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(54) **Title:** COMPOUND FOR ACTIVATING 5-HT2C RECEPTORS IN COMBINATION WITH AN AMPHETAMINE COMPOUND

(57) **Abstract:** A method of treating or preventing obesity in a subject comprising administering to the subject identified as in need thereof a combination of a PAT compound and an amphetamine compound, wherein the PAT compound is capable of selectively activating 5- HT2c relative to 5-HT2a or 5HT2b.

COMPOUND FOR ACTIVATING 5-HT2C RECEPTORS IN COMBINATION WITH AN AMPHETAMINE COMPOUND

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application Serial Number 61/002,002, filed November 6, 2007, the contents of which are incorporated by reference in their entirety.

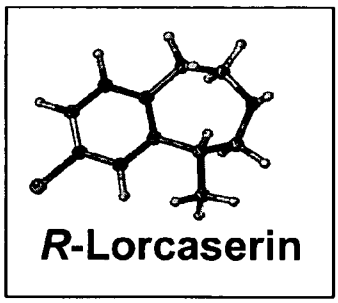
STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This work was supported in part by United States Public Health Service Grant MH068655. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Serotonin (5-hydroxytryptamine, 5HT) mediates a wide variety of central and peripheral psychological and physiological effects through 14 mammalian 5HT receptor subtypes that are grouped into the $5HT_1$–$5HT_7$ families (Sanders-Bush and Mayer, 2006). The $5HT_2$ family consists of the $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ membrane-bound G protein-coupled receptors (GPCRs) that signal primarily through $G\alpha_q$ to activate phospholipase (PL) C and formation of inositol phosphates (IP) and diacylglycerol (DAG) second messengers (Raymond et al., 2001). The human $5HT_{2C}$ receptor (Saltzman et al., 1991) apparently is found exclusively in brain 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; Rocha et al., 2002; Muller and Huston, 2006), sleep homeostasis (Frank et al., 2002), anxiety (Kennett et al., 1994; Sard et al., 2005; Heisler et al., 2007), depression (Tohda et al., 1989; Palvimaki et al., 1996), epilepsy (Heisler et al., 1998), Alzheimer's disease (Arjona et al., 2002; Stein et al., 2004), motor function (Heisler and Tecott, 2000; Segman et al., 2000), psychosis (Marquis et al., 2007; Siuciak et al., 2007) and response to antipsychotic drugs (Veenstra-VanderWeele et al., 2000; Reynolds et al., 2005). Thus, the importance of the $5HT_{2C}$ receptor as a pharmacotherapeutic target has been apparent for about 10 years, however, no $5HT_{2C}$-specific drugs have been developed.

One challenge regarding drug discovery targeting the $5HT_{2C}$ receptor is that this GPCR shares a transmembrane domain (TMD) sequence identity of about 80% with the $5HT_{2A}$ receptor and about 70% with the $5HT_{2B}$ receptor (Julius et al., 1988; 1990). The highly conserved TMDs and similar second messenger coupling has made development of agonist ligands selective for the $5HT_{2C}$ receptor especially difficult. Nevertheless, there is compelling evidence that activation of $5HT_{2C}$ receptors reduces food intake and leads to anti-obesity effects. For example, 5-HT_{2C} knockout mice demonstrate increased feeding and obesity, and, they are resistant to the anorectic effects of *S*-(+)-fenfluramine (Tecott et al., 1995; Vickers et al., 1999; 2001; Heisler et al., 2002). Moreover, activation of brain serotonin 5-HT_{2C} receptors is responsible for the pharmacotherapeutic effects of the anti-obesity drug *S*-(+)-fenfluramine in humans (Weintraub et al., 1992; Tecott et al., 1995; Vickers et al., 1999; 2001; Heisler et al., 2002). *S*-(+)-Fenfluramine is a serotonin transporter substrate that promotes release of serotonin by transporter-mediated exchange and disruption of vesicular serotonin storage (Rothman et al., 1999). Racemic- as well as *S*-(+)-fenfluramine alone, or, in combination with the amphetamine analog phentermine (i.e., fen-phen), was banned by the United States Food and Drug Administration in 1997 because the drug and/or its metabolite *S*-(+)-norfenfluramine causes indirect activation of 5-HT_{2B} receptors (presumably, via released serotonin) that can lead to valvular heart disease (Connolly et al., 1997; Fitzgerald et al., 2000; Rothman et al., 2000; Setola et al., 2005; Roth, 2007) and pulmonary hypertension (Pouwels et al., 1990; Launay et al., 2002) – fatalities have resulted from these effects.



The pharmacotherapeutic relevance of the $5HT_{2C}$ receptor in obesity has stimulated intense interest by pharmaceutical companies to develop a selective $5HT_{2C}$ agonist, however, all $5HT_{2C}$ agonists reported so far also activate $5HT_{2A}$ and/or $5HT_{2B}$ receptors (Nilsson, 2006). Nevertheless, the $5HT_2$ agonist lorcaserin recently went to Phase III clinical trials for obesity treatment (Jensen, 2006; Smith et al., 2006). Lorcaserin: is a benzazepine compound with a molecular structure very different from the PAT/APT-type compounds described in this application. *R*-lorcaserin (formerly APD356) was forwarded by Arena Pharmaceuticals to Phase 3 clinical trials for obesity. In June 2006, it was reported that, over a 12-week period, one-third of patients receiving the highest dose (20mg/day) lost about 5% of body weight (Smith et al., 2006; Maffuid et al., 2006; Jensen, 2006). Relative to 5HT, lorcaserin is a partial agonist (70% vs. 5HT) at $5HT_{2A}$ receptors and a full agonist at $5HT_{2B}$ and $5HT_{2C}$ receptors. The EC_{50} for lorcaserin activation of $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ receptors is ~ 190, 1,000 and 11 nM, respectively (Smith et al., 2005); Ki data is not reported. No cardiovascular toxicity was observed for the 12-week anti-obesity study, maybe, because of the

100-fold selectivity for activation of $5HT_{2C}$ over $5HT_{2B}$ receptors. No ($5HT_{2A}$-mediated) psychiatric side effects were reported for the 12 week study, however, the modest 15-fold selectivity of lorcaserin for activation of $5HT_{2C}$ over $5HT_{2A}$ receptors suggests there is potential for lorcaserin to cause psychotomimetic effects. It is important to note that the limited in vitro pharmacological results and clinical results for lorcaserin were divulged at an industry meeting and on a website for investors – no pharmacological data and no clinical data for lorcaserin has undergone the peer scientific review process, nor, has it been published in the peer-reviewed literature. Thus, it is prudent to assume that the pharmacology for lorcaserin is reported in the best possible light – even so, lorcaserin activates $5HT_{2A}$ and $5HT_{2B}$ receptors (less potently than it activates $5HT_{2C}$ receptors) and this activity is known to lead to hallucinations and other psychomimetic effects ($5HT_{2A}$), and, cardiovascular and pulmonary toxicity ($5HT_{2B}$). . In contrast, one of the lead molecules designed and synthesized in our laboratories, (1*R*,3*S*)-(–)-*trans*-1-phenyl-3-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT), is a full efficacy agonist at human $5HT_{2C}$ receptors, plus, it is an antagonist at $5HT_{2A}$ and $5HT_{2B}$ receptors. Thus, compared to lorcaserin and all other $5HT_{2C}$ agonist compounds so far reported, (–)-*trans*-PAT does not possess potentially deleterious $5HT_{2A}$ and $5HT_{2B}$ receptor activation liability. Meanwhile, (–)-*trans*-PAT activates $5HT_{2C}$ receptors, a pharmacological property that is desired in an anti-obesity drug. Moreover, as is the case for the combination of *S*-(+)-fenfluramine plus the amphetamine analog phentermine, preliminary in vivo data indicate that (–)-*trans*-PAT in combination with an amphetamine is particularly efficacious with regard to weight loss effects, thus, PAT-Phen could be a non-toxic alternative to Fen-Phen.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity comprising administering to subject in need thereof a therapeutically effective amount of a compound capable of activating 5-HT2C receptors in combination with an amphetamine compound. In one embodiment, the compound is capable of activating 5-HT2C receptors. In another embodiment, the compound is capable of activating 5-HT2C, while antagonizing 5-HT2A and/or 5-HT2B receptors. In another embodiment, the compound is capable of activating 5-HT2C, and/or antagonizing 5-HT2A and/or 5-HT2B receptors. In another embodiment, the compound is capable of antagonizing 5-HT2A and/or 5-HT2B receptors.

In one aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity. The method includes administering to a subject in need thereof a therapeutically effective amount of a 5-HT2c activating compound in combination with an amphetamine compound.

In another aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity. The method includes administering to a subject in need thereof a therapeutically effective amount of a compound capable of modulating 5-HT2 binding interactions by directly modulating 5-HT2c, preferably selectively relative to 5-HT2a and /or 5-HT2b, in combination with an amphetamine compound.

In another embodiment, the invention provides a method of treating a subject suffering from or susceptible to obesity. The method includes administering to a subject identified as in need thereof (i.e., an obese subject) a therapeutically effective amount of a 5-HT2c agonizing compound or a 5-HT2c selective compound, in combination with an amphetamine compound.

In another aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity. The method includes administering to a subject an agonizing 5-HT2c compound in combination with an amphetamine compound such that the obesity is prevented, ameliorated or treated (e.g., weight loss is observed, weight gain is prevented).

In another aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity, comprising administering to the subject an effective amount of a compound capable of activating 5-HT2c, selectively (alone or in combination with an amphetamine), relative to 5-HT2a and/or 5-HT2b activation, in combination with an amphetamine compound such that the subject is treated.

In another aspect, the invention provides a method of treating a subject suffering from or susceptible to obesity, comprising administering to the subject an effective amount of a compound capable of agonizing 5-HT2c (including selectively relative to 5-HT2a and/or 5-HT2b) while not (or to a lesser extent) agonizing (and/or antagonizing) 5-HT2a or 5-HT2b receptors, in combination with an amphetamine compound such that the subject is treated.

In another aspect, the invention provides a packaged composition including a therapeutically effective amount of a 5-HT2c agonist compound in combination with an amphetamine compound and a pharmaceutically acceptable carrier or diluent. The composition may be formulated for treating a subject suffering from or susceptible to obesity, and packaged with instructions to treat a subject suffering from or susceptible to obesity.

In one aspect, the invention a kit for treating a obesity in a subject is provided and includes a compound herein, in combination with an amphetamine compound, and pharmaceutically acceptable esters, salts, and prodrugs thereof, and instructions for use. In further aspects, the invention provides kits for activating (e.g., agonizing) 5-HT2c in combination with an amphetamine compound, assessing the efficacy of an anti-obesity treatment in a subject, monitoring the progress of a subject being treated with a 5-HT2c activating compound (e.g, agonist) in combination with an amphetamine compound, selecting a subject with a obesity for treatment with 5-HT2c activating compound (e.g, agonist) in combination with an amphetamine compound, and/or treating a subject suffering from or susceptible to a obesity. In certain embodiments, the invention provides: a kit for treating a obesity in a subject, the kit comprising a compound capable of modulating (e.g., agonizing) 5-HT2c activating activity in combination with an amphetamine compound. In other aspects the compound selectively activates 5-HT2c relative to 5-HT2a and/or 5-HT2b. In other aspects the compound selectively antagonizes 5-HT2a and/or 5-HT2b.

The invention also provides pharmaceutical compositions of the compounds described herein, comprising a compound capable of activating 5-HT2c; a compound capable of activating 5-HT2c selectively relative to 5-HT2a and/or 5-HT2b; a compound capable of activating 5-HT2c and antagonizing 5-HT2a and/or 5-HT2b; or a compound capable of antagonizing 5-HT2a and/or 5-HT2b;or a pharmaceutically acceptable ester, salt, or prodrug thereof, each in combination with an amphetamine compound together with a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further described below with reference to the following non-limiting examples and with reference to the following figures, in which:

FIG 1. depicts the effect of (-)-trans-PAT (compound 1)on basal body weight.

FIG 2. depicts the effect of (-)-trans-PAT administered with amphetamine-type stimulants on basal body weight.

FIG 3. depicts the effect of (-)-trans-PAT administered with amphetamine-type stimulants on food consumption.

