

# Lorcaserin, a Novel Selective Human 5-Hydroxytryptamine<sub>2C</sub> Agonist: in Vitro and in Vivo Pharmacological Characterization<sup>§</sup>

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## ABSTRACT

5-Hydroxytryptamine (5-HT)<sub>2C</sub> receptor agonists hold promise for the treatment of obesity. In this study, we describe the in vitro and in vivo characteristics of lorcaserin [(1*R*)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-3 benzazepine], a selective, high affinity 5-HT<sub>2C</sub> full agonist. Lorcaserin bound to human and rat 5-HT<sub>2C</sub> receptors with high affinity ( $K_i = 15 \pm 1$  nM,  $29 \pm 7$  nM, respectively), and it was a full agonist for the human 5-HT<sub>2C</sub> receptor in a functional inositol phosphate accumulation assay, with 18- and 104-fold selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors, respectively. Lorcaserin was also highly selective for human 5-HT<sub>2C</sub> over other human 5-HT receptors (5-HT<sub>1A</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4C</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub>), in addition to a panel of 67 other G protein-coupled receptors and ion channels. Lorcaserin did not compete for binding of ligands to serotonin, dopamine, and norepinephrine transporters, and it did not alter their function in vitro. Behavioral observations indicated that unlike

the 5-HT<sub>2A</sub> agonist ( $\pm$ )-1-(2,5-dimethoxy-4-phenyl)-2-aminopropane, lorcaserin did not induce behavioral changes indicative of functional 5-HT<sub>2A</sub> agonist activity. Acutely, lorcaserin reduced food intake in rats, an effect that was reversed by pretreatment with the 5-HT<sub>2C</sub>-selective antagonist 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl]indoline (SB242,084) but not the 5-HT<sub>2A</sub> antagonist (*R*)-(+)- $\alpha$ -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (MDL 100,907), demonstrating mediation by the 5-HT<sub>2C</sub> receptor. Chronic daily treatment with lorcaserin to rats maintained on a high fat diet produced dose-dependent reductions in food intake and body weight gain that were maintained during the 4-week study. Upon discontinuation, body weight returned to control levels. These data demonstrate lorcaserin to be a potent, selective, and efficacious agonist of the 5-HT<sub>2C</sub> receptor, with potential for the treatment of obesity.

Serotonin mediates its physiological effects through at least 14 different receptors. The serotonin 5-HT<sub>2</sub> receptor subfamily contains three distinct receptor subtypes, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub>, all of which share considerable sequence homology (>80% in transmembrane spanning regions) and activate common signaling pathways, including G<sub>q</sub> $\alpha$ -mediated stimulation of phospholipase-C $\beta$ , elevation of

intracellular inositol phosphates, and elevation of intracellular calcium (Roth et al., 1998). Human 5-HT<sub>2C</sub> receptors are predominately expressed in the CNS, and they are highly enriched in choroid plexus, prefrontal cortex, hippocampus, basal ganglia, and other brain regions associated with the control of mood, cognition, and appetite (Roth et al., 1998). Thus, 5-HT<sub>2C</sub> receptors have been proposed as a therapeutic target for the treatment of CNS disorders, including epilepsy, obsessive compulsive disorder, Parkinson's disease, schizophrenia, depression and anxiety, sleep disorders, and drug abuse (Tecott et al., 1995; Roth et al., 1998; Frank et al., 2002; Rocha et al., 2002; Meltzer et al., 2003; Isaac, 2005; Millan, 2005; Di Giovanni et al., 2006).

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**ABBREVIATIONS:** 5-HT, 5-hydroxytryptamine (serotonin); CNS, central nervous system; Ro60-0175, (S)-2-(6-chloro-5-fluorindol-1-yl)-1-methylethylamine; YM348, (S)-2-(7-ethyl-1*H*-furo[2,3-*g*]indazol-1-yl)-1-methylethylamine; WAY-161503, 8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one; WAY-163909, [(7*bR*,10*aR*)-1,2,3,4,8,9,10,10a-octahydro-7*bH*-cyclopenta-*b*][1,4]diazepino-[6,7,1*h*]indole]; VER2692, 1-methyl-2-pyrrolo[2,3-*f*]quinolin-1-yl-ethylamine; RS102221, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethyl-phenylsulfonamido)phenyl)-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione; HEK, human embryonic kidney; DMEM, Dulbecco's modified Eagle's medium; DOI, ( $\pm$ )-1-(2,5-dimethoxy-4-phenyl)-2-aminopropane; IP, inositol phosphate; MDL 100,907, (*R*)-(+)- $\alpha$ -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol; SB242,084, [6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl-carbamoyl]indoline]; GBR 12909, 1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine; ANOVA, analysis of variance; GPCR, G protein-coupled receptor; AUC, area under the curve.

A wealth of data also indicates that 5-HT<sub>2C</sub> agonists may provide an effective treatment for obesity, a disease that has reached epidemic proportions in many developed nations worldwide. 5-HT<sub>2C</sub> receptors are distributed in brain areas associated with regulation of food intake, including the nucleus of the solitary tract, dorsomedial hypothalamus, paraventricular hypothalamic nucleus, and the amygdala (Berthoud, 2002). 5-HT<sub>2C</sub> receptor knockout mice are hyperphagic, leading to obesity, partial leptin resistance, increased adipose deposition, insulin resistance, and impaired glucose tolerance (Tecott et al., 1995; Heisler et al., 1998; Nonogaki et al., 1998; Rosenzweig-Lipson et al., 2000; Tecott and Abdallah, 2003). Several studies have demonstrated that 1-(*m*-chlorophenyl)piperazine- and *d*-fenfluramine-induced weight loss in both rodents and humans is primarily due to activation of 5-HT<sub>2C</sub> receptors (Kennett and Curzon, 1991; Sargent et al., 1997; Vickers et al., 2001, 2003), a finding consistent with the observation that the weight-lowering actions of both mCPP and *d*-fenfluramine are absent in 5-HT<sub>2C</sub> knockout mice (Tecott et al., 1995; Vickers et al., 1999). Likewise, other 5-HT<sub>2C</sub> agonists (Ro 60-0175, YM348, WAY-161503, WAY-163909, and VER2692), also reduce food intake in animals and enhance satiety (Clifton et al., 2000; Rosenzweig-Lipson et al., 2000; Bentley et al., 2004; Hayashi et al., 2004; Dunlop et al., 2005). Rats fed a high fat diet have lower levels of hypothalamic 5-HT<sub>2C</sub> mRNA (Schaffhauser et al., 2002), and recent reports suggest that certain atypical antipsychotic drugs such as risperidone may cause weight gain via blockade of the 5-HT<sub>2C</sub> receptor (Reynolds et al., 2005). Last, the psychoactive substance 3,4-methylenedioxyamphetamine (ecstasy), promotes appetite suppression that is reversed by the 5-HT<sub>2C</sub>-selective antagonist RS102221 (Conductier et al., 2005). Several reviews regarding the utility of selective 5-HT<sub>2C</sub> agonists for the treatment of obesity have been published recently (Bickerdike, 2005; Miller, 2005; Halford, 2006; Nilsson, 2006; Smith et al., 2006).

Historically, a major obstacle limiting the development of 5-HT<sub>2C</sub> agonists has been a lack of selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors. Activation of 5-HT<sub>2A</sub> receptors can induce hallucinations (Nichols, 2004). The activation of 5-HT<sub>2B</sub> receptors has been implicated in cardiac valvular insufficiency and possibly in pulmonary hypertension associated with dexfenfluramine use (Fitzgerald et al., 2000; Rothman et al., 2000; Launay et al., 2002; Roth, 2007). Thus, discovery of selective 5-HT<sub>2C</sub> receptor agonists could lead to the development of efficacious antiobesity drugs that have the possibility of avoiding side effects associated with older classes of nonselective serotonergic modulators. In this communication, we describe the *in vitro* and *in vivo* characteristics of lorcaserin [(1*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-3 benzazepine], a selective and potent 5-HT<sub>2C</sub> agonist.

## Materials and Methods

### Drugs

The chemical structure of lorcaserin (molecular mass 195.69 Da) is shown in Fig. 1. The synthesis has been described previously (Smith et al., 2005). The following reagents were purchased from commercial suppliers: HEK293 and COS7 cells (American Type Culture Collection, Manassas, VA); fetal calf serum, Dulbecco's modified

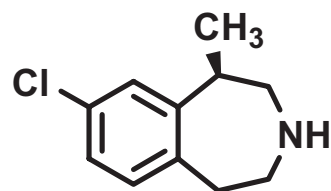


Fig. 1. Structure of lorcaserin (molecular mass 195.69 Da).

