Published in final edited form as:

Eur J Pharmacol. 2009 August 1; 615(1-3): 1–9. doi:10.1016/j.ejphar.2009.04.035.

(1*R*, 3*S*)-(-)-*Trans*-PAT: A novel full-efficacy serotonin 5-HT_{2C} receptor agonist with 5-HT_{2A} and 5-HT_{2B} receptor inverse agonist/antagonist activity

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Abstract

The serotonin 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} G protein-coupled receptors signal primarily through G_{αq} to activate phospholipase C (PLC) and formation of inositol phosphates (IP) and diacylglycerol. The human 5-HT_{2C} receptor, expressed exclusively in the central nervous system, is involved in several physiological and psychological processes. Development of 5-HT_{2C} agonists that do not also activate 5-HT_{2A} or 5-HT_{2B} receptors is challenging because transmembrane domain identity is about 75% among 5-HT₂ subtypes. This paper reports 5-HT₂ receptor affinity and function of (1*R*,3*S*)-(-)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT), a small molecule that produces anorexia and weight-loss after peripheral administration to mice. (-)-*Trans*-PAT is a stereoselective full-efficacy agonist at human 5-HT_{2C} receptors, plus, it is a 5-HT_{2A}/5-HT_{2B} inverse agonist and competitive antagonist. The *K_i* of (-)-*trans*-PAT at 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors is 410, 1200, and 37 nM, respectively. Functional studies measured activation of PLC/[³H]-IP formation in clonal cells expressing human 5-HT₂ receptors. At 5-HT_{2C} receptors, (-)-*trans*-PAT is an agonist (EC₅₀ = 20 nM) comparable to serotonin in potency and efficacy. At 5-HT_{2A} and 5-HT_{2B} receptors, (-)-*trans*-PAT is an inverse agonist (IC₅₀ = 490 and 1,000 nM, respectively) and competitive antagonist (*K_B* = 460 and 1400 nM, respectively) of serotonin. Experimental results are interpreted in light of molecular modeling studies indicating the (-)-*trans*-PAT protonated amine can form an ionic bond with D3.32 of 5-HT_{2A} and 5-HT_{2C} receptors, but, not with 5-HT_{2B} receptors. In addition to probing 5-HT₂ receptor structure and function, (-)-*trans*-PAT is a novel lead regarding 5-HT_{2C} agonist/5-HT_{2A} inverse agonist drug development for obesity and neuropsychiatric disorders.

Keywords

Drug addiction; Drug discovery; Obesity; Psychiatric disorder; Serotonin 5-HT₂ receptor

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1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) mediates its central and peripheral psychological and physiological effects through 14 mammalian serotonin receptor subtypes grouped into the 5-HT₁–5-HT₇ families. The 5-HT₂ receptor family consists of the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} membrane-bound G protein-coupled receptors that signal primarily through Gα_q to activate phospholipase C (PLC) and formation of inositol phosphates (IP) and diacylglycerol second messengers (Raymond et al., 2001). The human 5-HT_{2C} receptor (Saltzman et al., 1991) is found exclusively in the central nervous system where it is widely expressed and putatively involved in several (patho)- physiological and psychological processes, including, ingestive behavior (Tecott et al., 1995), cocaine addiction (Fletcher et al., 2002), psychosis (Siuciak et al., 2007), anxiety (Heisler et al., 2007), depression (Rosenzweig-Lipson et al., 2007), and motor function (Fox and Brotchie, 2000a, 2000b). Thus, the importance of the 5-HT_{2C} receptor as a pharmacotherapeutic target has been apparent for about 10 years, however, 5-HT_{2C} receptor-selective drugs still are not available.

Drug discovery targeting the 5-HT_{2C} receptor is challenging because it shares about 75% transmembrane sequence identity with 5-HT_{2A} and 5-HT_{2B} receptors. The highly conserved transmembrane domains and same second messenger signaling complicates development of selective 5-HT_{2C} agonists, in particular. Meanwhile, there is compelling evidence that 5-HT_{2C} receptor activation reduces food intake and leads to anti-obesity effects in lab animals and humans. For example, 5-HT_{2C} knockout mice demonstrate increased feeding and obesity, and, they are resistant to the anorectic effects of the now banned weight-loss drug *S*-(+)-fenfluramine (Tecott et al., 1995; Vickers et al., 2001). *S*-(+)- and (±)-fenfluramine promote serotonin release (Rothman et al., 1999) and both were banned by the US Food and Drug Administration in 1997 because fenfluramine and its metabolite *S*-(+)-norfenfluramine cause activation of 5-HT_{2B} receptors that can lead to valvular heart disease (Fitzgerald et al., 2000; Setola et al., 2005) and/or pulmonary hypertension (Launay et al., 2002)—fatalities have resulted.

There also is convincing preclinical evidence that activation of brain 5-HT_{2C} receptors could be pharmacotherapeutic for cocaine and other psychostimulant addiction (Bubar and Cunningham, 2008). For example, the non-selective 5-HT₂ agonist RO60175 reduces the rate of cocaine self-administration in rats and this effect is not blocked by M100907 (Fletcher et al., 2002), a 5-HT_{2A} antagonist with 500-times and 15-times selectivity over 5-HT_{2B} and 5-HT_{2C} receptors, respectively (Knight et al., 2004). In addition to psychostimulant addiction, evidence for the pharmacotherapeutic relevance of 5-HT_{2C} receptor activation in other neuropsychiatric disorders continues to come forth (e.g., Heisler et al., 2007; Siuciak et al., 2007; Rosenzweig-Lipson et al., 2007), and, there is intense interest by pharmaceutical companies to develop a genuinely selective 5-HT_{2C} agonist—no 5-HT_{2C} agonist devoid of 5-HT_{2A} and/or 5-HT_{2B} agonism has been reported previously (e.g., Siuciak et al., 2007; Rosenzweig-Lipson et al., 2007; Thomsen et al., 2008). Results reported in this paper, however, document that a novel compound synthesized in our laboratories, (1*R*,3*S*)-(–)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT; Fig.1), is a full-efficacy agonist at human 5-HT_{2C} receptors, plus, it is an inverse agonist at 5-HT_{2A} and 5-HT_{2B} receptors and competitively antagonizes 5-HT activation of these receptors. Recent studies (Rowland et al., 2008; Bivens et al., 2007) document that (–)-*trans*-PAT produces anorexia and weight-loss in mice after peripheral administration with potency and efficacy comparable to the commercially-available but nonselective 5-HT_{2C} agonist WAY-161503 (Rosenzweig-Lipson et al., 2006). The PAT molecule exists as four stereoisomers (Fig. 1) and 5-HT_{2C} receptor agonist activity resides only in the (1*R*,3*S*)-(–)-*trans*-PAT isomer. This striking PAT stereoselective functional activity is relevant not only for pharmacotherapeutic possibilities involving selective 5-HT_{2C} activation, but, also, for delineating the 3-dimensional molecular

determinants for ligand binding and function at 5-HT₂ receptors. Accordingly, G protein-coupled receptor homology-based molecular models were constructed for the 5-HT₂ receptor subtypes and (–)-*trans*-PAT docking experiments were undertaken to characterize molecular determinants for selective binding and inferences regarding agonism vs. inverse agonism functional activity.

