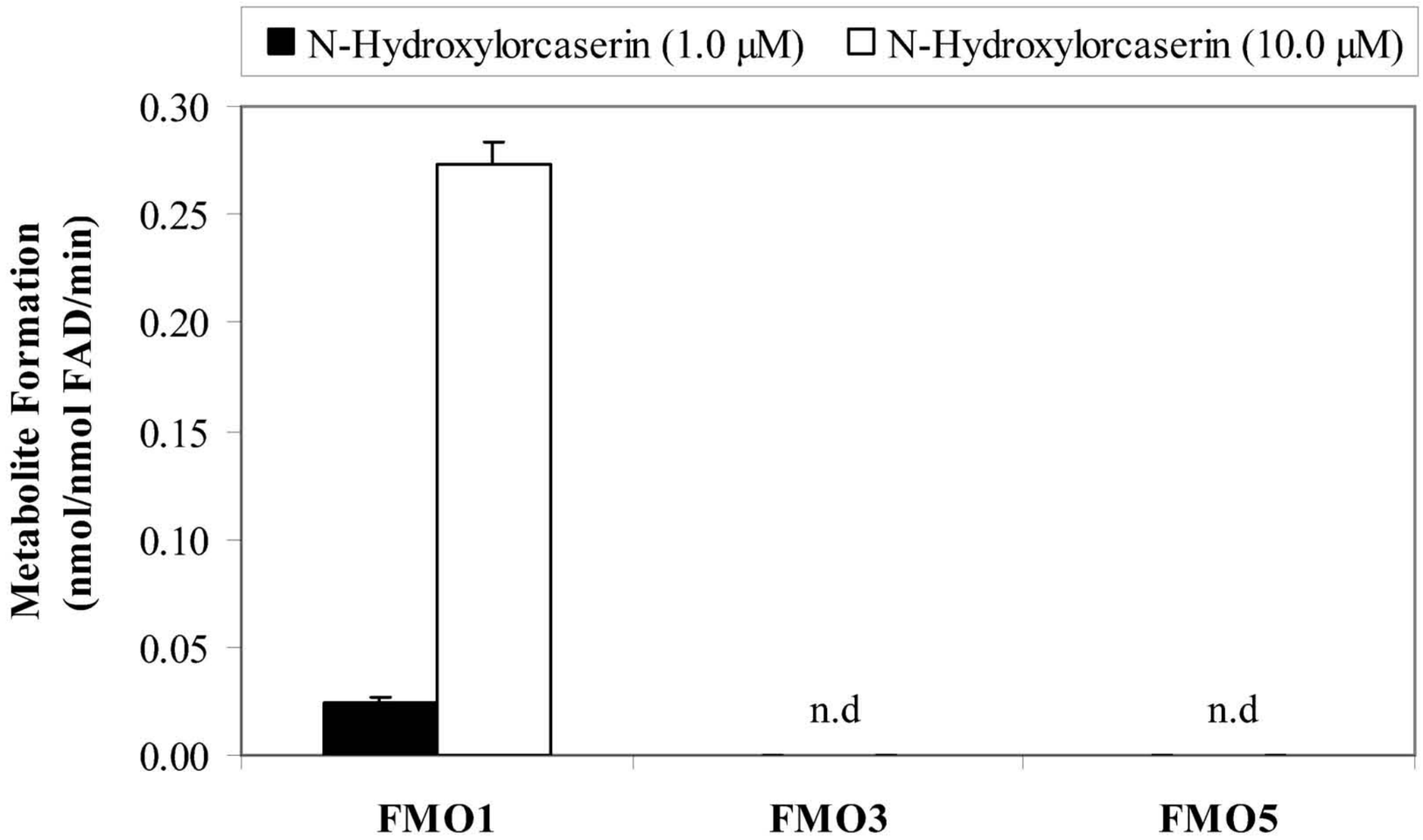
Fig. 7 (F) **(F) CYP3A4 Inhibitor Ketoconazole (10.0 µM Lorcaserin)**