Eagle's medium (DMEM), Opti-MEM I, and Lipofectamine (Invitrogen, Carlsbad, CA); *myo*-[<sup>3</sup>H]inositol, and [<sup>125</sup>I]DOI (PerkinElmer Life and Analytical Sciences, Boston, MA). Reference 5-HT<sub>2</sub> compounds were either purchased from Tocris Cookson Inc. (Ellisville, MO) or Sigma/RBI (Natick, MA).

### In Vitro Pharmacology Experiments

**Cell Growth and Transient Transfection of Recombinant Human and Rat 5-HT<sub>2</sub> Receptors.** For development of functional human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> inositol accumulation assays, recombinant 5-HT<sub>2</sub> receptors were transiently expressed in HEK293 cells using the Lipofectamine method. In brief, cells were incubated in 15-cm plates with DMEM, and they were grown to at least 60 to 80% confluence, rinsed with Opti-MEM I media, followed by addition of 2 ml of Opti-MEM I containing 60  $\mu$ l of Lipofectamine and 16  $\mu$ g of cDNA for human and rat 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors or 15  $\mu$ l of Lipofectamine and 16  $\mu$ g of cDNA for human and rat 5-HT<sub>2C</sub> receptors. Cells were then incubated for 5 h at 37°C in a 5% CO<sub>2</sub> incubator. Next, cells were aspirated, and 30 ml of DMEM was added. Cells were then used for assays 24 h after transfection. Under these conditions, cells expressing human 5-HT<sub>2C</sub> receptor displayed constitutive elevation of inositol phosphate accumulation that was reversed by the inverse agonist mianserin, whereas cells expressing human 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors display considerably less constitutive activity (data not shown).

For development of human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptor radioligand binding assays, recombinant receptors were stably expressed in HEK293 cells using standard protocols, and crude plasma membranes were prepared from these cells. For development of rat 5-HT<sub>2</sub> radioligand binding assays, rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were transiently expressed in HEK293 cells using the Lipofectamine method as described above.

**Preparation of Plasma Membranes from Cells Expressing Recombinant 5-HT<sub>2</sub> Receptors.** HEK293 cells stably expressing recombinant human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were collected, washed with ice-cold phosphate-buffered saline, pH 7.4, and then they were centrifuged at 48,000g for 20 min at 4°C. The cell pellet was then suspended in ice-cold wash buffer containing 20 mM HEPES, pH 7.4, and 0.1 mM EDTA, homogenized on ice using a Polytron (Brinkman Instruments, Westbury, NY), and centrifuged at 48,000g for 20 min at 4°C. The resultant pellet was then suspended in ice-cold 20 mM HEPES, pH 7.4, homogenized on ice, and centrifuged (48,000g for 20 min at 4°C). Crude membrane pellets were stored at -80°C until they were used for radioligand binding assays.

**[<sup>125</sup>I]DOI Binding to Recombinant Human and Rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> Receptors.** Radioligand binding assays for human and rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> receptors were developed using the 5-HT<sub>2</sub> agonist [<sup>125</sup>I]DOI as radioligand, and nonspecific radioligand binding was determined in the presence of 10  $\mu$ M unlabeled DOI. Competition experiments consisted of addition of 95  $\mu$ l of assay buffer (20 mM HEPES, pH 7.4, and 10 mM MgCl<sub>2</sub>), 50  $\mu$ l of membranes (5–25  $\mu$ g of protein), 50  $\mu$ l of [<sup>125</sup>I]DOI (0.5 nM final assay concentration), and 5  $\mu$ l of test compound diluted in assay buffer (final concentrations ranging from 1 pM to 10  $\mu$ M) to 96-well GF/C microtiter plates (PerkinElmer Life and Analytical Sciences), and incubations were performed for 1 h at room temperature. Each lorcaserin radioligand competition study consisted of testing eight different concentrations in which triplicate determinations were per-

formed for each test compound concentration. Assay incubations were terminated by rapid filtration of microtiter plates under vacuum pressure using a cell harvester (Brandel Inc., Gaithersburg, MD), followed by washing filter plates several times with ice-cold wash buffer (50 mM Tris-HCl, pH 7.4). Plates were then dried at 45°C in an oven for a minimum of 2 h, 25  $\mu$ l of BetaScint scintillation cocktail was added to each well, and microtiter plates were counted in a Packard TopCount scintillation counter (PerkinElmer Life and Analytical Sciences).

**Radioligand Binding Assays for Additional Human 5-HT Receptors and Neurotransmitter Transporters.** Evaluations of lorcaserin competition for radioligand binding to human 5-HT<sub>3</sub>, 5-HT<sub>4C</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors as well as serotonin, dopamine, and norepinephrine transporters were performed at Cerep, Inc. (Portiers, France) (see Supplemental Table 1 for general assay conditions) except for 5-HT<sub>1A</sub>, which was performed at Arena Pharmaceuticals using the same protocol described in Supplemental Table 1 except that assay buffer contained 50 mM Tris-HCl, pH 7.4, 2.5 mM MgCl<sub>2</sub>, and 1 mM EDTA. In particular, competition studies consisted of testing of lorcaserin at eight different concentrations in triplicate determinations were made at each concentration.

**[<sup>3</sup>H]5-Hydroxytryptamine, [<sup>3</sup>H]Norepinephrine, and [<sup>3</sup>H]-Dopamine Uptake and Release from Rat Synaptosomes.** These studies were performed at Cerep, Inc. (see Supplemental Table 2 for general assay conditions). Lorcaserin was tested at eight different concentrations in which duplicate determinations were made at each test concentration.

**5-HT<sub>2</sub> Inositol Phosphate Accumulation Assays.** On the day before experiments, cells expressing receptors of interest were removed from cell culture plates by treatment with 2 ml of trypsin, additional growth medium was added to dilute the trypsin, and then cells were diluted to a final concentration of 550,000 cells/ml using growth medium. Subsequently, 55,000 cells in total were added to 96-well microtiter plates pretreated with poly-D-lysine, and they were incubated for 5 h at 37°C with 5% CO<sub>2</sub>. One hundred microliters of inositol-free Dulbecco's modified Eagle's medium (titrated to pH 7.4 with sodium bicarbonate) containing 40  $\mu$ Ci of [<sup>3</sup>H]inositol was then added to each well, and plates were incubated overnight at 37°C with 5% CO<sub>2</sub>.

The next day, unincorporated [<sup>3</sup>H]inositol was carefully removed from wells by aspiration, and it was replaced with 90  $\mu$ l of inositol-free Dulbecco's modified Eagle's medium (titrated to pH 7.4 with sodium bicarbonate) also containing 10 mM LiCl and 10  $\mu$ M pargyline followed by addition of 10  $\mu$ l of test compound (1 pM–10  $\mu$ M, triplicate determinations at each concentration) containing a final concentration of 0.5% dimethyl sulfoxide. Assay plates were then incubated for 2 h at 37°C with 5% CO<sub>2</sub>, and then the reaction mixture was removed by gentle aspiration. Then, 160  $\mu$ l of stop solution containing 0.1 M formic acid was added to each well, and plates were frozen at –80°C overnight to promote cell lysis.

The following day, assay plates were thawed and [<sup>3</sup>H]inositol was separated from [<sup>3</sup>H]inositol phosphates by Dowex resin chromatography. Then, 200  $\mu$ l of activated resin slurry was added to 96-well Whatman Unifilter plates (Whatman, Florhampark, NJ), cell lysate was added to plates, and wells were washed twice with 200  $\mu$ l of water under vacuum pressure followed by elution of [<sup>3</sup>H]inositol phosphates with 180  $\mu$ l of low-salt elution buffer. The eluant was then transferred to clean 96-well microtiter plate, and sealed plates were then incubated overnight in an oven at 45°C. Plates were then counted in a Packard TopCount scintillation counter after addition of 50  $\mu$ l/well of HiSafe3 scintillation cocktail. Dose-response evaluations involved testing lorcaserin at eight different concentrations in which triplicate determinations were made at each test concentration.

**In Vitro Pharmacology Data Analysis.** For radioligand binding experiments, IC<sub>50</sub> values were obtained by fitting radioligand competition data to a sigmoidal function using a nonlinear least-squares program (Prism; GraphPad Software Inc., San Diego, CA).