2. Materials and Methods

2.1 Chemicals

The four stereoisomers of 1-phenyl-3-*N,N*-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT) stereoisomers (Fig. 1) were synthesized by modification of our previously reported procedure (Wyrick et al., 1993) and the details will be reported elsewhere. Briefly, (*E*)-1,4-diphenylbut-1-en-3-one was cyclized to the corresponding β-tetralone intermediate using polyphosphoric acid in toluene under reflux conditions (18 h) and the product was purified by flash column chromatography. The tetralone was reduced with sodium borohydride to a mixture of (±)-*cis*- and (±)-*trans*-tetralols that could be separated by recrystallization. To obtain (±)-*trans*-PAT, the (±)-*cis*-tetralol was stirred with *p*-toluenesulfonyl chloride in pyridine for 2 days at room temperature and purified by flash column to obtain the corresponding tosylate intermediate. Stirring the tosylate with sodium azide in *N,N*-dimethylformamide for 2 days at room temperature yields the stereochemically-inverted (±)-*trans*-azido derivative, which was reduced by catalytic hydrogenation to the free amine. The (±)-*trans*-amine was converted to the diastereomeric salt using (1*R*)-(–)-camphor-10-sulfonic acid and the diastereomers were separated by fractional recrystallization. The (1*R*, 3*S*)-(+)-*trans*- and (1*R*, 3*S*)-(–)-*trans*-amine enantiomers isolated were dimethylated using formic acid/formaldehyde and purified by column chromatography to obtain the (1*R*, 3*S*)-(+)-*trans*- and (1*R*, 3*S*)-(–)-*trans*-PAT products. The (1*R*, 3*R*)-(+)-*cis*- and (1*S*, 3*S*)-(–)-*cis*-PAT enantiomers were obtained from the (±)-*trans*-tetralol using methods analogous to those used to synthesize the (+)- and (–)-*trans* isomers. All products were converted to the hydrochloride salt for use in pharmacological experiments. Purity and absolute configuration were determined by elemental analysis, ¹H-NMR, mass spectrometry, melting point, X-ray crystallography, optical rotation, and HPLC using a polysaccharide-based chiral column (Kromasil, Akzo Nobel, Brewster, NY).

[³H]-Ketanserin (specific activity 72.2 Ci/mmol) and myo-[2-³H(N)]-inositol (specific activity 18.5 Ci/mmol) were purchased from Perkin-Elmer Life Science (Boston, MA) and [N⁶-methyl-³H]-mesulergine (specific activity 72.0 Ci/mmol) was purchased from Amersham Biosciences (GE healthcare, Piscataway, NJ). Unless otherwise noted, all other compounds were obtained in highest purity from Sigma-Aldrich (St. Louis, MO).

2.2 Clonal cell culture and transfection

Chinese hamster ovary K1 cells (CHO, ATCC CCL-61) were maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum and 1% sodium bicarbonate (Mediatech 25-035-CI), 10 IU/ml penicillin and 10 ug/ml streptomycin. Human embryonic kidney 293 cells (HEK, ATCC CRL-1573) were maintained in Eagle minimum essential medium with 10% fetal bovine serum and 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1.5 g/L sodium bicarbonate, 1.0 mM sodium pyruvate, 10 IU/ml penicillin, and 10 ug/ml streptomycin. Cells were grown in a humidified incubator at 37 °C with 5% carbon dioxide.

The cDNAs encoding the wild type human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors were obtained from UMR (Rolla, MO). Serotonin 5-HT_{2A} and 5-HT_{2B} receptors were transiently expressed in HEK cells (Setola et al., 2005) and 5-HT_{2C} receptors were transiently expressed in CHO cells (Porter et al., 1999). HEK cells were grown to 90-95% confluence in 100 mm dishes and transfected with 24 μg of plasmid DNA for the wild type 5-HT_{2A} or 5-HT_{2B} receptor

sequences using 40 μ l of Lipofectamine 2000 (Invitrogen, Carlsbad, CA) per dish. Transfection proceeded for 24 hrs, then, medium was replaced by fresh growth medium and cells were allowed to express 5-HT_{2A} or 5-HT_{2B} receptors for another 24 hrs. CHO cells were grown to 40% confluence in 100 mm dishes and transfected with 12 μ g of plasmid DNA for the wild type 5-HT_{2C} receptor sequence and 32 μ l of Lipofectamine 2000, then, transfection and expression proceeded as above.