FIG 4. depicts the effect of (-)-trans-PAT on weight loss in mice.

FIG 5. depicts the effect of (-)-trans-PAT on 5-HT_2 receptors.

FIGs 6A and 6B. depict (-)-trans-PAT antagonist activity at 5-HT_2 receptors.

FIG 7. depicts the affinity of (-)-trans-PAT stereoisomers at 5-HT_{2c} receptors.

FIG 8. depicts the affinity of (-)-trans-PAT stereoisomers at 5-HT_{2a} receptors.

FIG 9. depicts the affinity of (-)-trans-PAT stereoisomers at 5-HT_{2b} receptors.

FIGs 10A and 10B depict 5-HT_{2c} agonist activity of PAT compounds.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have now discovered a therapeutic strategy that addresses selective obesity treatment and prevention (i.e., having reduced or minimized adverse side effects) in a subject by selectively targeting activation of 5-HT2c receptors (e.g., using 5-HT2c agonists) in combination with an amphetamine compound. Such interactions are relevant for selective modulation of 5-HT2c mediated disorders, particularly in obesity where 5-HT2 mechanisms play a significant role.

1. DEFINITIONS

Before further description of the present invention, and in order that the invention may be more readily understood, certain terms are first defined and collected here for convenience.

The term "administration" or "administering" includes routes of introducing the compound of the invention(s) to a subject to perform their intended function. Examples of routes of administration that may be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, intrathecal), oral, inhalation, rectal and transdermal. The pharmaceutical preparations may be given by forms suitable for each administration route. For example, these preparations are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred. The injection can be bolus or can be continuous infusion. Depending on the route of administration, the compound of the invention can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally effect its ability to perform its intended function. The compound of the invention can be administered alone, or in conjunction with either another agent as described above or with a pharmaceutically-acceptable carrier, or both. The compound of the invention can be administered prior to the administration of the other agent, simultaneously with the agent, or after the administration of the agent. Furthermore, the compound of the invention

can also be administered in a pro-drug form which is converted into its active metabolite, or more active metabolite *in vivo*.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, e.g., oxygen, nitrogen, sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chain, C_3-C_{30} for branched chain), preferably 26 or fewer, and more preferably 20 or fewer, and still more preferably 4 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 3, 4, 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and sentences is intended to include both "unsubstituted alkyls" and "substituted alkyls," the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)). The term "alkyl" also includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six, and still more preferably from one to four carbon atoms in its backbone structure, which may be straight or branched-chain. Examples of lower alkyl groups include methyl, ethyl, n-propyl, i-propyl, tert-butyl, hexyl, heptyl, octyl and so forth. In preferred embodiment, the term

"lower alkyl" includes a straight chain alkyl having 4 or fewer carbon atoms in its backbone, e.g., C1-C4 alkyl.

The terms "alkoxyalkyl," "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, e.g., oxygen, nitrogen or sulfur atoms.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond, respectively. For example, the invention contemplates cyano and propargyl groups.

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles," "heteroaryls" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin).

The term "associating with" refers to a condition of proximity between a chemical entity or compound, or portions thereof, and a binding pocket or binding site on a protein. The association may be non-covalent (wherein the juxtaposition is energetically favored by hydrogen bonding or van der Waals or electrostatic interactions) or it may be covalent.

The term "binding pocket", as used herein, refers to a region of a molecule or molecular complex, that, as a result of its shape, favorably associates with another chemical entity or compound.

The language "biological activities" of a compound of the invention includes all activities elicited by compound of the inventions in a responsive cell. It includes genomic and non-genomic activities elicited by these compounds.

"Biological composition" or "biological sample" refers to a composition containing or derived from cells or biopolymers. Cell-containing compositions include, for example, mammalian blood, red cell concentrates, platelet concentrates, leukocyte concentrates, blood cell proteins, blood plasma, platelet-rich plasma, a plasma concentrate, a precipitate from any fractionation of the plasma, a supernatant from any fractionation of the plasma, blood plasma protein fractions, purified or partially purified blood proteins or other components, serum, semen, mammalian colostrum, milk, saliva, placental extracts, a cryoprecipitate, a cryosupernatant, a cell lysate, mammalian cell culture or culture medium, products of fermentation, ascites fluid, proteins induced in blood cells, and products produced in cell culture by normal or transformed cells (e.g., via recombinant DNA or monoclonal antibody technology). Biological compositions can be cell-free. In a preferred embodiment, a suitable biological composition or biological sample is a red blood cell suspension. In some embodiments, the blood cell suspension includes mammalian blood cells. Preferably, the blood cells are obtained from a human, a non-human primate, a dog, a cat, a horse, a cow, a goat, a sheep or a pig. In preferred embodiments, the blood cell suspension includes red blood cells and/or platelets and/or leukocytes and/or bone marrow cells.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "diastereomers" refers to stereoisomers with two or more centers of dissymmetry and whose molecules are not mirror images of one another.

The term "effective amount" includes an amount effective, at dosages and for periods of time necessary, to achieve the desired result, e.g., sufficient to treat a disorder delineated herein. An effective amount of compound of the invention may vary according to factors such as the disease state, age, and weight of the subject, and the ability of the compound of the invention to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. An effective amount is also one in which any toxic or detrimental effects (e.g., side effects) of the compound of the invention are outweighed by the therapeutically beneficial effects. A therapeutically effective amount of compound of the invention (i.e., an effective dosage) may range from about 0.01 to 100 mg/kg body weight, preferably about 0.1 to 50 mg/kg body weight, more preferably about 0.5 to 25 mg/kg body weight, and even more

preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The same dose range of novel therapeutic compound is expected to have synergistic anti-obesity effects in combination with 1.0 to 10.0 mg/kg amphetamine or therapeutically equivalent amount of amphetamine derivative. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a compound of the invention can include a single treatment or, preferably, can include a series of treatments. In one example, a subject is treated with a compound of the invention in the range of between about 0.01 to 100 mg/kg body weight/day, usually in 3-8 divided doses, for between about 1to 12 weeks, preferably between 2 to 10 weeks, more preferably between about 3 to 8 weeks, and even more preferably for about 4 to 6, or 5 weeks. It will also be appreciated that the effective dosage of a compound of the invention used for treatment may increase or decrease over the course of a particular treatment.

The term "enantiomers" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another. An equimolar mixture of two enantiomers is called a "racemic mixture" or a "racemate."

The term "haloalkyl" is intended to include alkyl groups as defined above that are mono-, di- or polysubstituted by halogen, e.g., fluoromethyl and trifluoromethyl.

The term "halogen" designates -F, -Cl, -Br or –I.

The term "hydroxyl" means -OH.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term "homeostasis" is art-recognized to mean maintenance of static, or constant, conditions in an internal environment.

The language "improved biological properties" refers to any activity inherent in a compound of the invention that enhances its effectiveness in vivo. In a preferred embodiment, this term refers to any qualitative or quantitative improved therapeutic property of a compound of the invention, such as reduced toxicity.

The term "obesity" includes ingestive disorders.

The language "5-HT2" refers to the serotonin receptors (including those delineated herein) such as 5-HT2a, 5-HT2b and 5-HT2c sub-types.

The term "optionally substituted" is intended to encompass groups that are unsubstituted or are substituted by other than hydrogen at one or more available positions, typically 1, 2, 3, 4 or 5 positions, by one or more suitable groups (which may be the same or different). Such optional substituents include, for example, hydroxy, halogen, cyano, nitro, C_1-C_8alkyl, C_2-C_8 alkenyl, C_2-C_8alkynyl, C_1-C_8alkoxy, C_2-C_8alkyl ether, C_3-C_8alkanone, C_1-C_8alkylthio, amino, mono- or di-(C1-C_8alkyl)amino, haloC_1-C_8alkyl, haloC_1-C_8alkoxy, C_1-C_8alkanoyl, C_2-C_8alkanoyloxy, C_1-C_8alkoxycarbonyl, -COOH, -$CONH_2$, mono- or di-(C_1 -C_8alkyl)aminocarbonyl, -SO_2NH_2, and/or mono or di(C_1-C_8alkyl)sulfonamido, as well as carbocyclic and heterocyclic groups. Optional substitution is also indicated by the phrase "substituted with from 0 to X substituents," where X is the maximum number of possible substituents. Certain optionally substituted groups are substituted with from 0 to 2, 3 or 4 independently selected substituents (i.e., are unsubstituted or substituted with up to the recited maximum number of substituents).

The term "isomers" or "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

The term "modulate" refers to an increase or decrease, e.g., in the ability of a compound inhibit activity of a target in response to exposure to a compound of the invention, including for example in an subject (e.g., animal, human) such that a desired end result is achieved, *e.g.*, a therapeutic result.

The term "obtaining" as in "obtaining a compound capable of modulating (agonizing, antagonizing) a target delineated herein and is intended to include purchasing, synthesizing or otherwise acquiring the compound.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, *e.g.*, the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy,

carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "prodrug" or "pro-drug" includes compounds with moieties that can be metabolized *in vivo*. Generally, the prodrugs are metabolized *in vivo* by esterases or by other mechanisms to active drugs. Examples of prodrugs and their uses are well known in the art (See, *e.g.*, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19). The prodrugs can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Hydroxyl groups can be converted into esters *via* treatment with a carboxylic acid. Examples of prodrug moieties include substituted and unsubstituted, branch or unbranched lower alkyl ester moieties, (*e.g.*, propionoic acid esters), lower alkenyl esters, di-lower alkyl-amino lower-alkyl esters (*e.g.*, dimethylaminoethyl ester), acylamino lower alkyl esters (*e.g.*, acetyloxymethyl ester), acyloxy lower alkyl esters (*e.g.*, pivaloyloxymethyl ester), aryl esters (phenyl ester), aryl-lower alkyl esters (*e.g.*, benzyl ester), substituted (*e.g.*, with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, di-lower alkyl amides, and hydroxy amides. Preferred prodrug moieties are propionoic acid esters and acyl esters. Prodrugs which are converted to active forms through other mechanisms *in vivo* are also included.

The language "a prophylactically effective amount" of a compound refers to an amount of a compound of the invention any formula herein or otherwise described herein which is effective, upon single or multiple dose administration to the patient, in preventing or treating a disorder herein.

The language "reduced toxicity" is intended to include a reduction in any undesired side effect elicited by a compound of the invention when administered *in vivo*.

The term "sulfhydryl" or "thiol" means –SH.

The term "subject" includes organisms which are capable of suffering from a disorder herein or who could otherwise benefit from the administration of a compound of the invention of the invention, such as human and non-human animals. Preferred humans include human patients suffering from or prone to suffering from obesity or associated state, as described herein. The

term "non-human animals" of the invention includes all vertebrates, *e.g.*, mammals, *e.g.*, rodents, *e.g.*, mice, and non-mammals, such as non-human primates, *e.g.*, sheep, dog, cow, chickens, amphibians, reptiles, etc.

The term "susceptible to obesity" is meant to include subjects at risk of developing obesity, e.g., including those delineated herein, i.e., subjects suffering from obesity or symptoms thereof, subjects having a family or medical history of obesity or symptoms thereof, and the like.

The phrases "systemic administration," "administered systemically", "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound of the invention(s), drug or other material, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The language "therapeutically effective amount" of a compound of the invention of the invention refers to an amount of an agent which is effective, upon single or multiple dose administration to the patient, treating or preventing obesity and/or symptoms of obesity, or in prolonging the survivability of the patient with such obesity beyond that expected in the absence of such treatment.

With respect to the nomenclature of a chiral center, terms "d" and "l", "R" and :S", and (+) and (–) configuration are as defined by the IUPAC Recommendations. As to the use of the terms, diastereomer, racemate, epimer and enantiomer will be used in their normal context to describe the stereochemistry of preparations.

2. COMPOUNDS OF THE INVENTION

In one aspect, the invention provides compounds capable of modulating (e.g., inhibiting or stimulating) (directly or indirectly) 5-HT binding activity each in combination with an amphetamine compound.

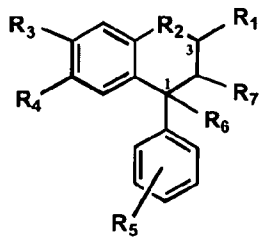
In one embodiment, the invention provides a compound capable of activating (e.g., agonizing) 5-HT2c; and pharmaceutically acceptable esters, salts, and prodrugs thereof, each in combination with an amphetamine compound.