In all cases, data produced a better fit to a single-site model than a two-site model (data not shown). The same nonlinear curve fitting program was used to fit [<sup>125</sup>I]DOI saturation data for 5-HT<sub>2</sub> receptors to a simple hyperbolic function for determination of  $K_d$  and  $B_{max}$  values (data not shown).  $K_i$  values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). For IP accumulation, EC<sub>50</sub> values were also determined by fitting data to sigmoidal function using the same nonlinear least-squares curve-fitting program.

## Pharmacokinetic Experiments

**Oral Plasma and Brain Pharmacokinetics in the Rat.** Male Sprague-Dawley rats (250–300 g) were obtained from Harlan (San Diego, CA). On the day of the experiment, rats received a single dose of lorcaserin in water via oral gavage. Dosing volume and dose were 5 ml/kg and 10 mg/kg, respectively. Lorcaserin was formulated in deionized water. The following sampling scheme was used ( $n = 3$ /time point): 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 40 h after dose. Rats were lightly anesthetized with isoflurane, blood was collected via cardiac puncture, and brains were removed from the cranium. Blood samples were dispensed into sodium-heparinized vials, and then vials were capped and stored at 4°C. Plasma was separated from formed elements in blood by centrifugation (10 min at 3000g), and then plasma was frozen. Brains were rinsed with ice-cold phosphate-buffered saline, blotted dry, weighed, and frozen. Plasma and brain samples were stored at –80°C before bioanalytical analysis.

**Bioanalytical Method.** Plasma samples were thawed on ice, and a 50- $\mu$ l aliquot was transferred to a 1-ml plastic tube. An acetonitrile (110  $\mu$ l) containing internal standard was added to the plasma, vortexed, and centrifuged (10 min at 3000g). A standard curve (1–2000 ng/ml) and quality control samples (3, 300, and 1500 ng/ml) were prepared in sodium-heparinized rat plasma in a similar manner. The resultant supernatants were analyzed for lorcaserin.

Brain samples were thawed on ice, placed into 50-ml plastic conical tubes, and 2 volumes of purified water per gram of brain was added. Brains were homogenized using a mechanical variable speed tissue homogenizer; 50  $\mu$ l of brain homogenate was transferred to a 1-ml plastic tube. An acetonitrile (110  $\mu$ l)-containing internal standard was added to brain homogenate, vortexed, and centrifuged (10 min at 3000g). A standard curve (1–2000 ng/ml) and quality control samples (3, 300, and 1500 ng/ml) were prepared in control brain homogenate in a similar manner. The resultant supernatants were analyzed for lorcaserin.

Processed plasma and brain samples were analyzed for lorcaserin and internal standard by liquid chromatography-tandem mass spectrometry. The LC separation used a binary gradient and C18 reverse phase column [Luna 3  $\mu$ m C18 (2), 50  $\times$  2 mm; Phenomenex, Torrance, CA] equipped with a C18 guard column (2  $\times$  4 mm; Phenomenex). The column was kept at a constant temperature of 35°C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. The flow rate was held constant at 0.5 ml/min. The following binary gradient was used for sample analysis: column equilibration for 1 min at 10% mobile phase B; after injection, mobile phase B was held constant for 0.3 min at 10%, increased linearly in 1.7 min to 90% B, and kept constant at 90% B for 1 min.

Detection of lorcaserin and internal standard was achieved using electrospray ionization (TurboIonSpray; Applied Biosystems, Foster City, CA) operating in positive ion mode. The source temperature was set to 350°C, with an ion spray voltage of 3400 V. Multiple reaction monitoring of the mass transition 196.1 atomic mass units to 176.1 atomic mass units was used for detection of lorcaserin. Lorcaserin and internal standard eluted within 1 min after injection.

The liquid chromatography-tandem mass spectrometric system consisted of an API4000 mass spectrometer (MDS Sciex, Concord, ON, Canada), a CTC HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), two single-channel LC10AD VP high-performance liquid chromatography pumps, DGU-14A degasser, and a SCL10A VP high-performance liquid chromatography controller (Shimadzu, Kyoto, Japan),



a Cheminert Divert Valve (Valco Instruments Co., Houston, TX), and PEEK mixing tee (Alltech Associates, Deerfield, IL).

**Pharmacokinetic Data Analysis.** The lorcaserin noncompartmental plasma and brain pharmacokinetics were determined using the program WinNonlin version 4.0.1 (Pharsight, Mountain View, CA). The area under the curve plasma concentration-time profile was established by linear trapezoidal rule. The terminal phase half-life was calculated using at least three data points in the terminal phase excluding the  $C_{max}$ . Standard deviations are represented by error bars on all pharmacokinetic figures.

## In Vivo Pharmacological Experiments

**Animals and Housing.** Male Sprague-Dawley rats (Harlan), weighing 250 to 300 g, were used for acute food intake and behavioral studies. Upon arrival at the test facility, animals were housed in groups of two within a holding room controlled for temperature and light (lights off 10:30 AM–6:30 PM for food intake studies; lights off 6:30 PM–6:30 AM for behavioral observations). All food intake studies were conducted during the dark phase, whereas behavioral observations were conducted during the light phase. Rats were allowed ad libitum access to food and water unless stated otherwise.

For chronic studies, male obesity prone Sprague-Dawley rats in the weight range of 150 to 200 g were obtained from Charles River (Indianapolis, IN), triple housed in shoebox cages, and maintained within a holding room controlled for temperature and light on reversed phase lighting, with lights off for 7 h from 9:00 AM. Animals were handled and given free access to a pelleted high fat diet (D12266; Research Diets, New Brunswick, NJ; 31.6 kilocalorie percentage of fat) for 8 weeks before, and for the duration of the study. At study onset subjects weighed between 350 and 500 g.

**Drug Administration.** Lorcaserin and DOI were dissolved in sterile water, whereas MDL 100,907 and SB242,084 were finely suspended in a 1% Tween 80 aqueous solution. Lorcaserin was administered p.o. in a volume of 2 ml/kg. DOI was administered s.c. in a volume of 1 ml/kg. MDL100,907 and SB242,084 were administered p.o. in a volume of 2 ml/kg. All drug doses are expressed as that of the salt.

**Study 1. Effect of Lorcaserin on Food Intake in Rats.** On the day of testing, animals were weighed, single housed in cages with grid floors at 8:30 AM, and they were allowed to acclimate to these cages for a 90-min period, with free access to water and no food. At 10:00 AM, rats were administered vehicle or lorcaserin (3, 6, 12, or 24 mg/kg) via oral gavage. Thirty minutes after administration (10:30 AM), lights were turned off, and animals were allowed access to food. Food and water were then weighed at 2, 4, 6, and 22 h after food exposure. Animals were weighed again at 22 h after food presentation.

**Study 2. Effects of 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> Antagonists on Lorcaserin-Induced Hypophagia.** Procedures were the same as described above (study 1) except that antagonists (vehicle; 1 mg/kg i.p. SB242,084; or 0.5 mg/kg i.p. MDL 100,907) were injected 15 min before p.o. administration of lorcaserin or vehicle. Food intake was then measured after a 60-min period after lights out (90 min after administration of lorcaserin).

**Study 3. Chronic Lorcaserin Administration in Male Diet-Induced Obesity Rats: Effect on Food Intake, Body Weight, and Body Composition during Treatment and Withdrawal.** At the start of the study, 56 male rats were weighed, single-housed, and allocated to one of seven weight-matched treatment groups, each containing eight animals. Rats then underwent a 1-week period of habituation to oral gavage dosing. Treatment groups were as follows: vehicle (p.o., b.i.d.) and lorcaserin (4.5, 9, and 18 mg/kg p.o., bi.d.). An additional two groups of rats received either vehicle or the highest dose of lorcaserin according to the same treatment regimen. These rats were later used to assess body weight and food intake during withdrawal (see below). All treatments were administered daily just before lights off, and again before lights on. Body weight and food and water intake were measured every day before morning injections.

After 28 days of dosing, all subjects except those in the withdrawal groups were sacrificed, and body composition was measured using dexascan analysis (see below). Food and water intake and body weight of the rats in the withdrawal groups were continuously monitored for a further period of 28 days subsequent to drug dosing.