2.3 Radioreceptor assays

Radioreceptor saturation and competition binding assays were performed using membrane homogenates prepared from transfected HEK (5-HT_{2A}, 5-HT_{2B}) or CHO (5-HT_{2C}) cells. [³H]-Ketanserin was used to radiolabel 5-HT_{2A} receptors and [³H]-mesulergine was used to label 5-HT_{2B} and 5-HT_{2C} receptors (Knight et al., 2004). Forty-eight hours following transfection, cells were harvested and homogenized in 50 mM Tris-HCl containing 0.1 % ascorbic acid and 4.0 mM calcium chloride at pH 7.4 (assay buffer). The homogenate was centrifuged at 35,000 $\times g$ for 25 min and the resulting membrane pellet was re-suspended in assay buffer. For saturation binding assays, membrane suspension containing 20 μ g (for 5-HT_{2A} receptor), 50 μ g (for 5-HT_{2B} receptor) or 100 μ g (for 5-HT_{2C} receptor) protein was incubated with 0.1 – 5.0 nM [³H]-ketanserin (5-HT_{2A} receptors) or 0.1 – 20 nM [³H]-mesulergine (5-HT_{2B} and 5-HT_{2C} receptors) in a total assay buffer volume of 250 μ l. Non-specific binding was determined in the presence of 10 μ M methysergide (5-HT_{2A} receptors) or 1.0 μ M mianserin (5-HT_{2B} and 5-HT_{2C} receptors). Competition binding assays were conducted under the same conditions using 1.0 nM [³H]-ketanserin (5-HT_{2A} receptors), 5.0 nM [³H]-mesulergine (5-HT_{2B} receptors), or 1.0 nM [³H]-mesulergine (5-HT_{2C} receptors) ($\sim K_d$ concentration). Incubation of radioreceptor binding assay mixtures was for 1.0 h at 37° C, with termination by rapid filtration through Whatman GF/B filters using a 96-well cell harvester (Tomtec, Hamden, CT). The membrane-bound [³H]-radioligand retained on the filter discs was quantified by liquid scintillation spectrometry. Data were analyzed by nonlinear regression using the sigmoidal curve-fitting algorithms in Prism 4.03 (GraphPad Software Inc., San Diego, CA), with the one-site model providing the best fit. Ligand affinity is expressed as an approximation of K_i values by conversion of the IC₅₀ data to K_i values using the equation $K_i = IC_{50}/1 + L/K_d$ where L is the concentration of radioligand having affinity K_d . Each experimental condition was performed in triplicate and each experiment was performed a minimum of three times to determine mean $K_d \pm SEM$.

2.4. Measurement of PLC Activation and [³H]-IP Formation

Functional activation of PLC was measured as [³H]-IP formation in HEK cells transiently expressing 5-HT_{2A} or 5-HT_{2B} receptors (Setola et al., 2005), or, CHO cells transiently expressing 5-HT_{2C} receptors (Porter et al., 1999). HEK cells expressing 5-HT_{2A} or 5-HT_{2B} receptors, or, CHO cells expressing 5-HT_{2C} receptors were seeded at 105 cells per well in 12-well plates in inositol-free Dulbecco's modified Eagle's medium (DMEM) for 12 – 24 hours with 1.0 μ Ci/ml myo-[2-³H]-inositol, the radiolabeled precursor of the PLC- β substrate phosphatidylinositol (with addition of 5% dialyzed fetal bovine serum to DMEM for HEK cells). Cells were subsequently washed and incubated in inositol-free DMEM containing 10 mM lithium chloride, 10 μ M pargyline, and various concentrations of test ligand for 45 – 60 min at 37 °C and 5% CO₂. After aspiration of media, cells were lysed by incubation with 50 mM formic acid (15 – 60 min). Formic acid was neutralized with ammonium hydroxide and contents from each well were added to individual AG1-X8 200-400 formate resin anion exchange columns. Ammonium formate/formic acid (1.2 M/0.1 M) was used to elute [³H]-IP directly into scintillation vials for counting of tritium by liquid scintillation spectrometry. Resulting data were analyzed using the nonlinear regression algorithms in Prism 4.03, with the one-site model providing the best fit. Data is expressed as mean percentage of basal control

[³H]-IP formation, with potency expressed as concentration required to stimulate (EC₅₀) or inhibit (IC₅₀) maximal basal (constitutive) [³H]-IP formation by 50% ± SEM. (n ≥ 3).

2.5 Molecular Modeling and Ligand Docking

Three-dimensional molecular models of the human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors were constructed by homology modeling according to the crystal structure of bovine rhodopsin (Protein Data Bank entry 1L9H) (Okada et al., 2002). The transmembrane (TM) portions were built using the Biopolymer module of Sybyl 7.3 by substituting the amino acid side chains in rhodopsin to the side chains of the amino acids in the respective 5-HT₂ receptor. The rhodopsin helix 7 structure that expands parallel to the membrane also was retained in the 5-HT₂ models. The resulting seven TM bundle was minimized with constrained C alpha atoms using Amber force field and atomic charges. The 5-HT₂ receptor intracellular and extracellular loops were added, however, the loop connecting TM 5 and 6 was truncated to include ten residues leading out of helix 5 and ten residues leading into helix 6. The conserved disulfide bridge formed between the cysteine amino acids in TM 3 and extracellular loop 2 also was included in the 5-HT₂ receptor models. Water molecules present in the rhodopsin crystal structure were not included. The crude 5-HT₂ models were minimized using the Powell method implemented in Sybyl with Amber force field and atomic charges and all Cα atoms restrained. The resulting structure was inserted into a rectangular box containing a pre-equilibrated dipalmitoylphosphatidylcholine (DPPC) bilayer. The DPPC bilayer was constructed with the “genconf” tool implemented with Gromacs v3.3.1 software (Lindahl et al., 2001) using coordinates available from the website of Dr. Peter Tieleman at the University of Calgary (http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies). The starting receptor-lipid bilayer system (180 DPPC molecules and 14,774 water molecules) was neutralized by adding chlorine ions and then subjected to 500 steps of energy minimization, followed by an equilibration run (500 ps) with receptor Cα atoms restrained (1000 kJ × mol⁻¹ × nm⁻¹) and 2 – 6 ns unrestrained production run. Minimization and molecular dynamics simulations were performed with Gromacs v3.3.1 software using the optimized potentials for liquid simulations all-atom force field for protein (Jorgensen et al., 1996) and Berger parameters for DPPC molecules (Berger et al., 1997). The LINCS algorithm was used to restrain bond lengths (Hess et al., 1997). Lipids topology files were from Dr. Tieleman's website and the water model was a simple rigid three-point charge model. All simulations are performed for an NPT ensemble (number of particles, pressure, temperature) using Berendsen's barostat and thermostat (Berendsen et al., 1981). The system was simulated with semiisotropic coupling, with the pressure at 1.0 bar and 300K (protein, phospholipids, solvent, and ions were coupled separately). Electrostatic interactions were calculated using particle mesh Ewald (Darden et al., 1993) with a cutoff of 0.9 nm. Cutoff for van der Waals interactions was set at 1.4 nm and the time step for integration was 2 fs.