In embodiments, the compounds described herein are referred are those having a tetrahydronaphthlenyl moiety substituted with an optionally substituted-aryl (e.g. phenyl) group and a substituted amino (e.g., alkylamino, dialkylamino)group. They are referred to herein as

PAT compounds and include the specific compounds delineated in the formulae, tables and examples herein.

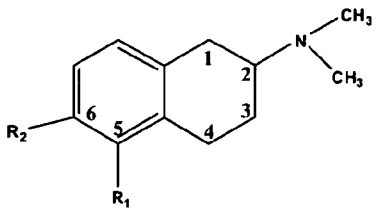
Certain preferred compounds include compounds specifically delineated herein:

Table 1: Compounds:



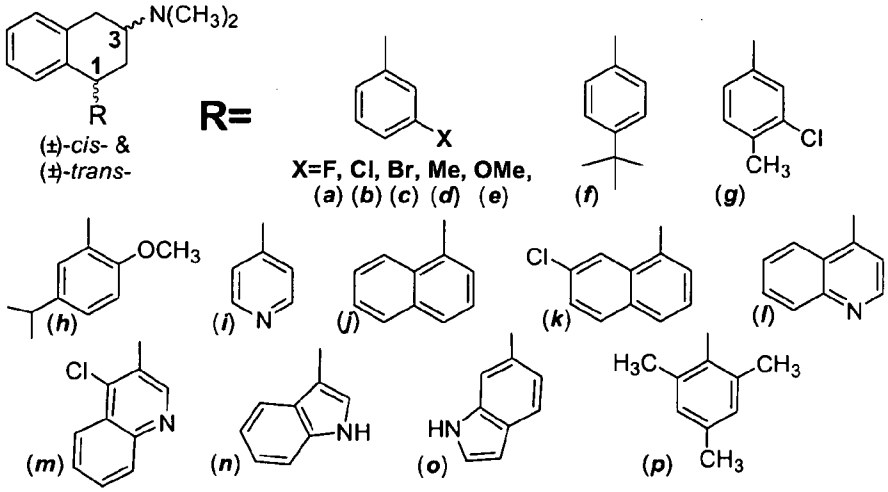
Compd	Config	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	(1*R*,3*S*)-(−)-*trans*	$N(CH_3)_2$	CH_2	H	H	H	H	H
2	(1*S*,3*R*)-(+)-*trans*	$N(CH_3)_2$	CH_2	H	H	H	H	H
3	(1*R*,3*R*)-(−)-*cis*	$N(CH_3)_2$	CH_2	H	H	H	H	H
4	(1*S*,3*S*)-(+)-*cis*	$N(CH_3)_2$	CH_2	H	H	H	H	H
5	(±)-*trans* (PAB)	$N(CH_3)_2$	$(CH_2)_2$	H	H	H	H	H
6	(±)-*cis* (PAB)	$N(CH_3)_2$	$(CH_2)_2$	H	H	H	H	H
7	(±)-*trans*	$N(CH_3)_2$	CH_2	Cl	OH	H	H	H
8	(±)-*cis*	$N(CH_3)_2$	CH_2	Cl	OH	H	H	H
9	(±)-*trans*	$N(CH_3)_2$	CH_2	OH	OH	H	H	H
10	(±)-*cis*	$N(CH_3)_2$	CH_2	OH	OH	H	H	H
11	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	H	CH_3	H
12	(±)-*cis*	$N(CH_3)_2$	CH_2	H	H	H	CH_3	H
13	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	*p*-Cl	H	H
14	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	*p*-F	H	H
15	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	*p*-CH_3	H	H
16	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	*o*-Cl	H	H
17	(±)-*trans*	$N(CH_3)_2$	CH_2	H	H	*o*-CH_3	H	H
18	(±)-*trans*	$N(CH_3)_3$	CH_2	H	H	H	H	H
19	(±)-*cis*	$N(CH_3)_3$	CH_2	H	H	H	H	H
20	(±)-*trans*	$NH(CH_3)$	CH_2	H	H	H	H	H
21	(±)-*cis*	$NH(CH_3)$	CH_2	H	H	H	H	H
22	(±)-*trans*	NH_2	CH_2	H	H	H	H	H
23	(±)-*cis*	NH_2	CH_2	H	H	H	H	H
24	(±)-*trans*	NH_2	CH_2	OH	OH	H	H	H
25	(±)-*cis*	NH_2	CH_2	OH	OH	H	H	H
26	(±)-*trans*	H	CH_2	H	H	H	H	$N(CH_3)_2$
27	(±)cis	H	CH_2	H	H	H	H	$N(CH_3)_2$
28	(±)-*trans*	$N(C_2H_5)_2$	CH_2	H	H	H	H	H
29	(±)-*trans*	$N(C_3H_5)_2$	CH_2	H	H	H	H	H
30	(±)-*trans*	$NCH_3(C_3H_5)$	CH_2	H	H	H	H	H
31	(±)-*trans*	$NH(C_3H_5)$	CH_2	H	H	H	H	H

Table 2 Compounds



PAT #	R_1	R_2
32 (5APT)	C_6H_5	H
33 (CF_3-5APT)	*m*-CF_3-C_6H_4	H
34 (OCH_3-5APT)	*m*-OCH_3-C_6H_4	
35 (Cl-5APT)	*m*-Cl-C_6H_4	H
36 (6APT)	H	C_6H_5
37 (CF_3-6APT)	H	*m*-CF_3-C_6H_4
38 (OCH_3-6APT)	H	*m*-OCH_3-C_6H_4
39 (Cl-6APT)	H	*m*-Cl-C_6H_4
40 (Br-6APT)	H	*m*-Br-C_6H_4

Table 3. Compounds 41(a)-(p)



The invention also relates to the pharmaceutically acceptable salts and esters of the above-mentioned compounds.

The compounds herein can be obtained directly from chemical compound sources or can be synthesized from readily available starting materials. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995) and subsequent editions thereof. Additionally, the following references are instructive: Wyrick, S.D., Booth, R.G., Myers, A.M., Owens, C.E., Kula, N.S., Baldessarini, R.J, Mailman, R.B., Synthesis and pharmacological evaluation of 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes as ligands for a novel receptor with sigma-like neuromodulatory activity. *Journal of Medicinal Chemistry* 36: 2542-2551 (1993); Wyrick, S.D., Booth, R.G., Myers, A.M., Owens, C.E., Bucholtz, E.C., Hooper, P.C., Kula, N.S., Baldessarini, R.J., and Mailman, R.B. 1-Phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes and related derivatives as ligands for the neuromodulatory σ_3 receptor: Further structure-activity relationships. *Journal of Medicinal Chemistry* 38:3857-3864 (1995); Bucholtz, E.C., Brown., R.L., Tropsha, A., Booth, R.G, and Wyrick, S.D. Synthesis, Evaluation and Comparative Molecular Field Analysis of 1-Phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes as Ligands for Histamine H_1 Receptors. *Journal of Medicinal Chemsitry.*42:3041-3054(1999); and Ghoneim OM, Legere JA, Glbraikh A, Tropsha A, Booth RG. Novel ligands for the human histamine H_1 receptor: Synthesis, pharmacology, and comparative molecular field analysis studies of 2-dimethylamino-5-(6)-phenyl-1,2,3,4-tetrahydronaphthalenes. *Bioorganic and Medicinal Chemistry*, 14:6640-6658 (2006).

Naturally occurring or synthetic isomers can be separated in several ways known in the art. Methods for separating a racemic mixture of two enantiomers include chromatography using a chiral stationary phase (see, *e.g.*, "Chiral Liquid Chromatography," W.J. Lough, Ed. Chapman and Hall, New York (1989)). Enantiomers can also be separated by classical resolution techniques. For example, formation of diastereomeric salts and fractional crystallization can be used to separate enantiomers. For the separation of enantiomers of carboxylic acids, the diastereomeric salts can be formed by addition of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, and the like. Alternatively, diastereomeric esters can be formed with enantiomerically pure chiral alcohols such as menthol, followed by separation of the diastereomeric esters and hydrolysis to yield the free, enantiomerically enriched carboxylic acid. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or

sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

3. USES OF THE COMPOUNDS OF THE INVENTION

The herein delineated compound combinations, compositions and methods are useful for treating or preventing obesity. In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human.

In this embodiment, the compounds of the invention may either directly or indirectly modulate (e.g., agonize, activate, stimulate) the activity of 5-HT2c or specific domains thereof. A cell can be contacted with a composition of the invention to agonize 5-HT2c and modulate 5-HT2c mediated activity. Contacting cells or administering the compounds of the invention to a subject is one method of treating a cell or a subject suffering from obesity that responds to activation of 5-HT2C receptors.

In one embodiment, a method of treating a subject suffering from or susceptible to obesity includes administering to a subject in need thereof a therapeutically effective amount of a compound capable of directly or indirectly modulating the activity of 5-HT2c alone or in combination with an amphetamine compound, in combination with an amphetamine compound to thereby treat the subject. Exemplary compounds include those compounds described herein (e.g., PATs, etc.). In addition to amphetamine itself, other exemplary amphetamine compounds include, for example, phentermine.

In certain embodiments, the methods of the invention include administering to a subject a therapeutically effective amount of a compound combination of the invention in combination with another pharmaceutically active compound. Other pharmaceutically active compounds that may be used can be found in *Harrison's Principles of Internal Medicine*, Thirteenth Edition, Eds. T.R. Harrison *et al.* McGraw-Hill N.Y., NY; and the Physicians Desk Reference 50th Edition 1997, Oradell New Jersey, Medical Economics Co., the complete contents of which are expressly incorporated herein by reference. The compound combination of the invention and the additional pharmaceutically active compound may be administered to the subject in the same pharmaceutical composition or in different pharmaceutical compositions (at the same time or at different times).

Certain 5-HT drugs (e.g., drugs that activate 5-HTa and/or 5-HTb receptors) have an undesirable side effect profile that make them unsuitable for most patients, that is, they demonstrate undesirable psychiatric and/or cardiovascular (e.g., valvular heart disease,

pulmonary hypertension, cardiotoxicity) side effects that may be life threatening. The combinations delineated herein provide therapeutics with improved functional selectivity (e.g., selectively activate 5-HT2c relative to activation of 5-HTa or 5-HTb subtypes), thus conferring improved side effect profiles compared to other 5-HT modulating compounds. In reference to functional selectivity of 5-HT2c over 5-HT2a and 5-HT2b subtypes, in aspects, the selectivity of PAT-type compounds is total, i.e., activation of 5-HT2c and no activation of 5-HT2a and 5-HT2b subtypes. In certain situations, it may be acceptable to treat certain patients with PAT-type compounds that are *at least* 10-fold selective for activation of 5-HT2c over 5-HT2a and/or 5-HT2b subtypes, or alternatively at least X-fold selective for activation of 5-HT2c over 5-HT2a and/or 5-HT2b subtypes, where X is any number between 2 and 5,000, e.g., at least 15-fold, 25-fold, 50-fold, 100-fold, 250-fold, 500-fold, 1,000-fold, 2,500-fold, or 5,000 fold.

Determination of a therapeutically effective amount or a prophylactically effective amount of the compound of the invention of the invention, can be readily made by the physician or veterinarian (the "attending clinician"), as one skilled in the art, by the use of known techniques and by observing results obtained under analogous circumstances. The dosages may be varied depending upon the requirements of the patient in the judgment of the attending clinician; the severity of the condition being treated and the particular compound being employed. In determining the therapeutically effective amount or dose, and the prophylactically effective amount or dose, a number of factors are considered by the attending clinician, including, but not limited to: the degree of obesity; pharmacodynamic characteristics of the particular agent and its mode and route of administration; the desired time course of treatment; the species of mammal; its size, age, and general health; the specific disease involved; the degree of or involvement or the severity of the disease; the response of the individual patient; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the kind of concurrent treatment (*i.e.*, the interaction of the compound of the invention with other co-administered therapeutics); and other relevant circumstances.

Treatment can be initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage may be increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired. A therapeutically effective amount and a prophylactically effective amount of a compound of the invention of the invention is expected to vary from about 0.01 milligram per kilogram of body weight per day (mg/kg/day) to about 100 mg/kg/day.