**Body Composition Analysis.** Postmortem body fat and nonfat mass were measured by dual-energy X-ray absorptiometry with the Prodigy Advance scanner (Prodigy Advance, GEMS Lunar; GE Healthcare, Madison, WI) using software designed for rats. The DEXA scanner was calibrated to quantify nonfat mass as lean tissue composed of 60% water, excluding bone mass.

**Study 4. Behavioral Observations after Lorcaserin Administration: Comparison with the 5-HT<sub>2A</sub> Agonist DOI.** For the measurement of behavioral signs, subjects were administered either lorcaserin (vehicle, 4.5, 9, or 18 mg/kg) via oral gavage, or DOI (vehicle or 1 mg/kg) via the subcutaneous route, and they were placed individually in Plexiglas observation chambers (15 × 22 × 36 cm), with a mirror placed behind the chamber to allow an all around view of behavior. Observations started immediately upon placement in the chamber. Over a 60-min period, the following behaviors were scored: penile grooming (number of distinct bouts of penile grooming accompanied by erection), wet dog shakes (total number), and back muscle contractions (total number). Scores of "active" or "resting/inactive" were also scored as present or absent within 5-min time bins, resulting in a total possible score of 12 for each. The experimenter was unaware of drug treatment at the time of testing.

**In Vivo Data Analysis.** *Study 1.* Cumulative food intake (grams) was subjected to separate one-way analyses of variance (ANOVAs) at each time point (2, 4, 6, and 22 h). Actual food intake data were subjected to two-way repeated measures ANOVA (dose and time as factors).

*Study 2.* Food intake over the 1-h consumption (grams) was subjected to two-way analysis of variance (lorcaserin and antagonist as factors). Separate ANOVAs were conducted for each antagonist.

*Study 3.* Food intake and body weight data for all rats over the 28-day dosing period were subjected to a two-way ANOVA (day and treatment factors). For rats that subsequently underwent withdrawal, food intake and body weight data were averaged for each week of withdrawal, and these data were subjected to a two-way ANOVA with week and treatment as factors.

*Study 4.* Data for which behaviors were counted (e.g., wet dog shakes) were subjected to a one-way ANOVA. Remaining data that were scored as present or absent within time bins (e.g., inactive) were subjected to square root transformation, and a one-way ANOVA. Because lorcaserin and DOI included their own vehicle controls, they were subjected to separate analyses. Where appropriate, significant main effects for all data were followed by post-hoc comparisons using the Newman-Keuls test.

## Results

### In Vitro Pharmacology

**Radioligand Binding and Functional Activity of Lorcaserin at 5-HT<sub>2</sub> Receptors.** The binding affinity of lorcaserin for recombinant human and rat 5-HT<sub>2</sub> receptors is summarized in Table 1. Lorcaserin exhibited higher affinity for human 5-HT<sub>2C</sub> than to 5-HT<sub>2A</sub> (7.5-fold selective) and 5-HT<sub>2B</sub> (11.6-fold selective) as indicated by  $K_i$  values of 15, 112, and 174 nM, respectively. Lorcaserin also bound to rat 5-HT<sub>2C</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2B</sub> receptors, with  $K_i$  values of 29, 159, and 190 nM. The (*S*)-isomer of lorcaserin had similar affinities as the (*R*)-isomer (data not shown). Furthermore, the major circulating metabolite of lorcaserin does not appreciably bind to human 5-HT<sub>2</sub> receptors (data not shown). To establish the affinity of lorcaserin for 5-HT<sub>2C</sub> receptors in a tissue naturally expressing endogenous human 5-HT<sub>2C</sub> re-

TABLE 1

Radioligand binding, functional potency, and efficacy of lorcaserin for human and rat 5-HT<sub>2</sub> receptors

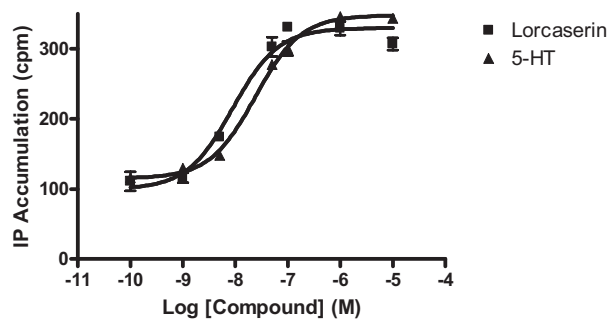
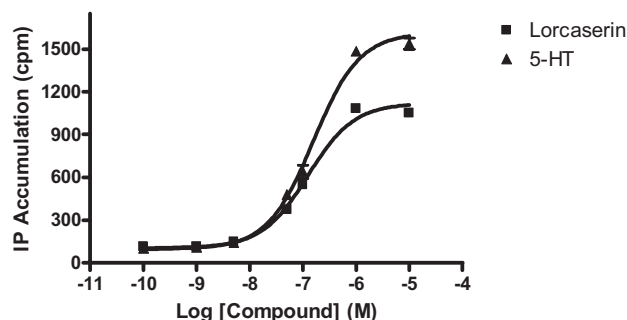
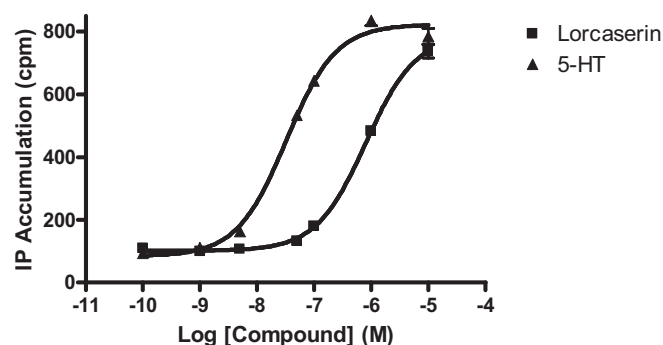
Values listed in this table are the mean  $\pm$  S.E.M. of the indicated number of independent IC<sub>50</sub> (radioligand binding assay) and EC<sub>50</sub> (IP accumulation assay) determinations (in parentheses). Triplicate determinations were performed at each of eight different test compound concentrations. K<sub>i</sub> values from radioligand competition studies with [<sup>125</sup>I]DOI were calculated from IC<sub>50</sub> values as described under *Materials and Methods*. Efficacy (the % 5-HT response column) was determined by comparison with 10  $\mu$ M 5-HT, which was assigned a value of 100%.

Receptor	Affinity K <sub>i</sub>	Potency EC <sub>50</sub>	Efficacy (% 5-HT Response)
	<i>nM</i>		
Human 5-HT <sub>2C</sub>	15 $\pm$ 1 (28)	9 $\pm$ 0.5 (40)	100
Human 5-HT <sub>2A</sub>	112 $\pm$ 7 (25)	168 $\pm$ 11 (34)	75
Human 5-HT <sub>2B</sub>	174 $\pm$ 32 (18)	943 $\pm$ 90 (35)	100
Rat 5-HT <sub>2C</sub>	29 $\pm$ 7 (7)	193 $\pm$ 12 (4)	100
Rat 5-HT <sub>2A</sub>	159 $\pm$ 27 (14)	676 $\pm$ 129 (3)	100
Rat 5-HT <sub>2B</sub>	190 $\pm$ 5 (3)	272 $\pm$ 87 (4)	100

ceptor, [<sup>125</sup>I]DOI competition binding experiments were performed using human choroid plexus membranes. A K<sub>i</sub> value of 9.4 nM was obtained for 5-HT<sub>2C</sub> from choroid plexus (data not shown).

Agonist activation of 5-HT<sub>2</sub> receptors increases intracellular inositol phosphates and calcium (Roth et al., 1998). The functional activity of lorcaserin for human 5-HT<sub>2C</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2B</sub> receptors was tested using inositol phosphate accumulation assays. Lorcaserin possesses agonist activity at all three human 5-HT<sub>2</sub> receptors (Fig. 2), but the potency for activating 5-HT<sub>2C</sub> receptors was substantially higher than 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors. Data from several independent evaluations are summarized in Table 1. Lorcaserin was 18-fold more potent at human 5-HT<sub>2C</sub> (EC<sub>50</sub> = 9 nM) than at human 5-HT<sub>2A</sub> (EC<sub>50</sub> = 168 nM), and it was 104-fold more potent at human 5-HT<sub>2C</sub> than human 5-HT<sub>2B</sub> (EC<sub>50</sub> = 943 nM). The functional selectivity of lorcaserin for rat 5-HT<sub>2C</sub> receptor compared with rat 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors was less than observed for human receptors. The efficacy of lorcaserin compared with serotonin indicates full agonist activity at the human 5-HT<sub>2C</sub> and 5-HT<sub>2B</sub> receptors and partial agonist activity at the human 5-HT<sub>2A</sub> receptor (75% of the maximal response elicited by serotonin). Taking these data together, both radioligand and functional data indicate that lorcaserin is a potent and selective full 5-HT<sub>2C</sub> agonist. For comparison, EC<sub>50</sub> values for several different reference 5-HT<sub>2</sub> agonists obtained in human 5-HT<sub>2</sub> IP assays are included in Supplemental Table 3.