Flexible ligand docking was performed with the FlexiDock utility in Sybyl 7.3 that uses a genetic algorithm to probe conformational space defining possible interactions between the ligand and its putative binding site. Ligand structures were built as monocations using the Sketch Molecule module in Sybyl 7.3, applying Gasteiger-Huckel atomic charges and Tripos force fields while Amber charges and atom types were used for 5-HT₂ receptors. The last 1 ns of dynamics trajectories was used to extract 5-HT₂ receptor structures in 250 ps increments. Each ligand was pre-positioned in the putative 5-HT₂ receptor binding pocket and all possible rotatable bonds in the ligand and side chains defining the putative binding site (120 rotatable bonds in total) were rotated during sampling of conformational space. Default FlexiDock parameters were set at 80,000-generation and the best docking solution, according to highest FlexiDock score, was minimized using an Amber force field (during minimization only backbone atoms of the receptor were constrained).

3. Results

3.1. Serotonin 5-HT₂ receptor radioligand saturation binding analysis

There was no measurable specific radioligand binding using membranes prepared from null-transfected CHO and HEK cells. Using membranes prepared from cells transiently transfected with 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} cDNA, however, saturable specific radioligand binding was observed. Fig. 2 shows representative binding data for [³H]-ketanserin labeled 5-HT_{2A} receptors and [³H]-mesulergine labeled 5-HT_{2B} receptors and 5-HT_{2C} receptors. [³H]-Ketanserin binds to an apparent single population of 5-HT_{2A} receptors ($B_{\max} = 1.73 \pm 0.11$ pmol/mg protein) with high affinity ($K_D = 0.80 \pm 0.03$ nM). [³H]-Mesulergine labels a single population of 5-HT_{2B} receptors with $B_{\max} = 1.13 \pm 0.39$ pmol/mg protein and $K_D = 5.19 \pm 0.36$ nM. Analogously, [³H]-mesulergine also labels an apparent single population of 5-HT_{2C} receptors ($B_{\max} = 8.37 \pm 0.15$ pmol/mg prot) with high affinity ($K_D = 0.88 \pm 0.03$ nM).

3.2. PAT binding to serotonin 5-HT₂-type radiolabeled receptors

Fig. 3 shows representative 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor radioligand displacement curves for the PAT isomers. Curves are sigmoidal in shape and span 3 to 4 log ligand concentration units to achieve complete radioligand displacement, characteristic of competitive displacement of $\sim K_d$ radioligand concentration from a single population of G protein-coupled receptors. Affinity data for PAT isomers at each 5-HT₂ receptor subtype is summarized in the Table.

The (–)-*trans*-PAT isomer has 10- to 30-times higher affinity ($K_i \sim 38$ nM) at 5-HT_{2C} receptors compared to the other three PAT stereoisomers—in fact, (–)-*trans*-PAT is the isomer with highest affinity at all three 5-HT₂ subtypes. For the other PAT isomers, rank order affinity potency is (+)-*trans*- > (+)-*cis*- > (–)-*cis*-PAT at 5-HT_{2A} and 5-HT_{2B} receptors, with the order reversed at 5-HT_{2C} receptors—overall, however, affinity differences between these isomers at any particular receptor subtype is relatively small (between 2- and 4-times), and, affinity is low ($K_i > 500$ nM) to nil ($K_i \sim 10$ μM) across 5-HT₂ receptor subtypes. Thus, the (–)-*trans*-PAT isomer demonstrated highest 5-HT_{2C} affinity and selectivity compared to the other isomers and detailed data for its 5-HT₂ receptor functional activity is shown in the next section.

3.3. PAT functional activity at serotonin 5-HT_{2C} receptors

The 5-HT₂ G protein-coupled receptor family is constitutively active when expressed in CHO and HEK cells, coupling primarily to G_{α_q} protein to activate PLC and IP formation (Raymond et al., 2001). In lysates of null-transfected CHO and HEK cells, no increase in PLC/[³H]-IP formation above basal activity was detected after incubation with up to 10 μM of the endogenous agonist serotonin for 45 min. In CHO cells expressing human 5-HT_{2C} receptors, however, serotonin produces a concentration-dependent increase in basal activity of PLC/[³H]-IP formation, with $EC_{50} = 7.30 \pm 0.55$ nM ($n_H = 1.04 \pm 0.20$) and $E_{\max} = 480 \pm 12.4$ % basal control activity (occurs at ~ 100 nM)—a representative graph of data is shown in Fig. 4. These results for serotonin activation of 5-HT_{2C} receptors are consistent with results reported in the literature (e.g., Porter et al., 1999).

Relative to serotonin, (–)-*trans*-PAT is a full-efficacy 5-HT_{2C} receptor agonist that produces a concentration-dependent increase in basal activity of PLC/[³H]-IP formation, spanning about 4 log concentration units to achieve maximal functional efficacy, characteristic of agonist activation of a single population of GPCRs, according to the ternary complex model (Fig. 4). For (–)-*trans*-PAT activation of 5-HT_{2C} receptors, $EC_{50} = 19.9 \pm 2.22$ nM ($n_H = 0.75 \pm 0.13$; not significantly different from unity, $P > 0.10$). The potency (EC_{50}) of (–)-*trans*-PAT to activate 5-HT_{2C} receptors appears to be about 2-times greater than its 5-HT_{2C} affinity (K_i), however, it is noted that the 5-HT_{2C} radioligand used to determine affinity, [³H]-mesulergine,

is an antagonist that likely does not selectively label the agonist-preferring conformation of the receptor (Knight et al., 2004). Regarding (–)-*trans*-PAT efficacy as a 5-HT_{2C} agonist, $E_{\max} = 464 \pm 10.8\%$ basal control activity (occurs at $\sim 10 \mu\text{M}$), and, there is no significant difference ($P = 0.2$) between the $E_{\max}$ values for (–)-*trans*-PAT and serotonin. None of the other three PAT isomers stimulated 5-HT_{2C} basal PLC/[³H]-IP formation at concentrations up to $10 \mu\text{M}$ (data not shown).

Having identified (–)-*trans*-PAT as an agonist for 5-HT_{2C} receptors, further studies were conducted with this isomer to determine its functional activity at 5-HT_{2A} and 5-HT_{2B} receptors. It was observed that (–)-*trans*-PAT does not stimulate PLC/[³H]-IP formation in cells expressing 5-HT_{2A} or 5-HT_{2B} receptors—in fact, beginning at concentrations of about $1.0 \mu\text{M}$, (–)-*trans*-PAT appeared to inhibit 5-HT_{2A} and 5-HT_{2B} receptor basal (constitutive) activity (Fig. 4). Accordingly, experiments were undertaken to evaluate antagonist, as well as, inverse agonist functional activity of (–)-*trans*-PAT at 5-HT_{2A} and 5-HT_{2B} receptors. In view of the very low affinity ($K_i \sim 0.5 - 10 \mu\text{M}$, Table) of the other PAT isomers at 5-HT_{2A} and 5-HT_{2B} receptors, further functional experiments were not conducted with these isomers.