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 8

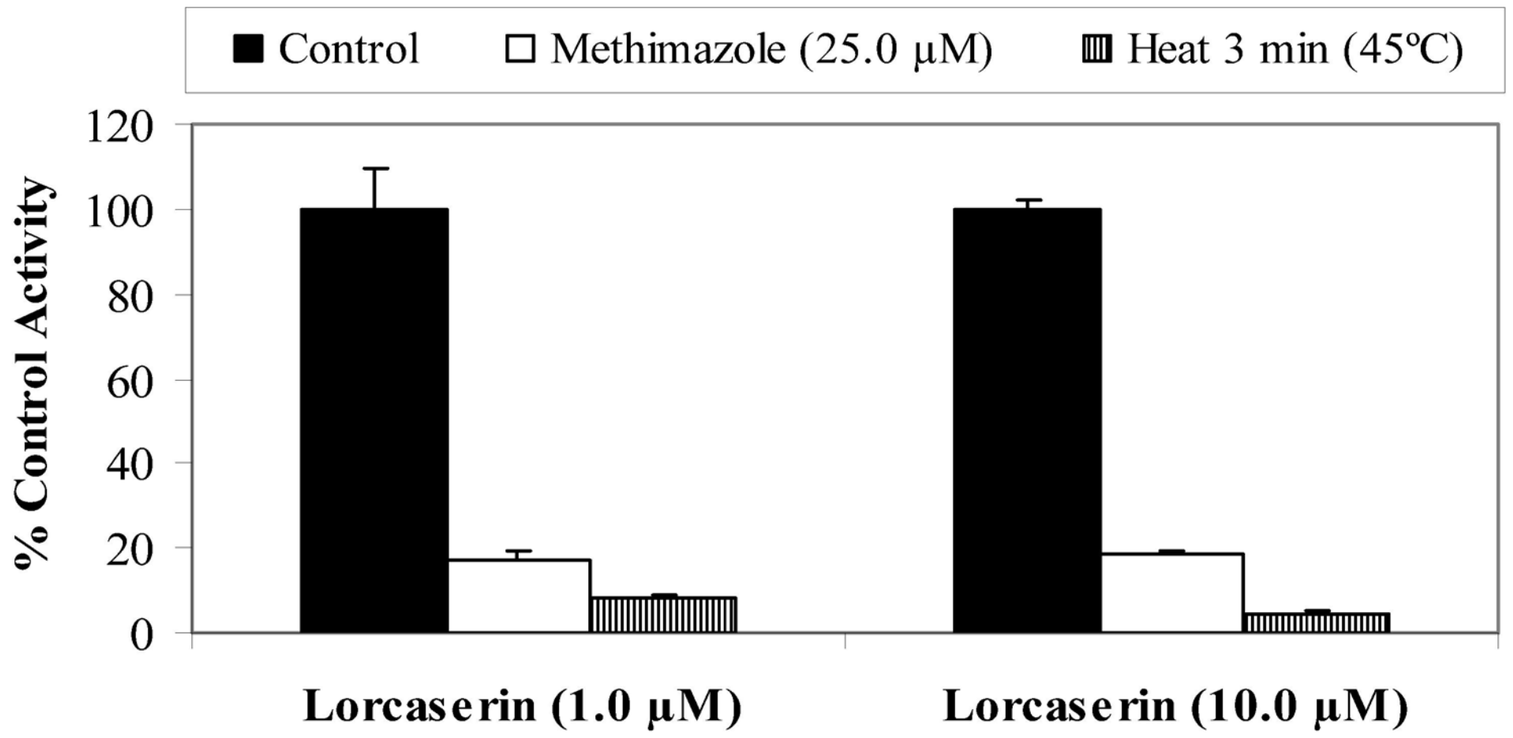


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 9 (A)

(A) Glycine buffer pH 8.5 (FMO)