Compounds determined to be effective for the prevention or treatment of obesity in animals, *e.g.*, dogs, chickens, and rodents, may also be useful in treatment of obesity in humans. Those skilled in the art of treating obesity generally, as well as obesity responsive to 5-HT2c receptor activation in humans will know, based upon the data obtained in animal studies, the dosage and route of administration of the compound to humans. In general, the dosage and route of administration in humans is expected to be similar to that in animals.

The identification of those patients who are in need of prophylactic treatment for obesity is well within the ability and knowledge of one skilled in the art. Certain of the methods for identification of patients which are at risk of developing obesity which can be treated by the subject method are appreciated in the medical arts, such as family history, and the presence of risk factors associated with the development of that disease state in the subject patient. A clinician skilled in the art can readily identify such candidate patients, by the use of, for example, clinical tests, physical examination and medical/family history.

A method of assessing the efficacy of a treatment in a subject includes determining the pre-treatment extent of obesity (overweight) by methods well known in the art and then administering a therapeutically effective amount of a compound delineated herein alone or each in combination with an amphetamine compound according to the invention to the subject. After an appropriate period of time after the administration of the compound (e.g., 1 day, 1 week, 2 weeks, one month, six months), the extent of obesity (overweightness) is determined again. The modulation (e.g., decrease) of the extent of overweightness indicates efficacy of the treatment. The extent or invasiveness of obesity may be determined periodically throughout treatment. For example, the extent or invasiveness of overweightness may be checked every few hours, days or weeks to assess the further efficacy of the treatment. A decrease in extent or invasiveness of the overweightness indicates that the treatment is efficacious. The method described may be used to screen or select patients that may benefit from treatment with a modulating compound capable of activating selectively 5-HT2c (vs. 5-HT2a,b) receptors in combination with an amphetamine compound.

As used herein, "obtaining a biological sample from a subject," includes obtaining a sample for use in the methods described herein. A biological sample is described above.

In another aspect, a compound of the invention is packaged in a therapeutically effective amount in combination with an amphetamine compound with a pharmaceutically acceptable carrier or diluent. The composition may be formulated for treating a subject suffering from or susceptible to obesity, and packaged with instructions to treat a subject suffering from or susceptible to obesity.

In one aspect, a method of monitoring the progress of a subject being treated with a combination composition herein includes determining the pre-treatment status (e.g., progression, target profile, Marker profile) of obesity, administering a therapeutically effective amount of a combination composition herein to the subject, and determining the status (e.g., progression, target profile, Marker profile) of obesity after an initial period of treatment with the combination composition, wherein the modulation of the status indicates efficacy of the treatment.

The subject may be at risk of obesity, may be exhibiting symptoms of obesity, may be susceptible to obesity and/or may have been diagnosed with obesity.

If the modulation of the status indicates that the subject may have a favorable clinical response to the treatment, the subject may be treated with the compound combination composition. For example, the subject can be administered therapeutically effective dose or doses of the combination composition.

Kits of the invention include kits for treating obesity in a subject. The kit may include a compound of the invention in combination with an amphetamine compound, for example, a compound described herein, pharmaceutically acceptable esters, salts, and prodrugs thereof, and instructions for use. The instructions for use may include information on dosage, method of delivery, storage of the kit, etc. The kits may also include, reagents, for example, test compounds, buffers, media (e.g., cell growth media), cells, etc. One or more of the kit of the invention may be packaged together, for example, a kit for assessing the efficacy of a treatment for obesity may be packaged with a kit for monitoring the progress of a subject being treated for obesity according to the invention.

The present methods can be performed on cells in culture, *e.g. in vitro* or *ex vivo*, or on cells present in an animal subject, *e.g., in vivo*. Compounds of the inventions can be initially tested *in vitro* using primary cultures of cells, e.g., transformed cells, and the like.

The present method can be performed on cells in culture, *e.g. in vitro* or *ex vivo*, or on cells present in an animal subject, *e.g., in vivo*. Compound of the invention can be initially tested *in vitro* using cells from the respiratory tract from embryonic rodent pups (*See e.g.* U.S. Patent No. 5,179,109 - fetal rat tissue culture), or other mammalian (*See e.g.* U.S. Patent No. 5,089,517 - fetal mouse tissue culture) or non-mammalian animal models.

Alternatively, the effects of compound of the invention can be characterized *in vivo* using animals models.

4. PHARMACEUTICAL COMPOSITIONS

The invention also provides a pharmaceutical composition, comprising an effective amount of a compound herein in combination with an amphetamine compound and a pharmaceutically acceptable carrier. In a further embodiment, the effective amount is effective to treat obesity, as described previously.

In an embodiment, the compound of the invention in combination with an amphetamine compound is administered to the subject using a pharmaceutically-acceptable formulation, *e.g.*, a pharmaceutically-acceptable formulation that provides sustained delivery of the compound of the invention to a subject for at least 12 hours, 24 hours, 36 hours, 48 hours, one week, two weeks, three weeks, or four weeks after the pharmaceutically-acceptable formulation is administered to the subject.

In certain embodiments, these pharmaceutical compositions are suitable for topical or oral administration to a subject. In other embodiments, as described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound.

The phrase "pharmaceutically acceptable" refers to those compounds of the present invention, compositions containing such compounds, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" includes pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers

include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Compositions containing a compound of the invention(s) include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, more preferably from about 10 per cent to about 30 per cent.

Methods of preparing these compositions include the step of bringing into association a compound of the invention(s) with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a

compound of the invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Compositions of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the invention(s) as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or

prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compound of the invention(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

In addition to inert diluents, the oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compound of the invention(s) may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compound of the invention(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of the invention(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound of the invention(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to compound of the invention(s) of the present invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of the invention(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The compound of the invention(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (*e.g.,* fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically-acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the invention(s) to the body. Such dosage forms can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the active ingredient across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredient in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of the invention.

Pharmaceutical compositions of the invention suitable for parenteral administration comprise one or more compound of the invention(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers, which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of compound of the invention(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters)

and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compound of the invention(s) are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically-acceptable carrier.

Regardless of the route of administration selected, the compound of the invention(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of the invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. An exemplary dose range is from 0.1 to 100 mg per day.

A preferred dose of the compound of the invention for the present invention is the maximum that a patient can tolerate and not develop serious side effects while still achieving weight loss. Preferably, the compound of the invention of the present invention is administered in amount of about 0.01 mg to about 100 mg per kilogram of body weight,. Ranges intermediate to the above-recited values are also intended to be part of the invention.

EXAMPLES

The invention is further illustrated by the following examples which are intended to illustrate the in vivo effectiveness of PAT–type compounds alone or in combination with amphetamine-type compounds as antiobesity pharmacotherapy but is not intended to limit the scope of the invention.

EXAMPLE 1

(–)-*Trans*-PAT alone reduces basal body weight (Fig 1)

Initially, we examined the acute effects of (–)-*trans*-PAT (1.0 and 10 mg/kg) on basal body weight of 129S6/SvEv mice. Mice were injected with vehicle or (–)-*trans*-PAT IP. The mice were returned to their home cage where they had *ad lib* access to food and water and reweighed

24 hours later. As shown in **Fig. 1**, vehicle treated mice (0 mg/kg (–)-*trans*-PAT) gained ~1% of their previous days body weight (~0.35 g). In contrast, mice receiving either 1 or 10 mg/kg (–)-*trans*-PAT lost ~0.5-0.7% of the previous day body weight. These differences were significant ($p < 0.05$; PAT vs Vehicle) but did not differ between doses of (–)-*trans*-PAT. The effect of (–)-*trans*-PAT is intriguing but preliminary. We have not yet ruled out that (–)-*trans*-PAT interaction with histamine H_1 receptors may have affected the mice, however, it is noted that H_1 affinity for typical and atypical antipsychotics is correlated with short-term weight gain (Kroeze et al., 2003). Thus, this acute experiment supports the hypothesis that (–)-*trans*-PAT exhibits anti-obesity properties *in vivo*. The experiments proposed in this application are designed to determine the receptor basis for this effect and will distinguish between various aspects of feeding behavior affected by (–)-*trans*-PAT (e.g., satiety vs reduced food seeking vs metabolic effects).

EXAMPLE 2

(–)-*Trans*-PAT interaction with amphetamine-type stimulants enhances weight loss (Fig 2). In this experiment, 10 mg/kg (–)-*trans*-PAT was administered to 129S6/SvEv mice in combination with amphetamine (3 mg/kg) IP and basal body weight measured 24 hours later. Mice treated with amphetamine and (–)-*trans*-PAT had nearly 3% loss of body weight (from baseline) that was significantly greater than effect of either agent alone (**Fig. 2**). This finding is of interest given the synergistic effects of the 5HT releasing agent/reuptake inhibitor *D*-fenfluramine with the stimulant phentermine (CH_3 replaces H on amphetamine α-carbon) on weight loss in humans (i.e., Fen-Phen).

EXAMPLE 3: **(–)-*Trans*-PAT reduces food consumption and this effect is enhanced by amphetamine-type stimulants (Fig 3).**

In this experiment, 129S6/SvEv mice were acclimated to a wet food mash and then food deprived for 20 hrs (~85% free-feeding body weight). Mice then received IP injection of one of the following treatments: vehicle; (–)-*trans*-PAT 3 mg/kg; (–)-*trans*-PAT 10 mg/kg; amphetamine 3 mg/kg + (–)-*trans*-PAT 3 mg/kg; amphetamine 3 mg/kg + (–)-*trans*-PAT 10mg/kg; fenfluramine 6 mg/kg; amphetamine 3 mg/kg. After 30 min, mice then were presented with the wet mash food and consumption over 60 min calculated. Results are shown in **Fig. 3**.

Using one-way ANOVA, we observed a treatment effect of $p<0.0001$. Using post-hoc testing, we determined that 10 mg/kg (but not 3mg/kg) (–)-*trans*-PAT inhibited food consumption compared to vehicle. Amphetamine alone had no effect on food consumption (hence combined with vehicle–treated mice), but, the combination of both doses of (–)-*trans*-PAT with amphetamine inhibited food consumption ($p <0.01$). The positive control fenfluramine (6 mg/kg) produced significant anorexic response ($p<0.01$).

The results of this experiment indicate that the effect of (–)-*trans*-PAT to reduce food consumption is enhanced by stimulants, such as, amphetamine and perhaps the structurally-related stimulant phentermine. It is proposed that (–)-*trans*-PAT may be a safe replacement for d-fenfluramine in combination with phentermine (i.e., PAT-Phen).

EXAMPLE 4

***In vivo* data showing that (–)-*trans*-PAT causes weight loss in mice (Figure 4).**

In these experments, mice (male, age 14-18 weeks; 129S6/SvEv from Taconic) were housed in groups of five. Animals were naïve to drug treatment. Animals were weighed and injected (0.1ml/10g i.p.) with either vehicle (0.9% NaCl with 0.028 mM acetic acid, 0.048 mM NaOH, and 1% DMSO), (–)-*trans*-PAT at 10 mg/kg, the $5HT_{2A}$ antagonist M100907 at 1.0 mg/kg, or, injected simultaneously with (–)-*trans*-PAT or M100907 issolved in saline at 0.6 mg/kg or saline control. Animals were placed for activity monitoring in Plexiglas chambers (43.2 cm long × 43.2 cm wide × 30.5 cm high) (model ENV-520; Med Associates, St. Albans, VT) in a novel open field assay. Mice were placed into the center of the open field and activity was recorded for 120 min with subtotals at 10 min increments. The first 30 min was separated from analysis as a habituation period. Testing was performed in darkness to minimize anxiety. At 22-26 hrs after injection, activity monitoring, and access to ad lib food and water in the home cage, weights were repeated for each animal. ANOVA was performed with Statview software. There were 9-10 animals in each treatment group, and testing for each treatment was performed on each of 4 testing days to accommodate the number of animals tested.