3.4. Assessment of (–)-*trans*-PAT antagonist activity at serotonin 5-HT_{2A} and 5-HT_{2B} receptors

In these experiments, the ability of (–)-*trans*-PAT to antagonize serotonin stimulation of 5-HT_{2A} and 5-HT_{2B} receptor-mediated activation of PLC/[³H]-IP formation was assessed in HEK cells expressing 5-HT_{2A} or 5-HT_{2B} receptors. Cells were incubated with 10^{-10} to 10^{-5} M serotonin alone, or, with 0.1 , 1.0 and $10 \mu\text{M}$ (–)-*trans*-PAT. Fig. 5 shows that serotonin stimulation of 5-HT_{2A} and 5-HT_{2B} receptor-mediated activation of PLC/[³H]-IP formation is competitively antagonized by (–)-*trans*-PAT, as indicated by a serotonin EC_{50} value that increases as concentration of (–)-*trans*-PAT increases (shift to the right in concentration–response curve). In contrast, the serotonin $E_{\max}$ values ($625.7 \pm 13.80\%$ and $549.9 \pm 15.36\%$ for 5-HT_{2A} and 5-HT_{2B} receptors, respectively) are not affected significantly ($P > 0.05$) by 0.1 , 1.0 or $10 \mu\text{M}$ of (–)-*trans*-PAT.

At 5-HT_{2A} receptors (Fig. 5A), the serotonin EC_{50} is $30.0 \pm 7.88 \text{ nM}$ in absence of (–)-*trans*-PAT, but, serotonin EC_{50} significantly increases to $77.8 \pm 0.20 \text{ nM}$ ($P = 0.0182$) and $570 \pm 70 \text{ nM}$ ($P = 0.0020$) in the presence of 1.0 and $10 \mu\text{M}$ of (–)-*trans*-PAT, respectively; $0.1 \mu\text{M}$ (–)-*trans*-PAT produced no significant change in the serotonin EC_{50} value ($37.4 \pm 7.15 \text{ nM}$; $P = 0.5677$), as could be expected given its relatively low 5-HT_{2A} receptor affinity ($K_i \sim 0.4 \mu\text{M}$, Table). The data in Fig. 5A yield an apparent $K_B = 0.46 \pm 0.11 \mu\text{M}$ ($pA_2 = 6.3$) for (–)-*trans*-PAT competitive antagonism of serotonin activation of 5-HT_{2A} receptors—the (–)-*trans*-PAT K_B and K_i values at 5-HT_{2A} receptors are in close agreement.

At 5-HT_{2B} receptors (Fig. 5B), the serotonin EC_{50} is $20.6 \pm 1.65 \text{ nM}$ in absence of (–)-*trans*-PAT, but, the serotonin EC_{50} significantly increases to $36.9 \pm 2.15 \text{ nM}$ ($P = 0.0172$) and $110 \pm 5.5 \text{ nM}$ ($P = 0.0001$) in the presence of 1.0 and $10 \mu\text{M}$ of (–)-*trans*-PAT, respectively; $0.1 \mu\text{M}$ (–)-*trans*-PAT produced no significant change in the serotonin EC_{50} value ($21.9 \pm 2.75 \text{ nM}$; $P = 0.6973$), as could be expected given its very low 5-HT_{2B} receptor affinity ($K_i \sim 1.2 \mu\text{M}$, Table). The data in Fig. 5B yield an apparent $K_B = 1.4 \pm 0.8 \mu\text{M}$ ($pA_2 = 5.8$) for (–)-*trans*-PAT competitive antagonism of serotonin activation of 5-HT_{2B} receptors—the (–)-*trans*-PAT K_B and K_i values for 5-HT_{2B} receptors are in close agreement.

3.5. Assessment of (–)-*trans*-PAT inverse agonist activity at serotonin 5-HT_{2A} and 5-HT_{2B} receptors

The functional data in Figs. 4 and 5 indicate that at higher concentrations (i.e., 1.0 and $10 \mu\text{M}$), (–)-*trans*-PAT inhibits 5-HT_{2A} and 5-HT_{2B} receptor constitutive (basal) activity,

suggesting, inverse agonism at these receptors. To assess (–)-*trans*-PAT inverse agonist activity, HEK cells expressing 5-HT_{2A} or 5-HT_{2B} receptors were incubated with 0.01 – 10 μM of (–)-*trans*-PAT and activity of PLC/[³H]-IP formation was measured. Results shown in Fig. 6 indicate (–)-*trans*-PAT is an inverse agonist at 5-HT_{2A} and 5-HT_{2B} receptors, with IC₅₀ values of 490 ± 96 nM (n_H = 0.85 ± 0.01) and 1000 ± 0.5 nM (n_H = 0.87 ± 0.23), respectively. The inverse agonist potency values (IC₅₀) for (–)-*trans*-PAT at 5-HT_{2A} and 5-HT_{2B} receptors are consistent with its affinity potency (K_i ~ 0.4 and 1.2 μM, respectively, Table) and competitive antagonist potency (K_B ~ 0.5 and 1.4, respectively, Fig. 5) at these receptors. (–)-*Trans*-PAT solubility limited the maximal test concentration to 10 μM, at which, (maximal) inhibition of constitutive signaling is 57.6 ± 5.66 and 35.3 ± 2.00 % at 5-HT_{2A} and 5-HT_{2B} receptors, respectively.

3.6. (–)-*Trans*-PAT molecular interaction with the 5-HT_{2A} receptor (Fig 7A)

When the energy minimized structure of (–)-*trans*-PAT is docked to the 5-HT_{2A} molecular model, the PAT dimethylamine group (pK_a ~ 9), protonated at physiological pH, can form an ionic bond with the 5-HT_{2A} residue D3.32 (distance between PAT proton and aspartate carboxylic acid side chain is 1.7 Å)—amino acid residues are numbered according to Ballesteros et al. (2001). The aromatic portion of the PAT tetrahydronaphthalene moiety is in close proximity to the 5-HT_{2A} residues V.3.33 (2.3 Å), S3.36 (3.2 Å) S5.43 (3.2 Å), and F6.52 (2.3 Å). The aliphatic portion of the PAT tetrahydronaphthalene and one of the methyl amine groups interact with 5-HT_{2A} F6.51 (distance is 2.3 and 3.4 Å, respectively) and the other PAT methyl group orients towards Y7.43 (4.4 Å). The PAT pendant (C1) phenyl moiety interacts with 5-HT_{2A} W6.48 (distance between centroids of PAT phenyl and tryptophan indole is 3.8 Å).