**Competition of Lorcaserin for Radioligand Binding to Several Other Human 5-HT Receptors and Neurotransmitter Transporters.** To further evaluate the selectivity of lorcaserin for human 5-HT<sub>2C</sub> relative to other additional human serotonin receptors and neurotransmitter transporters (serotonin, dopamine, and norepinephrine transporters), K<sub>i</sub> values were obtained from competitive radioligand binding experiments for the receptors and transporters shown in Table 2 (see Supplemental Material for general assay conditions). The affinity of lorcaserin for each of these targets was low relative to the reference compounds used in the same assay. The selectivity of lorcaserin for 5-HT<sub>2C</sub> receptor relative to an additional 67 human GPCRs was also evaluated. Lorcaserin did not inhibit radioligand binding by more than 31% to any of these additional receptors at a final assay concentration of

A Human 5-HT<sub>2C</sub>B Human 5-HT<sub>2A</sub>C Human 5-HT<sub>2B</sub>

**Fig. 2.** Representative EC<sub>50</sub> curves for 5-HT- and lorcaserin-mediated stimulation of inositol phosphate accumulation in HEK293 cells transiently expressing human 5-HT<sub>2C</sub> (A), 5-HT<sub>2A</sub> (B), and (C) 5-HT<sub>2B</sub> receptors. EC<sub>50</sub> values of 8 nM (5-HT<sub>2C</sub>), 157 nM (5-HT<sub>2A</sub>), and 804 nM (5-HT<sub>2B</sub>) were obtained for lorcaserin compared with EC<sub>50</sub> values of 24 nM (5-HT<sub>2C</sub>), 124 nM (5-HT<sub>2A</sub>), and 32 nM (5-HT<sub>2B</sub>) obtained for 5-HT.

1  $\mu$ M (see Supplemental Tables 4 and 5 for assay conditions and results, respectively).

**Influence of Lorcaserin on Functional Activity of Human Monoamine Transporters.** Lorcaserin was also tested to determine whether it alters the functional activity of serotonin, dopamine, and norepinephrine transporters in rat synaptosomal preparations (Table 3; see Supplemental Material for assay conditions) in comparison with reference compounds tested in the same assay. Lorcaserin did not stimulate [<sup>3</sup>H]norepinephrine, [<sup>3</sup>H]dopamine, or [<sup>3</sup>H]serotonin release from rat synaptosomes at submicro-

TABLE 2

Summary of  $K_i$  values of lorcaserin using radioligand binding competition with human recombinant 5-HT receptors and neurotransmitter transporters

Data represent  $K_i$  values derived from one determination in which eight test lorcaserin concentrations and eight reference compound concentrations were used. Triplicate determinations were made at each compound concentration.  $K_i$  values were determined for lorcaserin and the respective reference compounds.

Receptor	Lorcaserin $K_i$	Reference Compound ( $K_i$ )
		<i>nM</i>
Human 5-HT <sub>1A</sub>	700	8-Hydroxy-2-dipropylaminotetralin (0.9)
Human 5-HT <sub>3</sub>	4430	MDL 72222 (7)
Human 5HT <sub>4e</sub>	18,900	5-HT (330)
Human 5-HT <sub>5A</sub>	3710	5-HT (69)
Human 5-HT <sub>6</sub>	1980	5-HT (110)
Human 5-HT <sub>7</sub>	636	5-HT (0.4)
Human norepinephrine transporter	1400	Protriptyline (15)
Human dopamine transporter	33,000	1-[1-(2-Benzo[ <i>b</i> ]thienyl)cyclohexyl]piperidine (6.8)
Human 5-HT transporter	990	Imipramine (1.3)

TABLE 3

Effect of lorcaserin on release and uptake of 5-HT, norepinephrine, and dopamine from rat brain synaptosomes

Data represent  $EC_{50}$  (neurotransmitter release) or  $IC_{50}$  (neurotransmitter uptake) values derived from one determination in which eight concentrations of lorcaserin or reference compound were used. Duplicate determinations were made at each compound concentration.

Neurotransmitter	Stimulation of Release		Inhibition of Uptake	
	Lorcaserin $EC_{50}$	Reference Compound ( $EC_{50}$ )	Lorcaserin $IC_{50}$	Reference Compound ( $IC_{50}$ )
			<i>nM</i>	
5-HT	49,000	Fenfluramine (470)	1400	Imipramine (34)
Norepinephrine	>100,000	Amitriptyline (35,000)	12,000	Protriptyline (15)
Dopamine	23,000	Amphetamine (1000)	2500	GBR 12909 (6)

molar concentrations. In particular, lorcaserin was approximately 100-fold less potent than fenfluramine at promoting serotonin release. Lorcaserin was also evaluated for its ability to inhibit neurotransmitter uptake into rat brain synaptosomes. Lorcaserin weakly inhibited norepinephrine ( $EC_{50} = 2500$  nM), serotonin ( $EC_{50} = 1400$  nM), and dopamine ( $EC_{50} = 12,000$  nM) uptake. In each case, lorcaserin was much less effective than the reference compound tested in the same assay, and it displayed activity only at concentrations 100 times the lorcaserin  $K_i$  value for the 5-HT<sub>2C</sub> receptor.

## Pharmacokinetics

### Oral Plasma and Brain Pharmacokinetics in the Rat.

Lorcaserin brain and plasma pharmacokinetics were evaluated after a single oral administration of 10 mg/kg in male Sprague-Dawley rats ( $n = 3$ /time point) (Fig. 3; Table 4). Lorcaserin absorption from the gastrointestinal tract into the systemic circulation was rapid, resulting in a mean maximum concentration ( $C_{max}$ ) of 0.760  $\mu$ g/ml at 0.25 h (the first time point collected). The time to brain maximal exposure was 1 h. The mean maximum brain concentrations were 8.0-fold greater than plasma (e.g., 6.1  $\mu$ g/g for brain at 1 h and 0.760  $\mu$ g/ml for plasma at 0.25 h). This ratio changed only slightly throughout the time course (after 0.25 h), where brain/plasma ratios at each time point ranged from 11.5 to 17.3. The ratio for brain-to-plasma exposure ( $AUC_{INF}$ ) was 13.3. The elimination half-life and mean residence time of lorcaserin in the brain (4.7 and 6.1 h, respectively) were similar to the values for lorcaserin in the systemic circulation (4.9 and 5.4 h, respectively).

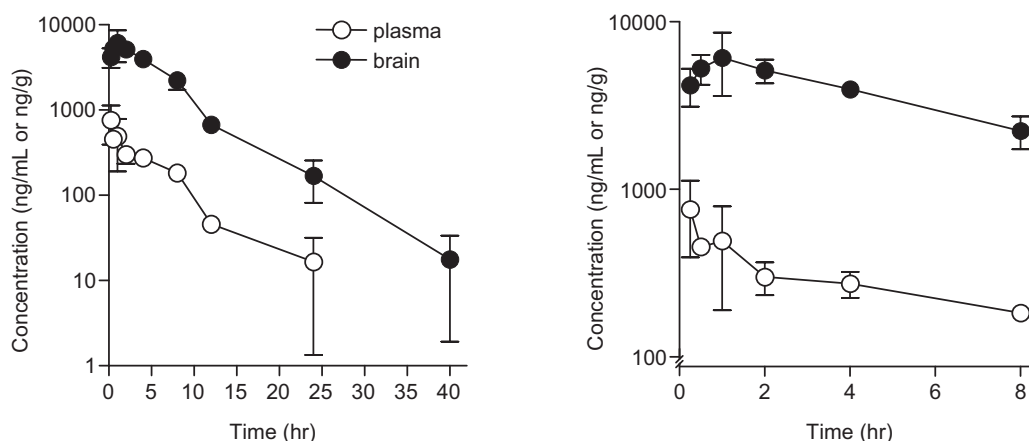
## In Vivo Pharmacology

**Study 1: Effect of Lorcaserin on Acute Food Intake in Rats.** Food intake was measured in rats after a single oral administration of lorcaserin (Fig. 4). Lorcaserin decreased