The data in Fig **4** below confirm the 24-hr. weight-loss effects of (–)-*trans*-PAT. The 100-fold selective serotonin 5-HT_{2A} receptor antagonist M100907 (Kehne et al., JPET 277:968, 1996) does not produce a change in weight different from vehicle. Given that our *in vitro* data indicate (–)-*trans*-PAT is a full-efficacy $5HT_{2C}$ agonist and a $5HT_{2A}$ antagonist, these results suggest that the weight loss effects of (–)-*trans*-PAT shown are due to $5HT_{2C}$ receptor activation and not 5HT2a antagonism..

EXAMPLE 5

Assessment of (–)-trans-PAT agonist activity at serotonin 5-HT_2 receptors **(Figure 5)**

The serotonin 5-HT_2 GPCR family is constitutively active when expressed in CHO and HEK cells, coupling to $G\alpha_q$ protein to activate PLC and IP formation (Raymond et al., 2001). In lysates of null-transfected CHO and HEK cells, no increase in PLC/[^{3}H]-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 serotonin 5-HT_{2C} receptors, however, serotonin produces a concentration-dependent increase in basal activity of PLC/[^{3}H]-IP formation, with EC_{50} = 6.30 ± 0.55 nM (n_H = 1.3 ± 0.2) and E_{max} = 480 ± 12.4 % basal control activity (occurs at ~ 0.1 μM), as shown in the Fig. 5 inset. The results for serotonin here are

consistent with results reported in the literature (e.g., Rosendorff et al., 2000). Relative to serotonin, (–)-*trans*-PAT is a full-efficacy serotonin 5-HT_{2C} receptor agonist that produces a concentration-dependent increase in basal activity of PLC/[^{3}H]-IP formation, with EC_{50} = 21.4 ± 2.22 nM (n_H = 0.66 ± 0.11) and E_{max} = 464 ± 10.8% basal control activity (occurs at ~ 10 μM), as shown in Fig. 4. There is no significant difference (p = 0.2) between the serotonin and (–)-*trans*-PAT E_{max} values.

In CHO cells expressing human serotonin 5-HT_{2A} receptors, (–)-*trans*-PAT does not stimulate basal PLC/[^{3}H]-IP formation at concentrations up to 10 μM (~25-times K_i) (Fig. 5), whereas, serotonin produces a concentration-dependent increase in basal activity of PLC/[^{3}H]-IP formation, with EC_{50} = 30 ± 2 nM and E_{max} = 300 ± 7 % basal control activity (data not shown). In HEK cells expressing human serotonin 5-HT_{2B} receptors, (–)-*trans*-PAT also does not stimulate basal PLC/[^{3}H]-IP formation at concentrations up to 10 μM (75-times K_i) (Fig. 5), whereas, serotonin increases basal PLC/[^{3}H]-IP formation, with EC_{50} = 19.7 ± 9.21 nM and E_{max} = 900 ± 10 % basal control activity (data not shown).

EXAMPLE 6

3.4. Assessment of (–)-trans-PAT antagonist activity at serotonin 5-HT_2 receptors **(Fig 6A and 6B)**

Given that (–)-*trans*-PAT binds with moderate affinity to serotonin 5-HT_{2A} and 5-HT_{2B} receptors but does not activate these 5-HT_2 receptor subtypes, the ability of (–)-*trans*-PAT to act as an antagonist of serotonin-induced activation of PLC/[^{3}H]-IP formation mediated by 5-HT_{2A} and 5-HT_{2B} receptors was assessed. In CHO cells expressing human serotonin 5-HT_{2A} receptors, serotonin (1.0 μM) stimulated PLC/[^{3}H]-IP formation (~ 250% basal control) and this effect was fully blocked by (–)-*trans*-PAT (Fig. 6A). In HEK cells expressing human serotonin 5-HT_{2B} receptors, serotonin (0.01 μM) stimulated PLC/[^{3}H]-IP formation (~ 350% basal control) and this effect was fully blocked by (–)-*trans*-PAT (Fig. 6B).

Competition binding experiments:

Affinity of *trans*-(–)-PAT stereoisomers at $5HT_{2c}$ receptors (Fig7, Table 4).

The $5HT_{2C}$ receptor affinity of (–)-*trans*-PAT and its stereoisomers, (+)-*trans*-PAT, (–)-*cis*-PAT, and (+)-*cis*-PAT, was assessed in competitive radioligand displacement assays using ~K_D concentration of radioligand (as determined above). Curves (**Fig 7**) are sigmoidal and span 3-4 log concentration units to achieve complete radioligand displacement, characteristic of competitive displacement of ~K_D radioligand concentration. The Hill coefficient (n_H) for the slope of the competitive displacement curve for (–)-*trans*-PAT is 0.9, characteristic of agonist ligand binding at a GPCR, according to the ternary complex model with limiting availability of G protein, and, subpopulation(s) of receptor in an agonist-preferring conformation(s). The n_H values for the other PAT stereoisomers range 0.8-1.0 (antagonists theoretically should have n_H=1). Except for (–)-*trans*-PAT, none of the other PAT stereoisomers activate $5HT_{2C}$ receptors at concentrations up to 10 μM (i.e., at least 10-times Ki) – see Aim 2 results. The stereoselectivity of $5HT_{2C}$ receptors for PAT isomers has significant applications to delineate the 3D structure of the $5HT_{2C}$ active site and molecular determinants for receptor activation.

Table 4

PAT Stereoisomer	$5HT_{2C}$ Ki ± SEM	n_H
(1*R*,3*S*)-(–)-*trans*-PAT	37.6 ± 3.0 nM	0.9
(+/-)-*trans*-PAT (not shown in Fig 5 for clarity)	75.0 nM ± 2.2 nM	0.7
(1*S*,3*R*)-(+)-*trans*-PAT	1270 ± 84.8 nM	1.0
(1*S*,3*S*)-(–)-*cis*-PAT	433 ± 4.8 nM	0.8
(1*R*,3*R*)-(+)-*cis*-PAT	975 ± 7.8 nM	0.8

1e. Affinity of *trans*-(–)-PAT stereoisomers at $5HT_{2A}$ receptors (Fig 8).

Concentration–response curves for radioligand displacement by (–)-*trans*-PAT and its stereoisomers at $5HT_{2A}$ receptors is shown in **Fig 8.** The $5HT_{2A}$ affinity of (–)-*trans*-PAT (Ki~400 nM) is 10-fold lower than at $5HT_{2C}$ receptors. Table 5 summarized Ki and n_H values for all 4 PAT stereoisomers. The rank order for affinity of PAT stereoisomers at $5HT_{2A}$ receptors is different than at $5HT_{2C}$ receptors; rank order at $5HT_{2A}$ also differs from histamine H_1 receptors. The n_H value for (–)-*trans*-PAT at $5HT_{2A}$ is 0.9, however, it is a $5HT_{2A}$ antagonist (Figs 10,11). The functional assay is not sensitive enough to detect inverse agonism, but, such activity is likely. Functional assessment of other PAT stereoisomers at $5HT_{2A}$ (n_H=0.9-1.0) is not complete.

1f. Affinity of *trans*-(–)-PAT stereoisomers at $5HT_{2B}$ receptors (Fig 9).

Concentration–response curves for radio-ligand displacement by (–)-*trans*-PAT and its stereoisomers at $5HT_{2B}$ receptors is shown in **Fig 9**. The $5HT_{2B}$ affinity of (–)-*trans*-PAT (Ki~1 μM) is 20-fold lower than at $5HT_{2C}$ receptors.

Ki and n_H values for all 4 PAT stereoisomers are summarized in Table 5. Rank order of PAT stereoisomer binding at $5HT_{2B}$ receptors is different and overall much lower than at $5HT_{2A}$ and $5HT_{2C}$ receptors. Thus, although amino acid sequence is very similar for members of the $5HT_2$ family, the 3D arrangement of amino acids that form the PAT ligand binding site appears to be different (especially for the $5HT_{2B}$ receptor). Thus the PAT stereochemical scaffold can be used as a template for molecular modeling (structural) studies and optimized to provide drugs with selective actions at $5HT_2$ subtypes.

Table 5

PAT Stereoisomer	$5HT_{2A}$ Ki ± SEM	n_H	$5HT_{2B}$ Ki ± SEM	n_H	$5HT_{2C}$ Ki ± SEM	n_H
(1*R*,3*S*)-(–)-*trans*-PAT	**407.3 ± 38.4 nM**	0.9	**1,168.8 ± 6.3 nM**	1.0	**37.6 ± 3.0 nM**	0.93
(1*S*,3*R*)-(+)-*trans*-PAT	520.1 ± 0.29 nM	1.0	~ 2500 nM	1.0	1270 ± 84.8 nM	1.0
(1*S*,3*S*)-(–)-*cis*-PAT	1452.4 ± 0.23 nM	1.0	> 5000 nM		433 ± 4.8 nM	0.80
(1*R*,3*R*)-(+)-*cis*-PAT	776.8 ± 0.20 nM	0.9	> 5000 nM		975 ± 7.8 nM	0.76

EXAMPLE 10

5HT2C Agonist activity of other PAT analogs (Figs 10A & 10B).

Fig. 10A shows that in CHO-$5HT_{2C}$ cells, (–)-*trans*-PAT is a $5HT_{2C}$ agonist (EC_{50}=21.4 ± 2.22 nM, n_H=0.66) with full efficacy relative to 5HT (EC_{50}= 6.30 ± 0.55nM, n_H=1.3; [consistent with Rosendorff et al., 2000]). **Fig 10A** also shows that Cl-6APT (Ki~300nM; Fig 11), is a full efficacy $5HT_{2C}$ agonist, but, it has very low potency (EC_{50} = 4,630 ± 312 nM; n_H=0.63). Results (not shown) suggest that Cl-6APT docks to our $5HT_{2C}$ homology model differently than (–)-*trans*-PAT – the tetralin probably interacts with W6.48 & F6.51 as is the case for (–)-*trans*-PAT

(below), but, the Cl-substituted pendant phenyl of Cl-6APT likely interacts with $5HT_{2C}$ W3.28 & N7.36 (H-bond with Cl).

Figure 10B shows preliminary data is available from the PDSP (2005) regarding functional activity of 5APT and Cl-6APT (Table 2) at $5HT_{2C}$ vs. $5HT_{2A}$ receptors. 5APT is a moderate potency partial agonist at $5HT_{2C}$; EC_{50}~80 nM; E_{max}~75% 5HT value (5HT E_{max}~80,000 cpm; not shown). Thus, the location of the pendant phenyl ring appears to have a significant effect on ability of PAT analogs to activate $5HT_{2C}$ receptors – potency decreases as the phenyl moiety is moved from the 1-position of (–)-*trans*-PAT (EC_{50}~20nM), to the 5-position of 5APT (EC_{50}~80 nM), to the 6-position of Cl-6APT (EC_{50}~4 μM). At concentrations to 10 μM, 5APT and Cl-6APT did not activate $5HT_{2A}$ receptor-mediated PLC/[^{3}H]-IP formation (PDSP, 2005; not shown).

METHODS.