3.7. (–)-*Trans*-PAT molecular interaction with the 5-HT_{2B} receptor (Fig. 7B)

There are significant differences in binding mode of (–)-*trans*-PAT to the 5-HT_{2B} receptor in comparison to 5-HT_{2A} (Fig. 7A) and 5-HT_{2C} (Fig. 7C, below). Most importantly, there is no interaction between the PAT protonated dimethylamine moiety and the 5-HT_{2B} residue D3.32 (the PAT amine proton orients away from D3.32 at a distance of 5.5 Å). Instead, the PAT dimethylamine group is much closer to residue F6.51 (2.3 Å). The two aromatic moieties of (–)-*trans*-PAT sandwich residue V3.33 midway between them (2.4 Å). The PAT tetrahydronaphthalene phenyl is close (2.7 Å) to 5-HT_{2B} residue S3.36, as is the case for binding at 5-HT_{2A}, however, no interaction of (–)-*trans*-PAT with 5-HT_{2B} residue S5.43 is observed (distance between closest atoms is 6.9 Å). The PAT tetrahydronaphthalene phenyl interacts with the 5-HT_{2B} W6.48 residue, likely, via π–π electron T-stacking interaction (distance 2.9 Å)—note that at the 5-HT_{2A} receptor the PAT pendant (C1) phenyl interacts with W6.48. At 5-HT_{2B}, the PAT pendant (C1) phenyl interacts with F6.52 (4.4 Å)—recall that at 5-HT_{2A} (above), it is the PAT tetrahydronaphthyl phenyl that interacts with F6.52. Meanwhile, there is the same interaction between the methyl group of (–)-*trans*-PAT and 5-HT_{2B} Y7.43 (4.9 Å) that is apparent for the 5-HT_{2A} receptor.

3.8 (–)-*Trans*-PAT interaction with the 5-HT_{2C} receptor (Fig. 7C)

The PAT basic nitrogen of the dimethylamine moiety apparently forms an ionic bond with 5-HT_{2C} residue D3.32 (distance 1.7 Å), similar to the result for 5-HT_{2A}. At the 5-HT_{2C} receptor, the PAT methyl groups orient differently than at 5-HT_{2A}, interacting with 5-HT_{2C} residues V. 3.33 (distance 2.5 Å) and F6.51 (2.2 Å). The PAT tetrahydronaphthyl system is situated between the indole ring of 5-HT_{2C} residue W6.48 (2.7 Å) and S3.36 (3.5 Å), which, is quite different than its orientation at 5-HT_{2A}. In addition, S5.43 of the 5-HT_{2C} receptor is further away (4.9 Å) from the PAT tetrahydronaphthalene moiety as compared to (–)-*trans*-PAT binding at the 5-HT_{2A} receptor (3.2 Å). Meanwhile, There is no interaction between (–)-*trans*-PAT and 5-HT_{2C} residue F6.52 (the distance between closest atoms is 6.2 Å), whereas,

the PAT tetrahydronaphthyl system interacts relatively closely (2.3 Å) with this residue at the 5-HT_{2A} receptor. Another important difference in (–)-*trans*-PAT at the 5-HT_{2C} vs. 5-HT_{2A} receptors is with regard to the pendant phenyl (C1) moiety—it is in close proximity to 5-HT_{2C} residues S3.36 (2.5 Å) and Y7.43 (3.3 Å), but, it interacts mainly with W6.48 at the 5-HT_{2A} receptor.

4. Discussion

The results presented establish (–)-*trans*-PAT as a stereoselective agonist at human 5-HT_{2C} receptors with full-efficacy relative to the endogenous agonist serotonin. The selectivity of (–)-*trans*-PAT for activation of 5-HT_{2C} versus 5-HT_{2A} and 5-HT_{2B} receptors is unequivocal in light of its inverse agonist activity and competitive antagonism of serotonin at 5-HT_{2A} and 5-HT_{2B} receptors. The results for docking of (–)-*trans*-PAT to molecular models of the 5-HT₂ receptor subtypes help to rationalize (–)-*trans*-PAT selectivity for receptor binding, albeit, only clues can be gleaned to explain its unique 5-HT₂-type multi-functional activity.

Regarding molecular determinants for 5-HT₂ receptor binding, an obvious correlation is that (–)-*trans*-PAT has very low affinity ($K_i \sim 1.2 \mu\text{M}$) at 5-HT_{2B} receptors and ligand docking results indicate (–)-*trans*-PAT does not interact with the 5-HT_{2B} D3.32 residue. The corresponding D3.32 is conserved among aminergic GPCRs and mutagenesis studies indicate D3.32 interacts with positively charged amines of endogenous agonists and other ligands, including, for all three 5HT₂ receptor subtypes (Muntasir et al., 2000a,b; 2007; Wang et al., 2003). In fact, for the human histamine H₁ GPCR, phylogenetically closely related to the 5-HT₂ family, D3.32 is required for [³H]-(–)-*trans*-PAT binding (Bakker et al., 2004). At 5-HT_{2A} and 5-HT_{2C} receptors, differences in the binding mode of (–)-*trans*-PAT are subtle, and, correspondingly, the difference in (–)-*trans*-PAT affinity at these receptors is modest (10-times). The major difference in (–)-*trans*-PAT binding at 5-HT_{2A} and 5-HT_{2C} receptors involves the S5.43 and F6.52 residues—(–)-*trans*-PAT interacts with these residues at the 5-HT_{2A} but not at the 5-HT_{2C} receptor. Regarding the change in (–)-*trans*-PAT functional activity from inverse agonism at 5-HT_{2A}/5-HT_{2B} receptors to agonism at 5-HT_{2C} receptors, the modeling results are not very informative. Currently, there is not 5-HT₂ G protein-coupled receptor structure–function information available to link PAT–5HT₂ differential binding to stabilization of receptor conformations that lead to activation (5-HT_{2C}) versus inactivation (5-HT_{2A} and 5-HT_{2B}), however, we have recently initiated mutagenesis studies to this end.