cumulative food intake at 2, 4, 6, and 22 h [ $F(4,35) = 42.9$ , 13.2, 10.2, and 6.1, respectively;  $p < 0.01$ ] (Fig. 4A), with a significant decrease continuing throughout the 22-h duration of the study at the highest dose (24 mg/kg) only. Using the cumulative food intake data, a single dose  $ED_{50}$  of 18 mg/kg was derived at the 6-h time point (Fig. 4B). A two-way ANOVA of absolute (noncumulative) food intake over time demonstrated a significant effect of lorcaserin [ $F(4,35) = 6.06$ ;  $p < 0.01$ ], and a significant interaction of lorcaserin with time [ $F(12,105) = 9.1$ ;  $p < 0.01$ ] (data not shown). Post-hoc analysis at each time point indicated that the decreased cumulative food intake over the duration of the study was accounted for by a significant decrease during the first 2 h only; food intake over subsequent measurement times was not different from that of vehicle-treated rats, and the maintained effect on cumulative intake was due to a lack of compensatory hyperphagia.

**Study 2: Selective Inhibition of Lorcaserin-Induced Hypophagia with a 5-HT<sub>2C</sub> Antagonist and Not a 5-HT<sub>2A</sub> Antagonist.** To establish receptor subtypes mediating the hypophagic effect of lorcaserin, the selective 5-HT<sub>2C</sub> antagonist SB242,084 and the selective 5-HT<sub>2A</sub> antagonist MDL 100,907 were tested for their ability to inhibit lorcaserin-induced (18 mg/kg, oral administration) reductions in food intake (Fig. 5). ANOVA revealed a significant main effect of SB242,084 [ $F(1,28) = 64.8$ ;  $p < 0.01$ ] and a significant interaction with lorcaserin [ $F(1,28) = 12.0$ ;  $p < 0.01$ ], but no effect for MDL 100,907 [ $F(1,28) = 2.7$ ; N.S.]. For SB242,084, post-hoc analyses revealed a significant attenuation of the decrease in food intake induced by lorcaserin, in addition to a small but significant ( $p = 0.049$ ) rise in baseline food intake with administration of SB242,084 alone. These data indicate that the reduction in food intake induced by lorcaserin is mediated by 5-HT<sub>2C</sub> and not 5-HT<sub>2A</sub> receptors.





**Fig. 3.** Lorcaserin plasma and brain concentrations after a single oral administration at 10 mg/kg to male rats. The concentrations were calculated per milliliter of plasma or per gram of brain, respectively. Mean  $\pm$  S.D.;  $n = 3$ /time point.

**TABLE 4**

Pharmacokinetic parameters for lorcaserin after a single oral dose (10 mg/kg) in male Sprague-Dawley rats

Parameter	Plasma	Brain
$\lambda_z$ (h <sup>-1</sup> )	0.142	0.148
$t_{1/2}$ (h)	4.9	4.7
AUC <sub>last</sub> (h · $\mu$ g/ml or g)	3.202	44.010
AUC <sub>INF</sub> (h · $\mu$ g/ml or g)	3.322	44.132
%AUC <sub>extrap</sub>	3.6	0.3
$T_{max}$ (h)	0.25	1
$C_{max}$ ( $\mu$ g/ml or $\mu$ g/g)	0.760	6.110
AUMC <sub>last</sub> (h · h · $\mu$ g/ml or g)	17.401	267.221
Mean residence time <sub>last</sub> (h)	5.4	6.1

AUMC<sub>last</sub>, area under the moment curve of the last point.

### Study 3: Chronic Lorcaserin Administration to Male Diet-Induced Obesity Rats.

A similar temporal pattern of effects on food intake and body weight gain emerged for lorcaserin-treated rats over time. Statistical analyses revealed significant reductions in both food intake [lorcaserin:  $F(3,43) = 29.3$ ;  $p < 0.01$ ] (Fig. 6, top) and body weight gain [lorcaserin:  $F(3,43) = 3.1$ ;  $p < 0.05$ ] (Fig. 6, bottom) over the 4 weeks of dosing. All doses of lorcaserin significantly reduced food intake over the first 13 days of dosing, after which time there was a progressive loss in food intake reduction, which was generally dose-dependent. For body weight gain, significant reductions relative to vehicle were apparent for the 18-mg/kg dose from day 8 onward, and day 19 onward for the 9-mg/kg group. For subjects in the 4.5-mg/kg group, there was a trend toward reduction in body weight gain that failed to reach statistical significance. Body composition analysis revealed that decreases in body weight were attributable to selective effects on fat mass [ $F(3,27) = 4.7$ ;  $p < 0.01$ ] (Fig. 7), rather than lean mass [ $F(3,27) = 0.9$ ; N.S.] (Fig. 7). After 28 days of treatment, a vehicle group and an 18-mg/kg group were monitored further for 4 weeks (Fig. 8). During this withdrawal period, an increase in food intake increased in the lorcaserin-treated group, resulting in recovery in body weight, such that body weights in the treated group did not differ from vehicle controls throughout the withdrawal period [ $F(1,14) = 0.1$ ; N.S.].

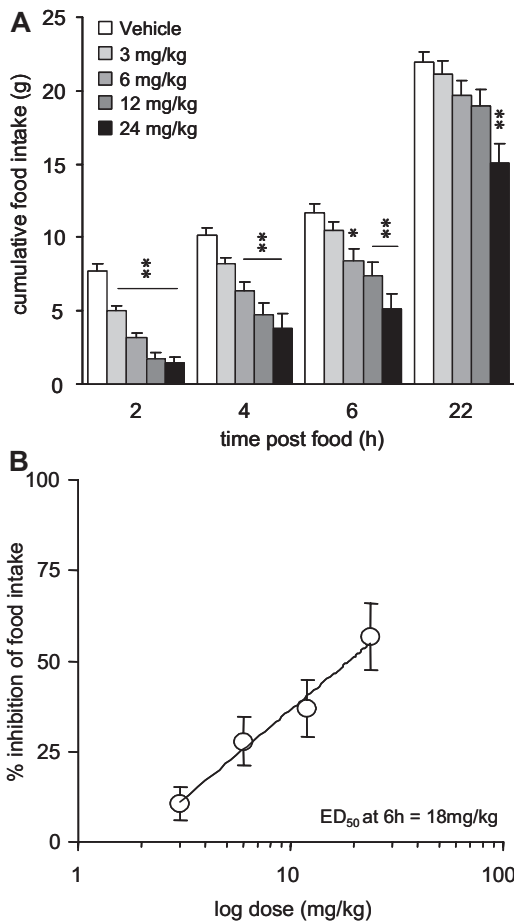
**Study 4: Behavioral Observations after Administration of Lorcaserin: Comparison with the 5-HT<sub>2A</sub> Agonist DOI.** The effects of lorcaserin on behaviors indicative of 5-HT<sub>2C</sub> or 5-HT<sub>2A</sub> receptor activation were also evaluated. (Fig. 9). Lorcaserin significantly reduced active, and in-

creased inactive counts [ $F(3,20) = 6.5$  and 29.7, respectively;  $p < 0.01$ ], whereas DOI was without effect [ $F(1,10) = 4.1$  and 4.3; N.S.]. Lorcaserin significantly increased the incidence of penile grooming [ $F(3,20) = 8.6$ ;  $p < 0.01$ ], an effect mediated by the 5-HT<sub>2C</sub> receptor, whereas DOI did not [ $F(3,20) = 0.1$ ; N.S.]. In contrast, DOI produced large increases in both wet dog shakes [ $F(3,20) = 170.0$ ;  $p < 0.01$ ] and back muscle contractions [ $F(3,20) = 30.0$ ;  $p < 0.01$ ], behavioral outcomes mediated by activation of the 5-HT<sub>2A</sub> receptor, whereas lorcaserin had no effect on either measures [ $F(3,20) = 1.1$  and 0.0; N.S.].