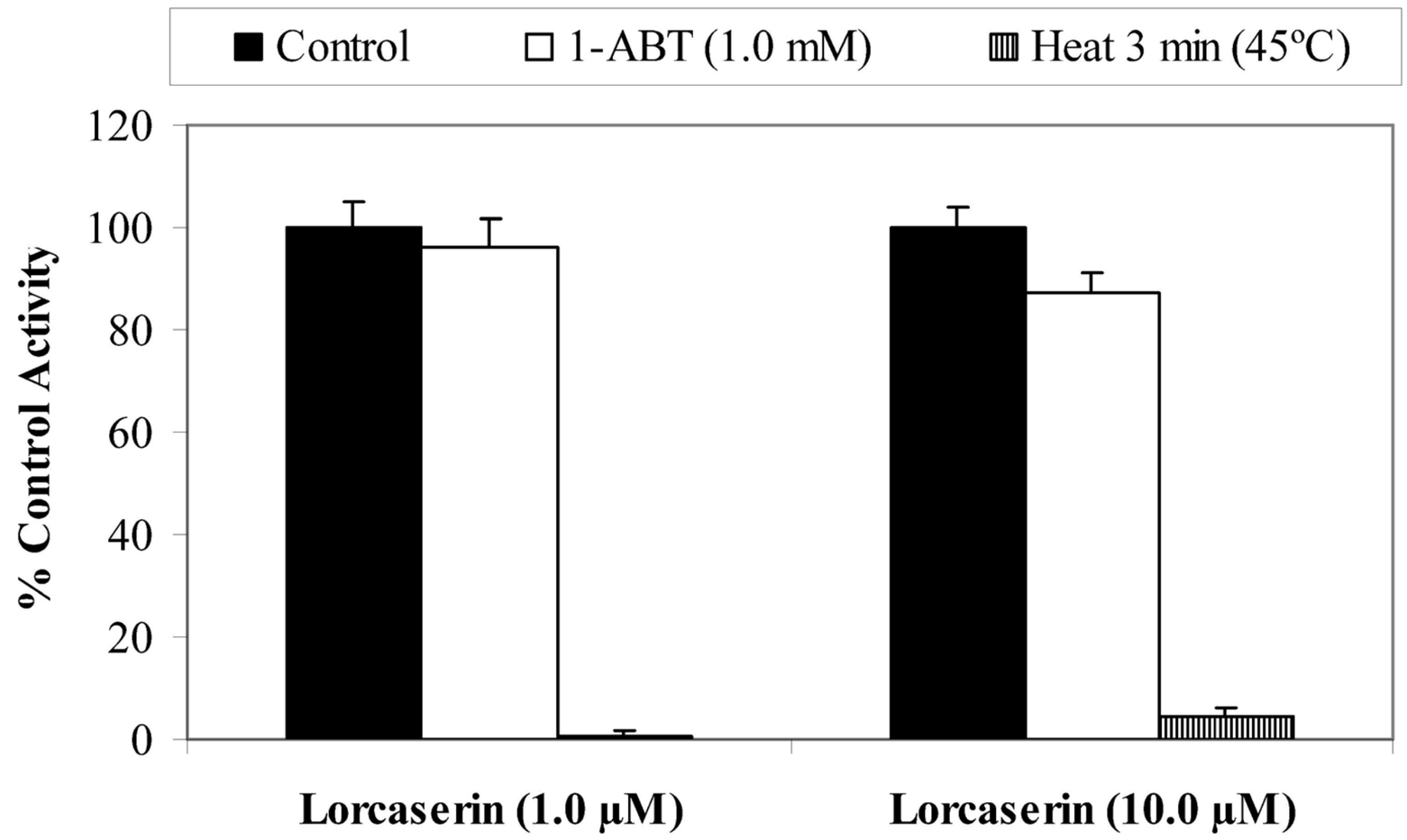


DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 9 (B)