Chemicals:

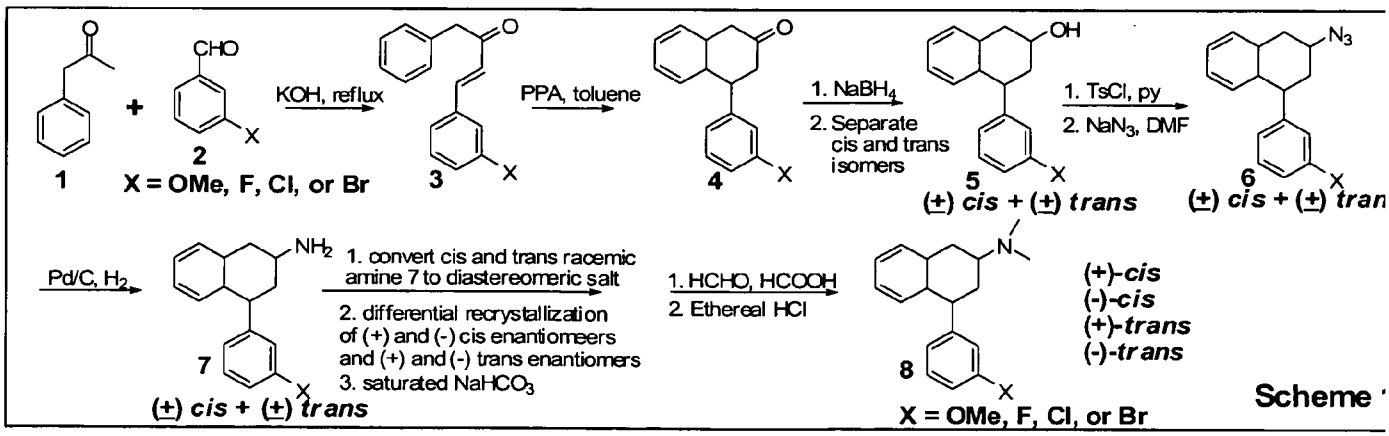
Synthesis of (1*R*,3*S*)-(–)-*Trans*-1-phenyl-3-*N*,*N*-dimethylamino-1,2,3,4-tetrahydronaphthalene (*trans*-PAT

(1*R*,3*S*)-(–)-*Trans*-1-phenyl-3-*N*,*N*-dimethylamino-1,2,3,4-tetrahydronaphthalene (*trans*-PAT,) was synthesized by modification of a procedure previously reported (Wyrick et al., 1993). Briefly, (*E*)-1,4-diphenylbut-1-en-3-one was cyclized to the corresponding 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. The (±)-*cis*-tetralol was stirred with *p*-toluenesulfonyl chloride in pyridine for 2 days at room temp to obtain the corresponding tosylate intermediate. Stirring the tosylate with sodium azide in *N*,*N*-dimethylformamide for 2 days at room temp yielded the (±)-*trans*-azido derivative, which was reduced by catalytic hydrogenation to the free amine. In order to resolve the enantiomers of (+/-)-trans-PAT, the free base was converted to the (1R)-(-)-camphor- 10-sulfonic acid diastereomeric salts and repeatedly recrystallized from acetonitrile/methanol at room temperature to afford the pure levorotatory isomer. In addition to optical rotation measurements,progress of the resolution was monitored by derivatization to the (R)-(-)-a-methoxy-a-[(trifluoromethyl)phenyl]acetamide2' diastereomers with subsequent 'H NMR or gas chromatographic (GC) analysis of the mixture. The proton on the chiral C-1 position that appears as a triplet at 4.35 ppm for the racemic primary amine, appeared **as** two completely resolved triplets of equal integration at 4.25 and 4.10 ppm for the diastereomers formed by derivatization of the racemic mixture. X-ray crystallographic analysis revealed the absolute stereochemistry of the levorotatory isomer of trans-PAT, that corresponds to the pharmacologically more active levorotatory isomer of trans-PAT, to be 1R,3S. The dextrorotatory isomer was obtained using (1s)-(+)-camphor-10-sulfonica cid as the resolving agent and has the 1S,3R configuration. The (±)-*trans*-amine compound was converted to a diastereomeric salt using *D*-(–)-tartaric acid and the diastereomers were separated by fractional recrystallization. The pure (1*R*,3*S*)-(–)-*trans*-amine was dimethylated using formic acid/formaldehyde and purified by flash column chromatography to obtain the pure (1*R*,3*S*)-(–)-*trans*-PAT product.

Synthesis of *meta*-substituted PAT analogs and separation of enantiomers (Schemes 1-3)

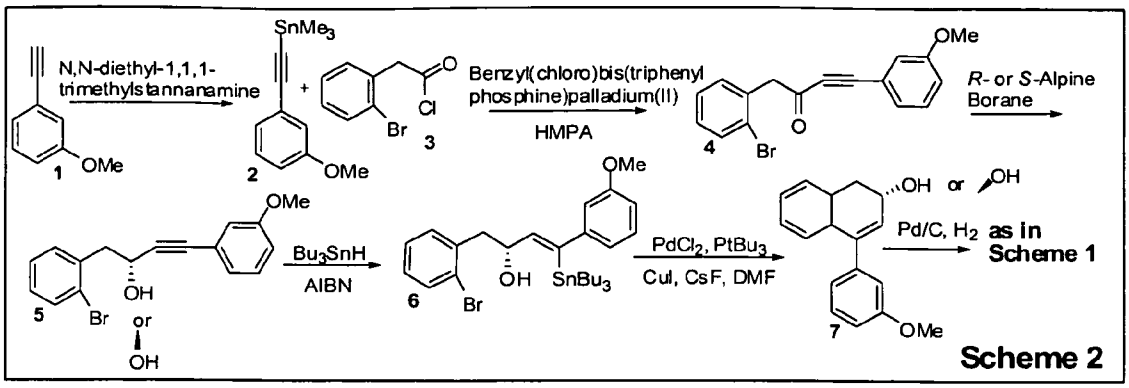
General synthetic methods: Details are described in our synthetic medicinal chemistry publications (e.g., Ghoneim et al., 2006; Bucholtz et al., 1999; Wyrick et al., 1993; 1995). *In vitro* pharmacological studies initially will use racemic *cis* and *trans* products. Racemic PATs with Ki<50 nM will be resolved to (+)- and (–)-enantiomers by derivatization to the diastereomeric salt followed by differential crystallization or synthesized *de novo* using a chiral reduction step (Scheme 3). Absolute configuration is assigned by single crystal *X*-ray crystallography or spectrophotometric methods (NMR, optical rotation) by comparison to pure enantiomers already synthesized. Products (as HCl salts) characterized for purity using NMR, elemental analysis, mass spectrometry, melting point and thin layer chromatography.

Scheme 1: *Meta*-substituted PATs: Methods are modified from our papers cited above and others (Agarwal et al., 2005). The Claisen-Schmidt reaction using *meta*-substituted aldehyde **2** gives the α,β-unsaturated ketone **3**, which is cyclized to the ketone **4** and reduced using $NaBH_4$. The (±)-*cis* and (±)-*trans* free base **7** is converted to the (1*R*)-(–)- or (1*S*)-(+)-camphor-10-sulfonic acid diastereomeric salt, which undergoes differential recrystallization to afford (+)- or (–)-enantiomer, that is alkylated to product **8**.



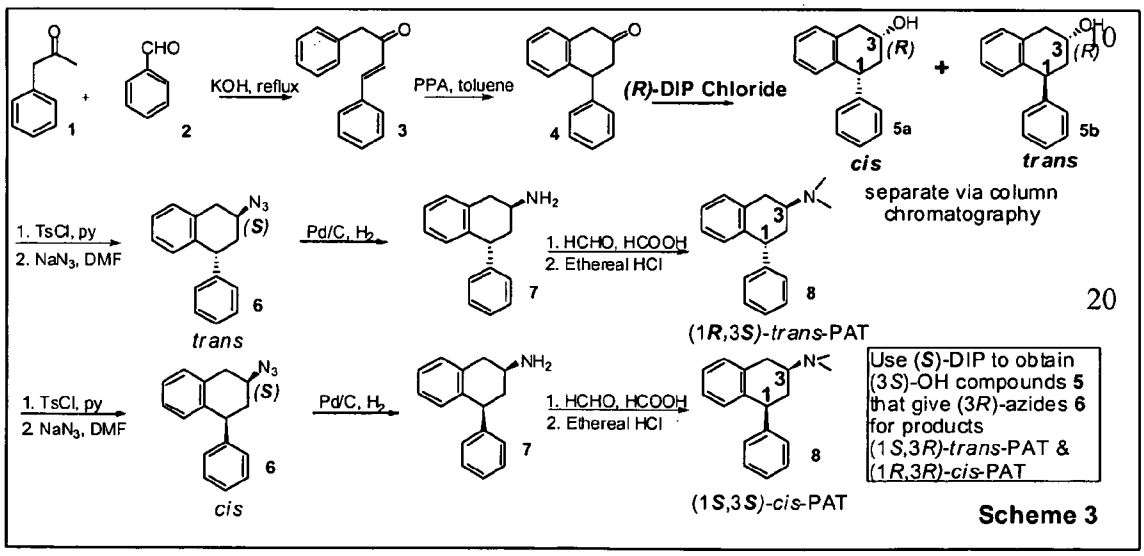
Scheme 2: Alternate synthetic strategy for *meta*-PATs: The PPA cyclization step (Scheme 1; **3** to **4**) sometimes gives poor yields. An alternative route utilizes Stille coupling for C–C bond formation (Labadie et al., 1983). This method determines stereochemistry at the C3

position in the straight chain (Scheme 2; **5**) before cyclization using chiral borane reduction (Brown et al., 1991; Rossi et al., 1999).

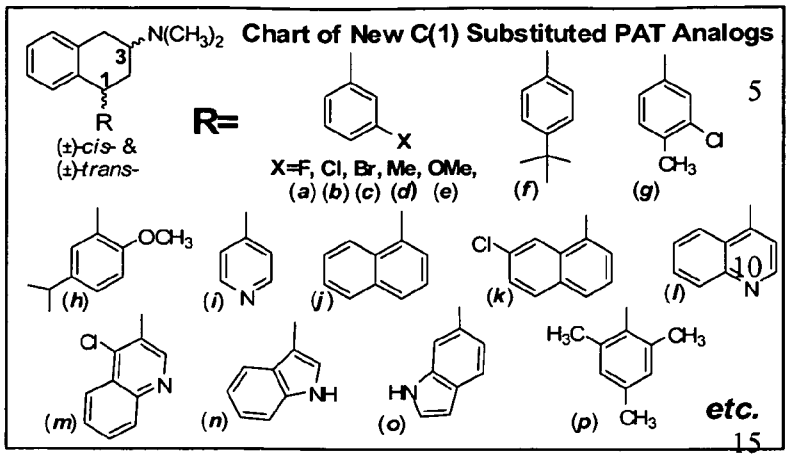


Scheme 3: Use of chiral reducing agent to obtain PAT analog stereoisomers:

Di-isopinocamphenyl-borane (DIP) analogs recently were reported as stereoselective reducing agents for ketones with structures similar to the PAT ketone **4** in Scheme 3 (Cha et al., 2005

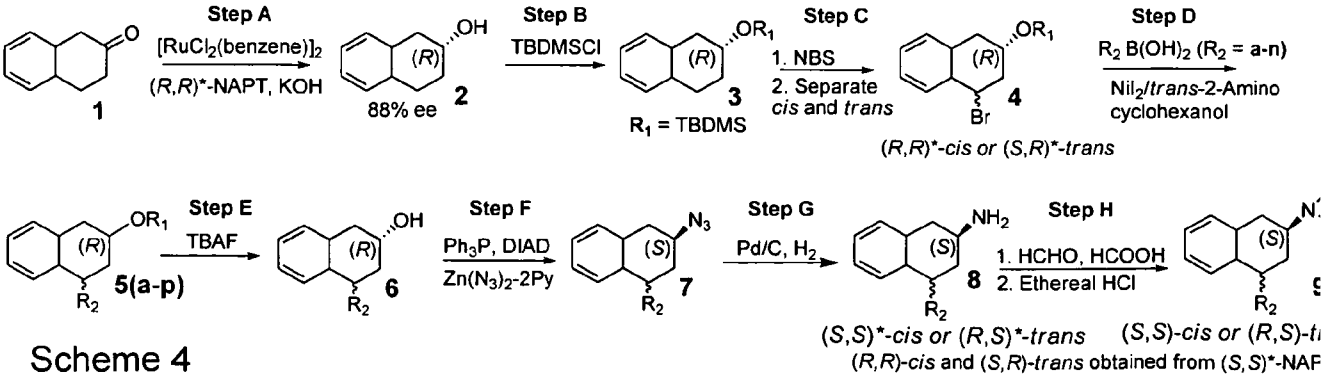


Synthesis of PAT analogs a-p in Chart



Based on binding, function, 3D QSAR, and molecular modeling results in Preliminary Data, we hypothesize the (–)-*trans*-PAT C(1) pendant phenyl moiety is critical to providing full-efficacy $5HT_{2C}$ agonist activity without activation of $5HT_{2A}$ and $5HT_{2B}$ receptors. Testing PAT pendant phenyl ring substitution and orientation will help to determine optimal steric and electrostatic binding interactions with $5HT_2$ active site amino acids to obtain $5HT_{2C}$ agonists and/or $5HT_{2A}/5HT_{2B}$ antagonists with higher affinity, potency, and/or selectivity. Based on recommendations made in the review of the initial version of this application, here, we have substantially diversified the diversity of C(1) substituents structures (**Chart**). Additional APT-type syntheses are not pursued here because affinity and functional activity for this series, so-far, is lame compared to the PAT scaffold, and, there is loss of the stereochemical position for the pendant phenyl, hypothesized to be key in $5HT_{2C}$ selective binding and activity.