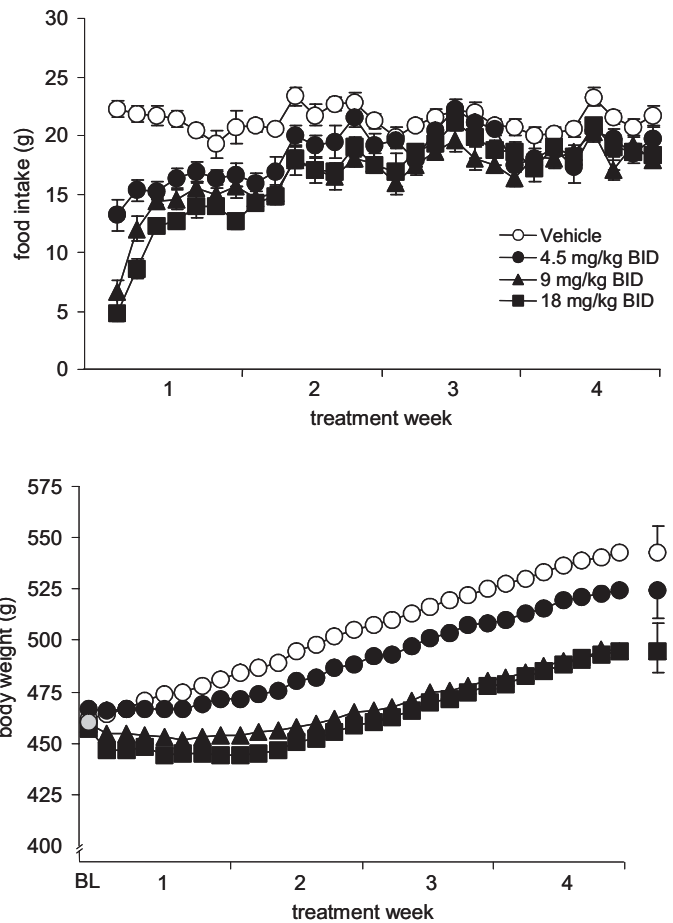
## Discussion

Lorcaserin is a potent and selective 5-HT<sub>2C</sub> agonist with rapid oral absorption and efficacy to decrease food intake and body weight gain in the rat. Functional activity assessed by inositol phosphate accumulation demonstrates lorcaserin to be a high-affinity full agonist at human 5-HT<sub>2C</sub> receptors, with selectivity over both 5-HT<sub>2A</sub> (18-fold) and 5-HT<sub>2B</sub> (104-fold) receptors at which it is a partial and a full agonist, respectively. This functional selectivity may be critical in the context of potential side effects associated with activation of both 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors. The differential 5-HT<sub>2</sub> selectivity of lorcaserin is somewhat lower when evaluated in radioligand binding studies rather than by functional assays (EC<sub>50</sub> values of 9, 168, and 943 nM obtained from functional assays compared with  $K_i$  values of 15, 112, and 174 nM obtained from radioligand competition studies). Regardless, we believe that activity derived from functional rather than radioligand binding data are more relevant to the in vivo effects of lorcaserin.

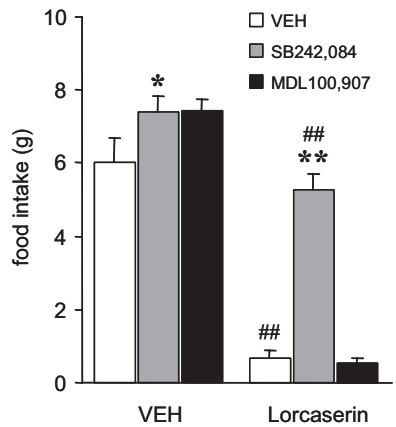
Lorcaserin was also evaluated for its ability to interact with a number of other human GPCRs and neurotransmitter transporters. Lorcaserin bound only weakly to other human 5-HT receptors (5-HT<sub>1A</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4C</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors), and it did not significantly compete for radioligand binding to serotonin, dopamine, or norepinephrine transporters until reaching micromolar concentrations. In addition, lorcaserin did not affect 5-HT, norepinephrine, or dopamine release from rat brain synaptosomes or synaptosomal neurotransmitter uptake until approaching micromolar concentrations. These data represent an important difference between lorcaserin and dexfenfluramine, which is a potent



**Fig. 4.** Cumulative food intake (A, top), and percentage of inhibition of food intake at 6 h (B, bottom) after a single-dose administration of lorcaserin (3–24 mg/kg p.o.). Food was removed from cages, and lorcaserin was administered 30 min before dark-cycle onset. At 30 min after dose, food was presented. Data represent mean  $\pm$  S.E.M. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus vehicle within each time point.

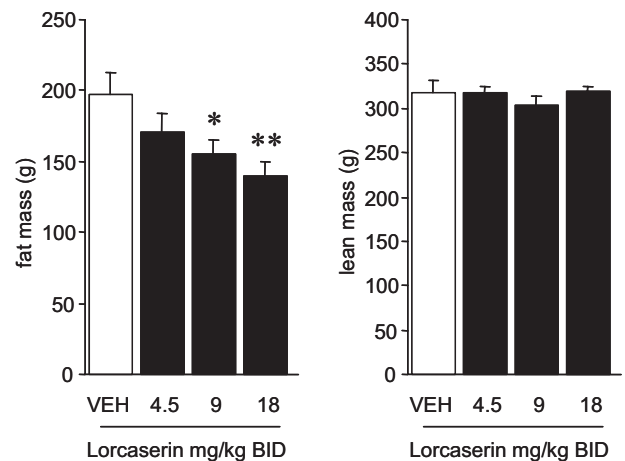


**Fig. 6.** Effect of lorcaserin (4.5–18 mg/kg p.o., b.i.d.) on food intake (top), and body weight (bottom) over 28 days of dosing to male Levin rats maintained on a high fat diet. Data are represented as means  $\pm$  S.E.M. (top) or means only (bottom). For clarity, S.E.M. for body weight data were averaged across the 28 days of dosing and are presented on the far right portion of the bottom figure.



**Fig. 5.** Inhibition of lorcaserin-induced hypophagia with a 5-HT<sub>2C</sub> antagonist but not a 5-HT<sub>2A</sub> antagonist. Rats were pretreated with the 5-HT<sub>2C</sub> antagonist SB242,084 (1 mg/kg i.p.) or the 5-HT<sub>2A</sub> antagonist MDL 100,907 (0.5 mg/kg) 15 min before oral administration of lorcaserin (18 mg/kg) or vehicle. Cumulative food intake was measured 60 min later. Bars represent the mean food intake  $\pm$  S.E.M. during a 60-min period. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus vehicle within lorcaserin; ##,  $p < 0.01$  versus appropriate control across lorcaserin.

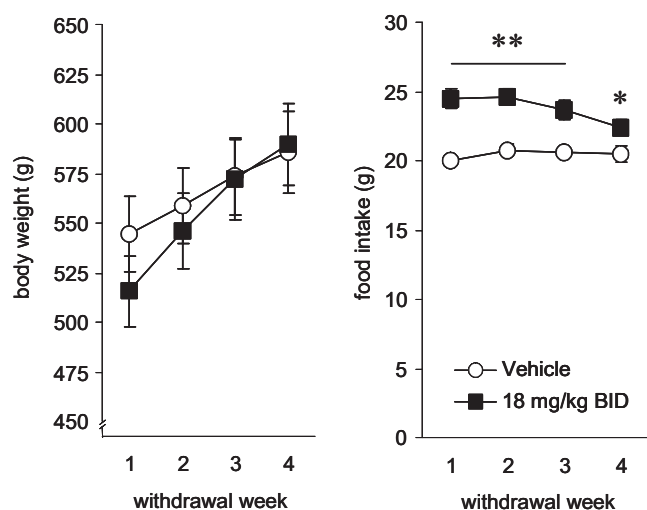
serotonin reuptake inhibitor and releaser, suggesting that therapeutic doses of lorcaserin will not promote monoamine release. Finally, lorcaserin did not compete for radioligand



**Fig. 7.** Fat and lean mass in male Levin rats as assessed by dual-energy X-ray absorptiometry after 28 days of dosing with either lorcaserin (4.5–18 mg/kg p.o., b.i.d.) or sibutramine (6 mg/kg p.o., daily). Bars represent mean  $\pm$  S.E.M. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus vehicle controls.

binding to an additional panel of 67 other human GPCRs and ion channels when tested at a final concentration of 1  $\mu$ M (see Supplemental Table 5). Taking these data together, our data





**Fig. 8.** Body weight (left) and food intake in male Levin rats over 4 weeks of withdrawal after a 28-day period of dosing with lorcaserin. Data points represent means  $\pm$  S.E.M. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus vehicle.

indicate that lorcaserin is a selective and potent agonist of the 5-HT<sub>2C</sub> receptor.