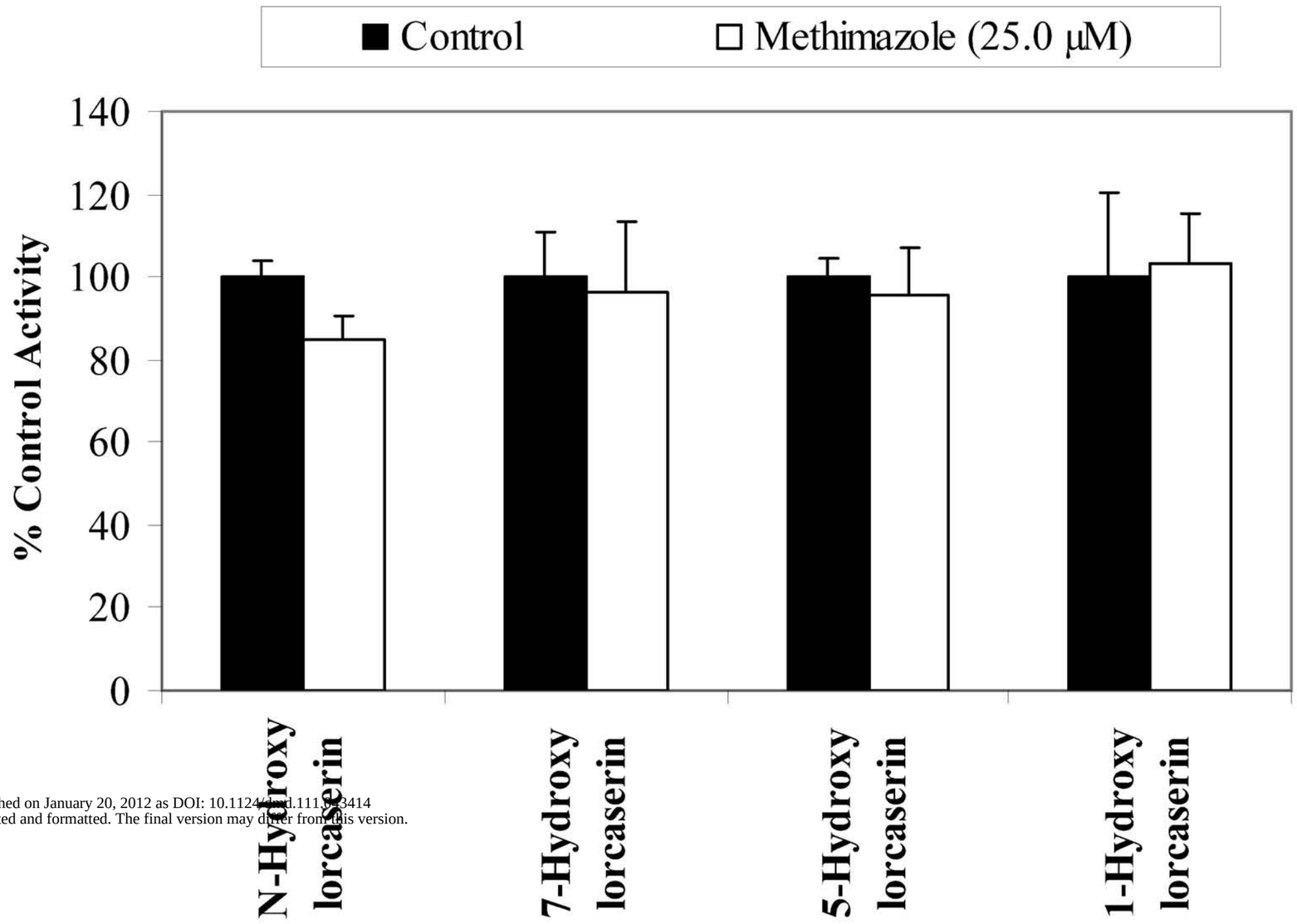
(B) Phosphate buffer pH 7.4 (P450)



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

Fig. 10



DMD Fast Forward. Published on January 20, 2012 as DOI: 10.1124/dmd.111.043414
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024