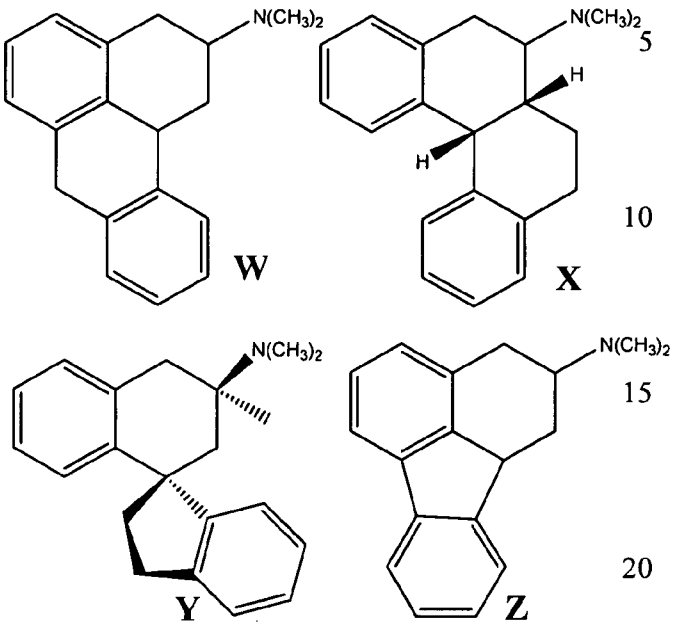
We have modified our synthetic approach (**Scheme 4**) from the previous application to be more efficient and allow for more structural diversity. The intermediate (**4**) in Scheme 1 can be used for synthesis all the PAT analogs. The C(1) structural diversity now is practically unlimited as there are myriad commercially available boronic acid analogs ($R_2B(OH)_2$, R_2 = ***a-p***, **Chart**) that can undergo Suzuki coupling with tetrahydronaphthyl. The initial 32 analogs (16-*cis*, 16-*trans*) in Chart 1 were chosen to probe steric, lipophilic, and electronic parameters for PAT–$5HT_{2C}$ binding, according to our QSAR/molecular modeling results – the process is iterative and other analogs likely will be synthesized. In addition, given that the PAT pendant phenyl ring has a large degree of rotation flexibility relative to the tetrahydronapthalene scaffold, we propose synthesis of 8 (4 [±]-*cis*, 4 [±]-*trans*) new PATs with the pendant phenyl ring in a locked conformation.



Scheme 4

Scheme 4 synthesis details: In Step A, β-tetralone (**1**) is refluxed with benzene ruthenium (II) chloride dimer and (*R*,*R*)-*N*-(2-amino-1,2-diphenylethyl)-*p*-toluenesulfonamide (NAPTS) to give the (*R*)-β-tetralol (**2**) (Mogi et al., 2004). In Step B, the (*R*)-β-tetralol (**2**) is converted to the tert-butyldimethylsilyl (TBDMS) derivative (**3**); TBDMS is a protecting group to prevent bromination at the adjacent benzylic position. In Step C, bromination with N-bromosuccinimide (NBS) under reflux in anhydrous CCl_4 (Agarwal et al., 1990) will give the common intermediate (**4**), separated to *cis* and *trans* bromo compounds by flash chromatography. In Step D, each *cis* and *trans* brominated intermediate (**4**) is reacted with a commercial boronic acid analog ($R_2B(OH)_2$, R_2 = ***a-p***, **Chart**) using the nickle (Nil) catalzyed Suzuki reaction with NiI_2/*trans*-2-aminocyclohexanol and sodium bis(trimethylsilyl)amide (Gonzalez et al., 2006). Step E is de-protection using tetrabutylammonium fluoride (TBAF) and in Step F, the *cis* and *trans* hydroxyl PAT analogs are converted in one-pot to the corresponding *trans* and *cis* azides (**7**) using a Mitsunobu reaction with zinc azide/bis-pyridine complex, diisopropyl azodicarboxylate (DIAD) and triphenylphosphene (Vorogushin et al., 2003). In Step G, the azido PATs are reduced to the amines (**8**) and in Step H, the diastereomeric *cis* and *trans* amines are converted to the dimethylated PAT analogs using Eschweiler-Clarke methylation with formic acid/formaldehyde under reflux.

2. Synthesis of PAT analogs with fixed phenyl ring (Analogs W-Z)



PATs with fixed pendant phenyl ring

The PAT pendant phenyl ring has a large degree of rotation flexibility relative to the tetrahydronapthalene scaffold, though it minimizes to a low energy orthogonal conformation. Analogous "pendant" phenyl ring systems are present in a fixed configuration in high affinity $5HT_{2C}$ antagonist ligands such as mesulergine and ketanserin, but not in any reported agonists. Fixing the PAT phenyl ring in an orthogonal configuration relative to the tetrahydronapthalene may enhance $5HT_{2C}$ affinity and provide information regarding PAT ligand–$5HT_{2C}$ receptor aromatic (π–π) binding interactions. Compounds such as benzo[*de*]anthracene **W** and benzo[c]phenanthrene **X** are rigid tetracyclic analogs with a slightly curved conformation. In addition, the spiroindane analog **Y** wherein the phenyl ring is held at torsion angle 61°, similar to the X-ray crystal structure of (–)-*trans*-PAT (Wyrick et al., 1993); the related planar indane tetracyclic system in the tetrahydrofluoranthene analog **Z** are additional examples of fixed phenyl ring compounds.

i. 4,11-dihydro-5-N,N-dimethylamino-6H-benzo[de]anthracene (W): Anthrone is subjected to a Wittig reaction to give the acetate (Spinella, 1997). After reduction of the alkene and ester group using $NaBH_4$ and PEG (Santaniello et al., 1981), the resultant alcohol is converted to 9-bromoethyl-10-hydroanthracene with hydrobromic acid. Reaction of the bromide with sodium cyanide is followed by hydrolysis to give the acid and Friedel Crafts ring closure produces the ketone 5,11-dihydro-4-keto-6*H*-benzo[*de*]anthracene (Itoh et al., 1984) that undergoes the same reactions as the corresponding PAT ketone **4** in Scheme 3 to give **W**.

ii. ***Cis*** **&** ***trans*** **(+) & (–)-6-(dialkyl)amino-5,6,7,8,12b,6a-hexahydrobenzo[c]-phenanthrene (X):** The procedure of Laus (1984) is used to prepare 1-phenyl-2-tetralol which is oxidized to the tetralone. Wittig reaction and reduction affords *cis* and *trans*-1-phenyl-2-carbethoxymethylindane. Saponification gives the corresponding acid which is cyclized to the ketone. Treatment of the ketone with isoamylnitrite (Pandit and Huisman, 1966) affords the corresponding α-carbonyl oxime. Reduction and methylation give **X**.

iii. ***Cis*** **&** ***trans*** **(+) & (–)-3'(dialkyl)-aminospiro[indan-2,1(2'H)-3,4-dihydro-naphthalene] (Y):** The procedure of Majerus (1967) is used to prepare bis(1-hydroxyindanyl) to 2-oxospiro[indan-2,1(2'H)-3,4-dihydronaphthalene. This ketone is treated with isoamyl nitrite to afford the α-oxime followed by reduction under acidic conditions to the *cis* and *trans* primary amines which are N,N-dialkylated to give product **Y**.

iv. ***R*****- &** ***S*****-2-Dimethylamino-10b,1,2,3,tetrahydrofluoranthene (Z):** 1-(Fluoren-9-yl)-propanoate is prepared by formylation of fluorene using potassium methoxide and ethyl formate followed by Wittig reaction to afford the olefin which is catalytically reduced and the ester saponified according to the procedure of Von and Wagner (1944). This acid is ring closed with PPA to afford 3-oxo-10b,1,2,3-tetrahydrofluoranthene. Reaction of this ketone with isoamyl nitrite affords the α oxime followed by catalytic reduction to the primary amine and *N,N*-alkylation to give product **Z**.

Clonal cell culture and transfection

All cell lines were maintained by following ATCC suggestion, Chinese Hamster Ovary cells (CHO-K1, ATCC CCL-61) in Ham's F-12 medium supplemented with 10% fetal bovine serum, 1% sodium bicarbonate (Mediatech 25-035-CI), 10 IU/ml Penicillin and 10ug/ml Streptomycin, and human embryonic kidney (HEK) 293 in minimum essential medium (Eagle) (MEM) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate (90%) with 10% fetal bovine serum, 10 IU/ml Penicillin and 10ug/ml Streptomycin. Cells were grown at 37oC in a humidified incubator with 5% CO2. The cDNAs encoding the human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (wild type) were purchased from UMR (Rolla, MO) for transient transfection of the clonal cells. For radioreceptor binding assays, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor membranes were prepared from transfected CHO-K1 cells. For functional assays measuring activity of PLC/IP formation, transfected CHO-K1 cells were used for 5-HT_{2A} and 5-HT_{2C} receptors. For $5HT_{2B}$ receptors, however, more robust and consistent results for the PLC/IP assay were obtained using transfected HEK cells (Setola et al., 2005). Twenty-four hours before transfection, cells were seeded at 40% confluence in 100 mm dishes for radioreceptor binding assays or at 10^5 cells per well in 12-well plates for functional assays. CHO-K1 cells were transiently transfected with 12 μg of plasmid and 32 μl of lipofectamine (Invitrogen) per 100 mm dish for radioreceptor binding assays, or, 0.8 μg plasmid and 4.0 μl of lipofectamine per well for functional assays. For 5-HT_{2B} functional assays using HEK cells, 24 μg plasmid DNA was mixed with 60 μl of Lipofectamine 2000 (Invitrogen) to transfect 1-2 x 10^6 cells in a 10-cm plate. Cells were allowed to express transfected receptors for another 24 hrs (Herrick, 1997).

Radioreceptor assays

Radioreceptor saturation and competition binding assays were performed using membrane homogenates, similar to our methods reported previously for the phylogenetically closely related histamine H_1 GPCR (Booth, 2002; Moniri et al., 2004). [^{3}H]-Ketanserin was used to radiolabel 5-HT_{2A} receptors and [^{3}H]-mesulergine for 5-HT_{2B} and 5-HT_{2C} receptors. Briefly, forty-eight hours following CHO cell transfection, cells were harvested and homogenized in 50 mM Tris-HCl containing 0.1 % ascorbic acid and 4.0 mM $CaCl_2$ at pH 7.4 (assay buffer). The homogenate was centrifuged at 35,000g for 25 min and the resulting membrane pellet was re-suspended in assay buffer. Protein concentration was determined by the method of Lowry et al. (Lowry, 1951). For saturation binding assays, membrane suspension containing 100 μg protein was incubated with

0.1 – 5.0 nM [^{3}H]-ketanserin (5-HT_{2A} receptors) or 0.1 – 20 nM [^{3}H]-mesulergine (5-HT_{2B} and 5-HT_{2C} receptors) in a total assay buffer volume of 250 µl. Non-specific binding was determined in the presence of 10 µM methysergide (5-HT_{2A} receptors) or 1.0 µM mianserin (5-HT_{2B} and 5-HT_{2C} receptors). Competition binding assays were conducted similarly with 1.0 nM [^{3}H]-ketanserin or [^{3}H]-mesulergine. 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 [^{3}H]-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). Ligand affinity is expressed as an approximation of K_i values by conversion of the IC_{50} data to $K_{0.5}$ values using the equation $K_{0.5} = IC_{50}/1 + L/K_D$, where L is the concentration of radioligand having affinity K_D (Cheng, 1973). Each experimental condition was performed in triplicate and each experiment was performed a minimum of three times to determine S.E.M.