Obtaining adequate selectivity versus the closely related human 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors has been a major challenge for the discovery and development of 5-HT<sub>2C</sub> agonists. Agonist activity at the 5-HT<sub>2A</sub> receptor has been associated with hallucinations (Nichols, 2004). Although many compounds with potent 5-HT<sub>2A</sub> receptor agonist activity, such as *d*-lysergic acid diethylamide or psilocybin, are hallucinogenic, other potent 5-HT<sub>2A</sub> agonists such as lisuride, are not. Thus, it is currently difficult to predict whether a drug with 5-HT<sub>2A</sub> agonist activity will be hallucinogenic. Therefore, to explore this further, we evaluated the ability of lorcaserin to induce behaviors that are well known to be mediated by central 5-HT<sub>2A</sub> receptor activation. In this regard, doses of lorcaserin that potentially reduced food intake and weight gain in rats did not induce wet dog shakes or back muscle contractions, but DOI, a potent 5-HT<sub>2A</sub> agonist, caused a robust stimulation of these responses (Fig. 9, C and D). These behaviors are indicative of 5-HT<sub>2A</sub> agonist activity, and importantly, they are induced by known hallucinogens such as DOI and *d*-lysergic acid diethylamide (Schreiber et al., 1995; Ouagazzal et al., 2001; Nichols, 2004) and also by nonselective serotonin modulators such as dexfenfluramine (Higgins et al., 2001). As such, the lack of induction of these behaviors by lorcaserin suggests that the functional consequences of 5-HT<sub>2A</sub> activation are minimal or absent, and they demonstrate that CNS exposures of the compound that result in potent reductions in food intake and weight gain do not induce behavioral effects that are typical of most potent 5-HT<sub>2A</sub> agonists. Thus, our preclinical pharmacology data suggest that clinically important agonist activity of 5-HT<sub>2A</sub> receptors at therapeutic doses of lorcaserin should be minimal or absent, and the impact of any such 5-HT<sub>2A</sub> receptor occupancy may be further lessened by partial agonism at this receptor.

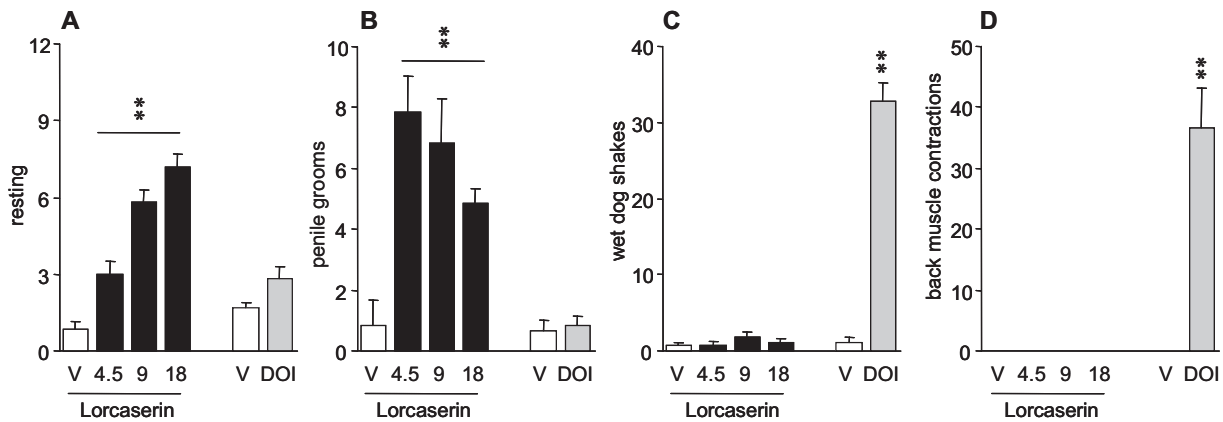
Other potential liabilities of agonist activity at human 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> receptors include cardiac valvular disease (Connolly et al., 1997; Devereux, 1998; Fitzgerald et al., 2000; Rothman et al., 2000; Horowski et al., 2004; Roth, 2007) and possibly pulmonary hypertension (Abenhaim et

al., 1996; Launay et al., 2002). These receptors are both expressed on cardiac valvular interstitial cells and smooth cells, and agonist activity at these receptors has been implicated in causing cardiac valvular insufficiency and possibly pulmonary hypertension. Although activation of both receptors has been suggested as a mechanism, a substantial body of evidence indicates that activation of the 5-HT<sub>2B</sub> receptor and not the 5-HT<sub>2A</sub> receptor is responsible (Fitzgerald et al., 2000; Rothman et al., 2000; Roth, 2007). With respect to pulmonary hypertension, the fenfluramines, through their primary metabolites, the norfenfluramines, have potent 5-HT<sub>2B</sub> activity with only modest 5-HT<sub>2A</sub> activity (Roth, 2007), and mice are protected from the development of hypoxia-induced disease either by genetic or pharmacological restriction of 5-HT<sub>2B</sub> activity (Esteve et al., 2007; Hauso et al., 2007).

As reported herein, lorcaserin has human functional receptor selectivity for 5-HT<sub>2C</sub> versus 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub>. Also reported herein, lorcaserin exposure in rodents was 13.3-fold higher in the brain versus peripheral blood. Using these facts and assuming that the rodent CNS-to-plasma ratio holds in humans, the predicted blood concentration required to activate peripheral 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> receptors are approximately 1400-fold (5-HT<sub>2B</sub>/5-HT<sub>2C</sub> ratio of 105, CNS/plasma ratio of 13.3) and 250-fold (5-HT<sub>2A</sub>/5-HT<sub>2C</sub> ratio of 19, CNS/plasma ratio of 13.3), respectively, above the required blood concentrations to stimulate CNS 5-HT<sub>2C</sub> receptors. Thus, these data suggest that the theoretical risk of cardiac valvulopathy with lorcaserin administration in humans is very low.

Lorcaserin exhibits rapid absorption and significant exposure in rat brain. Acutely, lorcaserin decreased food intake in rats in a dose-dependent manner, with efficacy observed at 3 mg/kg and above. Mediation by 5-HT<sub>2C</sub> receptors was confirmed by studies in which lorcaserin-induced decreases in food intake were reversed by the 5-HT<sub>2C</sub>-selective antagonist SB242,084, but not the 5-HT<sub>2A</sub> antagonist MDL 100,907. In the diet-induced obese Levin rat, efficacy to decrease food intake was maintained over repeated daily doses, although there was a dose-dependent tendency for food intake to return to control levels over time. However, despite apparent tolerance to the hypophagic effects of lorcaserin, there was no rebound hyperphagia, and decreased body weight gain compared with vehicle was maintained throughout the 28 days of dosing. It is noteworthy that this reduction in body weight gain reflected selective effects on fat rather than lean mass. Cessation of treatment in rats that had been dosed for 28 days with lorcaserin led to a rebound hyperphagia and a gradual return of body weight to control levels. This rebound hyperphagia coupled with maintenance of food intake at or below control levels during dosing suggests there are additional mechanisms of food intake suppression that require further elucidation. In addition to the study in diet-induced obese Levin rats, we have recently reported that chronic lorcaserin administration reduces food intake and weight gain in Sprague-Dawley rats fed a normal chow diet, demonstrating that lorcaserin has consistent efficacy across different strains and diets (Smith et al., 2008).

In conclusion, the present study demonstrates lorcaserin to be a potent and selective 5-HT<sub>2C</sub> agonist, with a 18- and 104-fold functional selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors, respectively. Lorcaserin reduced food intake acutely in



**Fig. 9.** Effect of lorcaserin (4.59 mg/kg p.o.) and DOI (1 mg/kg) on resting (A), penile grooming (B), wet dog shakes (C), and back muscle contractions (D) recorded over a 60-min test session.  $n = 6$  rats/group. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$  versus respective vehicle group.

the rat, an effect that was reversed by preadministration of a selective 5-HT<sub>2C</sub> antagonist. Administration of lorcaserin to the rat for 28 days resulted in transiently reduced food intake and sustained reductions in body weight gain compared with vehicle. These data show lorcaserin to have potential as a safe and efficacious treatment for obesity. Lorcaserin is currently undergoing evaluation in phase 3 clinical trials for the treatment of obesity.

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