In addition to characterization of 5-HT₂ G protein-coupled receptor structure and function, the unique 5-HT₂ multi-functional activity of (–)-*trans*-PAT is promising with regard to development of novel 5-HT₂ receptor-based pharmacotherapy. For example, activation of brain 5-HT_{2C} receptors is mainly responsible for the pharmacotherapeutic effects of the anti-obesity drug *S*-(+)-fenfluramine (Tecott et al., 1995; Vickers et al., 2001). As mentioned, however, fenfluramine also indirectly leads to activation of 5-HT_{2B} receptors that can produce heart valve damage (Fitzgerald et al., 2000; Setola et al., 2005) and/or pulmonary hypertension (Launay et al., 2002) and resulting fatality. In light of the unfortunate but informative experience with fenfluramine, evidence for lack of 5-HT_{2B} receptor activation should be unequivocal regarding development of a weight-loss drug that is intended to act via 5-HT_{2C} receptors. In this regard, (–)-*trans*-PAT shows no evidence of 5-HT_{2B} receptor activation, and, in fact, it is a 5-HT_{2B} inverse agonist as well as antagonist.

Although toxicity associated with 5-HT_{2B} receptor activation has (deservedly) received much attention with regard to development of 5-HT₂ receptor-based obesity pharmacotherapy, indiscriminant 5-HT_{2A} receptor activation also is problematic. For example, 5-HT_{2A} receptor activation is associated with psychomimetic (hallucinogenic) effects (Nichols, 2004) that obviously should be avoided, especially, given the potentially large patient population that may

use a weight-loss drug, and, the sometimes subtle and complex nature of psychiatric disturbances. In this regard, the unequivocal 5-HT_{2A} inverse agonist/antagonist activity demonstrated by (–)-*trans*-PAT suggests psychiatric side-effects linked to 5-HT_{2A} receptor activation would not be an issue for this compound in a clinical setting.

In contrast, the only 5-HT_{2C} agonist to undergo clinical trials, lorcaserin (APD356), also activates 5-HT_{2A} receptors (75% efficacy) and 5-HT_{2B} receptors (100% efficacy) (Jensen, 2006; Smith et al., 2006; Thomsen et al., 2008). Affinity of lorcaserin for 5-HT_{2C} receptors (K_i ~ 15 nM) is only about 7.5-times higher than its affinity at 5-HT_{2A} receptors (K_i ~ 112 nM), thus, activation of both receptors is likely *in vivo*, with possible 5-HT_{2A} receptor-mediated psychiatric effects. In a 12-week clinical trial, about one-third of patients taking lorcaserin showed approximately 5% weight loss, however, except for dizziness, incidence of other central nervous system or psychiatric side-effects (e.g., 5-HT_{2A}-mediated) was not specified. Affinity of lorcaserin for human 5-HT_{2C} over 5-HT_{2B} receptors (K_i ~ 174 nM) also is modest (about 12-times), however, echocardiograms performed at baseline and at the end of the trial suggested no drug effect on heart valves or pulmonary artery pressure. Nevertheless, the 12-week time-frame of the lorcaserin clinical trial is far short of the 5-month time period used to demonstrate *in vivo* valvular heart disease by serotonin in rats (Droogmans et al., 2007).

The 5-HT_{2C} functional potency of lorcaserin (EC₅₀ ~ 10 nM) (Thomsen et al., 2008) and (–)-*trans*-PAT (EC₅₀ ~ 20 nM) is similar regarding activation of PLC/IP formation in cells transiently expressing the cloned human 5-HT_{2C} receptor, and, both ligands are full efficacy 5-HT_{2C} agonists compared to serotonin. Preclinical *in vivo* studies with lorcaserin and (–)-*trans*-PAT are not directly comparable because they used different paradigms and species—oral single-dose administration of lorcaserin to rats reduced food intake maximally by about 60% at 24 mg/kg, with ED₅₀ = 18 mg/kg (Thomsen et al., 2008), and, intraperitoneal single-dose administration of (–)-*trans*-PAT to mice reduced food intake maximally by about 75% at 10 mg/kg, with ED₅₀ = 4.2 mg/kg (Rowland et al., 2008). For both cases, the *in vivo* results unequivocally demonstrate human 5-HT_{2C} receptor-mediated activation of PLC/IP signaling in clonal cells can predict anti-obesity activity, and, development of 5-HT_{2C} agonists can lead to efficacious anti-obesity drugs—safety of such drugs will be a paramount concern, and, in this regard, the 5-HT_{2A/2B} inverse agonist/antagonist activity of (–)-*trans*-PAT suggests it would not carry the potential psychiatric and cardiopulmonary toxicity liability that could result from activation of these receptors *in vivo*.

In addition to their role in treatment of obesity, 5-HT_{2C} agonists also show efficacy in rodent models of schizophrenia (Siuciak et al., 2007). In this regard, the multi-functional activity of (–)-*trans*-PAT at multiple 5-HT₂ receptor subtypes (5-HT_{2C} agonism and 5-HT_{2A/2B} inverse agonism/antagonism) is in line with new dogma that proposes drugs with a multi-faceted pharmacological profile may be more effective than single-target drugs in treatment of neuropsychiatric disorders—the qualified effectiveness of atypical antipsychotic drugs, such as, olanzapine supports this new treatment paradigm. Atypical antipsychotics generally have clinically-relevant affinity at several neurotransmitter receptor types, including, adrenergic (α₁ and α₂), dopamine (D₁, D₃ and D₄), histamine (H₁), muscarinic, and serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₆ and 5-HT₇), and, their functional activity includes partial-agonism, as well as, inverse agonism and antagonism at these receptors. While 5-HT_{2A} receptor inverse agonism/antagonism is thought to contribute to the efficacy of atypical antipsychotic drugs, most, such as olanzapine, also are about equi-potent at blocking 5-HT_{2C} receptors, which could contribute to their propensity to cause weight gain. Antagonist activity at the histamine H₁ G protein-coupled receptor also correlates with atypical antipsychotic-induced weight gain (Kroeze et al., 2003). In fact, histamine H₁ receptors and the serotonin 5-HT₂ receptor family are phylogenetically closely related and propensity of atypical antipsychotics to cause weight

gain is most highly correlated with affinity for both H₁ and 5-HT_{2C} receptors, as well as, adrenergic α_1 receptors (Kroeze et al., 2003).

Receptor profiling studies indicate (–)-*trans*-PAT has very low ($K_i > 1 \mu\text{M}$) or virtually no ($K_i > 5 \mu\text{M}$) affinity for 35 tested CNS receptor systems, including neurotransmitter (adrenergic; cholinergic; GABA_A; histamine H₂, H₃, H₄; serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{5A}, 5-HT₆, 5-HT₇), neuromodulator (adenosine, benzodiazepine, opiate, NMDA/PCP, sigma), neurotransporter (dopamine, norepinephrine, serotonin), ion channel (Ca⁺⁺, Cl[–], K⁺), and second messenger (adenylyl cyclase, PLC) (PDSP, 2005). In contrast, (–)-*trans*-PAT has high affinity ($K_i \sim 2 \text{ nM}$) at human H₁ receptors, where it is an agonist that activates H₁-linked adenylyl cyclase signaling and catecholamine (dopamine, norepinephrine) synthesis in mammalian brain and adrenal tissues (Moniri et al., 2004; Moniri and Booth, 2006). (–)-*Trans*-PAT activation of brain catecholamine synthesis via H₁ receptor activation may result in effects similar to norepinephrine—when injected into rat brain, norepinephrine inhibits feeding, putatively, by activation of hypothalamus α_1 receptors (Wellman and Davies, 1991). Selective brain-penetrating norepinephrine uptake inhibitors that inhibit feeding behavior in rats have been considered as potential drugs for obesity (Gehlert et al., 1998).

Regarding cocaine addiction, there is compelling evidence that 5-HT_{2C} receptor agonism can attenuate neurobehavioral effects of cocaine, as can 5-HT_{2A} receptor antagonists (Bubar and Cunningham, 2008). For example, the reinforcing effects of cocaine are reduced by 5-HT_{2C} agonists, whereas, discriminative stimulus and reinstating effects are sensitive to attenuation by both 5-HT_{2C} agonists and 5-HT_{2A} antagonists (Bubar and Cunningham, 2008). Both 5-HT_{2C} agonism and 5-HT_{2A} antagonism/inverse agonism are demonstrated by (–)-*trans*-PAT, suggesting, (–)-*trans*-PAT may be a lead for psychostimulant addiction pharmacotherapy.

An intriguing possibility for (–)-*trans*-PAT-type multi-functional drugs involves new treatment paradigms based on classification of over-eating and drug addiction as compulsive behavioral disorders directed toward different objects, food and drugs (Simansky, 2005). Although clinical efficacy of (–)-*trans*-PAT 5-HT₂ in obesity and/or neuropsychiatric disorders is highly speculative at this point, recent studies using rodents give cause for optimism. In addition to the *in vivo* anorexic and weight-loss effects of (–)-*trans*-PAT in mice (Rowland et al., 2008; Bivens et al, 2007), preliminary results indicate (–)-*trans*-PAT also reduces amphetamine-induced locomotion in mice after intraperitoneal administration (Bivens et al., 2007), indicative of antipsychotic neurobehavioral activity. Taken together, the *in vitro* and *in vivo* activity of (–)-*trans*-PAT suggests it may show activity as an antipsychotic drug without propensity for weight gain.

Acknowledgments

This research was supported by United States Public Health Service (NIH) Grants MH068655, MH081193, DA023928, and the University of Florida Opportunity Fund. Receptor binding profile data for (–)-*trans*-PAT was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # NO1MH32004 (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA.

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Fig. 1.
Structures of 1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT) stereoisomers.

Fig. 2.

Representative saturation binding curves for serotonin 5-HT₂ receptor radioligands used to calculate $B_{\max} \pm \text{SEM}$ (pmol/mg protein) and $K_d \pm \text{S.E.M}$ (nM) values.

- (A) [³H]-Ketanserin labeled 5-HT_{2A} receptors; $B_{\max} = 1.73 \pm 0.11$, $K_d = 0.80 \pm 0.03$.
- (B) [³H]-Mesulergine labeled 5-HT_{2B} receptors; $B_{\max} = 1.13 \pm 0.39$, $K_d = 5.19 \pm 0.36$.
- (C) [³H]-Mesulergine labeled 5-HT_{2C} receptors; $B_{\max} = 8.37 \pm 0.15$, $K_d = 0.88 \pm 0.03$.

Fig. 3. Representative concentration–response curves for PAT isomer displacement of 5-HT_2 receptor radioligands from $5\text{-HT}_{2\text{A}}$ (A), $5\text{-HT}_{2\text{B}}$ (B), and $5\text{-HT}_{2\text{C}}$ (C) receptors. K_i and n_H values are summarized in the Table.

Fig. 4. Representative concentration–response curves for serotonin (closed squares) and (–)-*trans*-PAT (closed circles) activation of PLC/[³H]-IP formation in clonal cells expressing 5-HT_{2C} receptors. Single concentration effect of (–)-*trans*-PAT at 5-HT_{2A} (open triangles) and 5-HT_{2B} (open squares) receptors also is shown. EC₅₀ and n_H values are given in the text.

Fig. 5.
Representative graphed data for (-)-*trans*-PAT competitive antagonism of serotonin activation of 5-HT_{2A} (A) and 5-HT_{2B} (B) receptors.

Fig. 6.
Representative graphed data for (-)-*trans*-PAT inverse agonist activity at serotonin 5-HT_{2A} (A) and 5-HT_{2B} (B) receptors.

Fig. 7.
Molecular models of the 5-HT_{2A} (A), 5-HT_{2B} (B), and 5-HT_{2C} (C) receptors with (-)-*trans*-PAT docked at the putative binding pocket.

Table 1

PAT Isomer	5-HT _{2A} Ki ± SEM (nM)	n _H ^a	5-HT _{2B} Ki ± SEM (nM)	n _H ^a	5-HT _{2C} Ki ± SEM (nM)	n _H ^a
(-)- <i>trans</i>	410 ± 38	1.1 ± 0.1	1,200 ± 6.8	1.0 ± 0.001	37.6 ± 3.02	0.9 ± 0.1
(+)- <i>trans</i>	520 ± 3.0	1.0 ± 0.2	~ 2,500	~ 1.0	1300 ± 80	1.0 ± 0.1
(+)- <i>cis</i>	780 ± 2.0	0.9 ± 0.2	~ 5,000	~ 1.0	980 ± 7.8	0.9 ± 0.1
(-)- <i>cis</i>	1500 ± 2.0	1.0 ± 0.1	~ 10,000	~ 1.0	430 ± 4.8	0.90 ± 0.04

^aNone of the Hill slope values differs significantly (P>0.10) from unity.