Measurement of [^{3}H]-IP formation in CHO-K1 and HEK cells

Functional activation of PLC was measured as [^{3}H]-IP formation in CHO cells transiently expressing 5-HT_{2A} or 5-HT_{2C} receptors and HEK cells transiently expressing $5HT_{2B}$ receptors, as previously reported by our lab (Booth, 2002; Moniri et al., 2004). Briefly, thirty-two hours following transfection, cells in inositol-free Dulbecco's modified Eagle's medium (DMEM) were labeled with 1 µCi/ml *myo*-[2-^{3}H]-inositol, a precursor of the PLC-β substrate phosphatidylinositol. Cells then were washed and incubated in DMEM containing 25 mM Hepes (pH 7.4), 10 mM LiCl, 10 µM pargyline (with addition of 5% dialyzed FBS for HEK cells), and various concentrations of test ligand for 45-60 min at 37 °C. After aspiration of media, wells were placed on ice and lysed by incubation with 50 mM formic acid (15-60 min). Formic acid was neutralized with ammonium hydroxide and all 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 [^{3}H]-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 and are expressed as mean percentage of control [^{3}H]-IP formation, with potency expressed as concentration required to produce 50% maximal [^{3}H]-IP formation (EC_{50}) ± S.E.M ($n \geq 3$).

Radioreceptor assays

Radioligand saturation binding analysis of 5HT-subtype receptors: There was no measurable specific radioligand binding using membranes prepared from null-transfected CHO and HEK cells. Using membranes prepared from CHO cells transiently transfected with 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} cDNA, however, saturable specific radioligand binding occurs – representative binding curves for [^{3}H]-ketanserin labeled $5HT_{2A}$ receptors and [^{3}H]-mesulergine labeled 5-HT_{2B} receptors and 5-HT_{2C} receptors are shown in Figures 2A-C. [^{3}H]-Ketanserin binds to an apparent single population of $5HT_{2A}$ receptors (B_{max} = 1.73 ± 0.11 pmol/mg protein) with high affinity (K_D = 0.80 ± 0.03 nM). Similarly, [^{3}H]-mesulergine labels a single population of $5HT_{2B}$ receptors with B_{max} = 1.13 ± 0.39 pmol/mg protein and K_D = 5.19 ± 0.36 nM. [^{3}H]-mesulergine also labels an apparent single population of $5HT_{2C}$ receptors (B_{max} = 8.37 ± 0.15 pmol/mg prot) with high affinity (K_D = 0.88 ± 0.03 nM).

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The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination of listed elements. The recitation of an element, or an embodiment herein includes that element or embodiment as any single element or embodiment or in combination with any other element, embodiments or portions thereof.

All references cited herein, whether in print, electronic, computer readable storage media or other form, are expressly incorporated by reference in their entirety, including but not limited to, abstracts, articles, journals, publications, texts, treatises, technical data sheets, internet web sites, databases, patents, patent applications, and patent publications.

Although the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of the invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

1. A method of treating or preventing obesity in a subject comprising administering to the subject identified as in need thereof a combination of a PAT compound and an amphetamine compound.

2. The method of claim 1, wherein the PAT compound and the amphetamine compound are administered concurrently.

3. The method of claim 1, wherein the PAT compound and the amphetamine compound are administered simultaneously.

4. The method of claim 1, wherein the PAT compound and the amphetamine compound are administered sequentially.

5. The method of claim 1, wherein the PAT compound is a compound in Tables 1-3.

6. The method of claim 1, wherein the amphetamine compound is phentermine.

7. The method of claim 1, wherein the PAT compound is *(–)*-trans-*PAT* and the amphetamine compound is phentermine.

8. The method of claim 1, wherein the PAT compound has 100-fold less potency to activate 5-HT2a and 5-HT2b compared to 5-HT2c.

9. A method of modulating 5-HT2c in a subject identified as in need of such treatment, comprising administering a combination of a 5-HT2c agonist in combination with an amphetamine.

10. The method of claim 9, wherein the amphetamine is phentermine.

11. The method of claim 9, wherein the PAT compound is *(–)*-trans-*PAT* and the amphetamine compound is phentermine.

12. A method of treating obesity in a subject comprising administering to the subject identified as in need thereof a compound capable of selectively activating 5-HT2c relative to 5-HT2a or 5-HT2b in combination with an amphetamine.

13. The method of claim 12, wherein the activating for 5-HT2c is 10-fold or more greater than for either 5-HT2a or 5-HT2b.

14. The method of claim 12, wherein the wherein the activating for 5-HT2c is 50-fold or more greater than for either 5-HT2a or 5-HT2b.

15. The method of claim 12, wherein the wherein the activating for 5-HT2c is 100-fold or more greater than for either 5-HT2a or 5-HT2b.

16. The method of claim 12, wherein the compound is a 5-HT2a antagonist.

17. The method of claim 12, wherein the compound is a 5-HT2b antagonist.

18. A method of treating obesity in a subject comprising administering to the subject identified as in need thereof a compound capable of antagonizing 5-HT2a or 5-HT2b and an amphetamine.

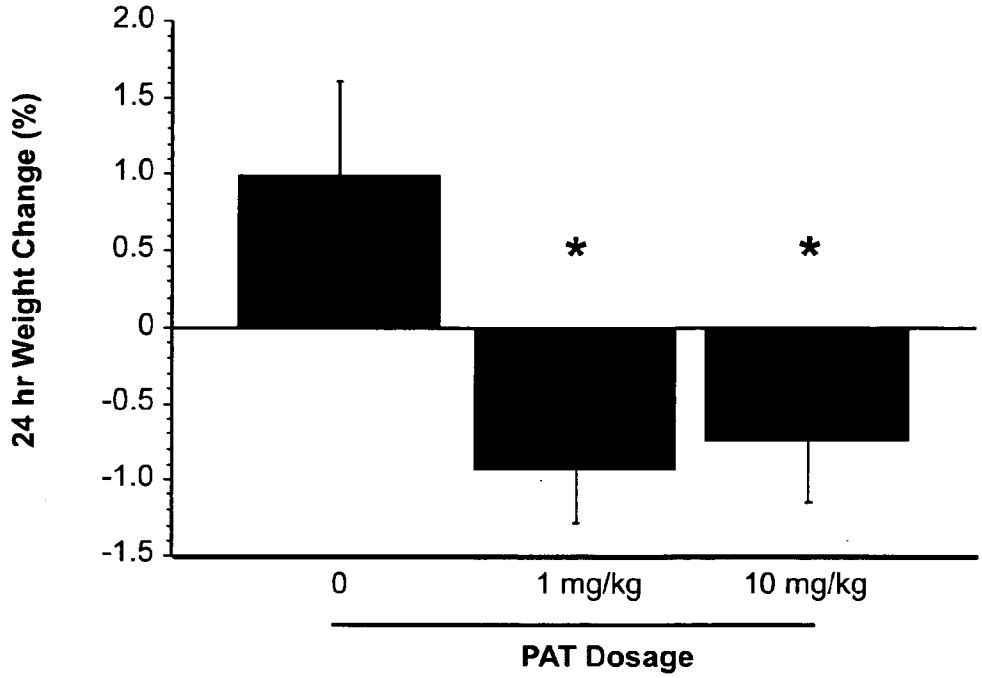
24 hr Weight Change (%)
2.0
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
*
*
0
1 mg/kg
10 mg/kg
PAT Dosage

FIG. 1

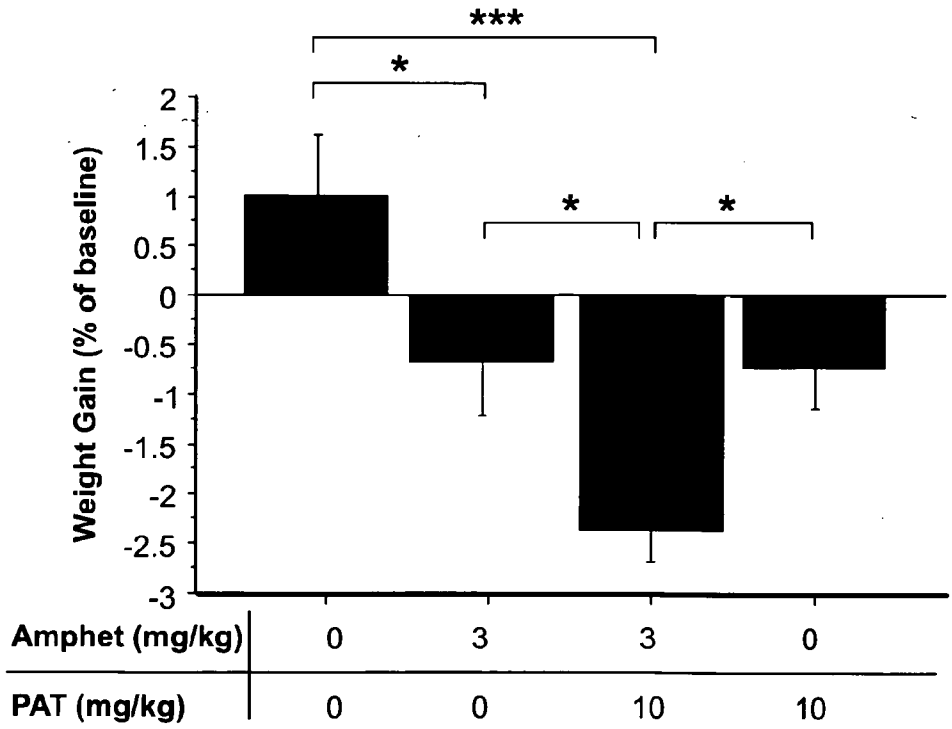
***
*
*
*
Weight Gain (% of baseline)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
Amphet (mg/kg) 0 3 3 0
PAT (mg/kg) 0 0 10 10

FIG. 2

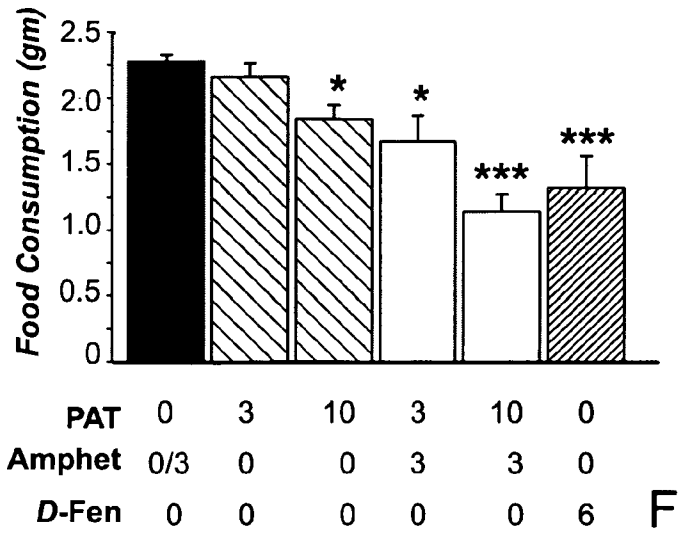
Food Consumption (gm)
2.5
2.0
1.5
1.0
0.5
0
*
*
***
***
PAT 0 3 10 3 10 0
Amphet 0/3 0 0 3 3 0
D-Fen 0 0 0 0 0 6

FIG. 3

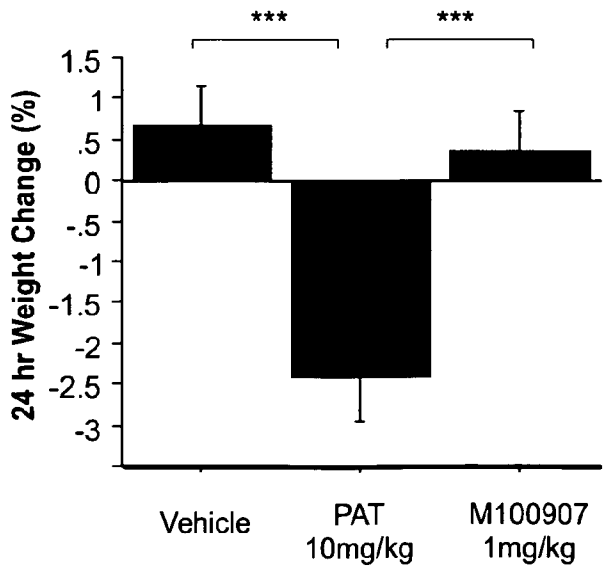
***
***
24 hr Weight Change (%)
1.5
1
.5
0
-.5
-1
-1.5
-2
-2.5
-3
Vehicle
PAT 10mg/kg
M100907 1mg/kg

FIG. 4

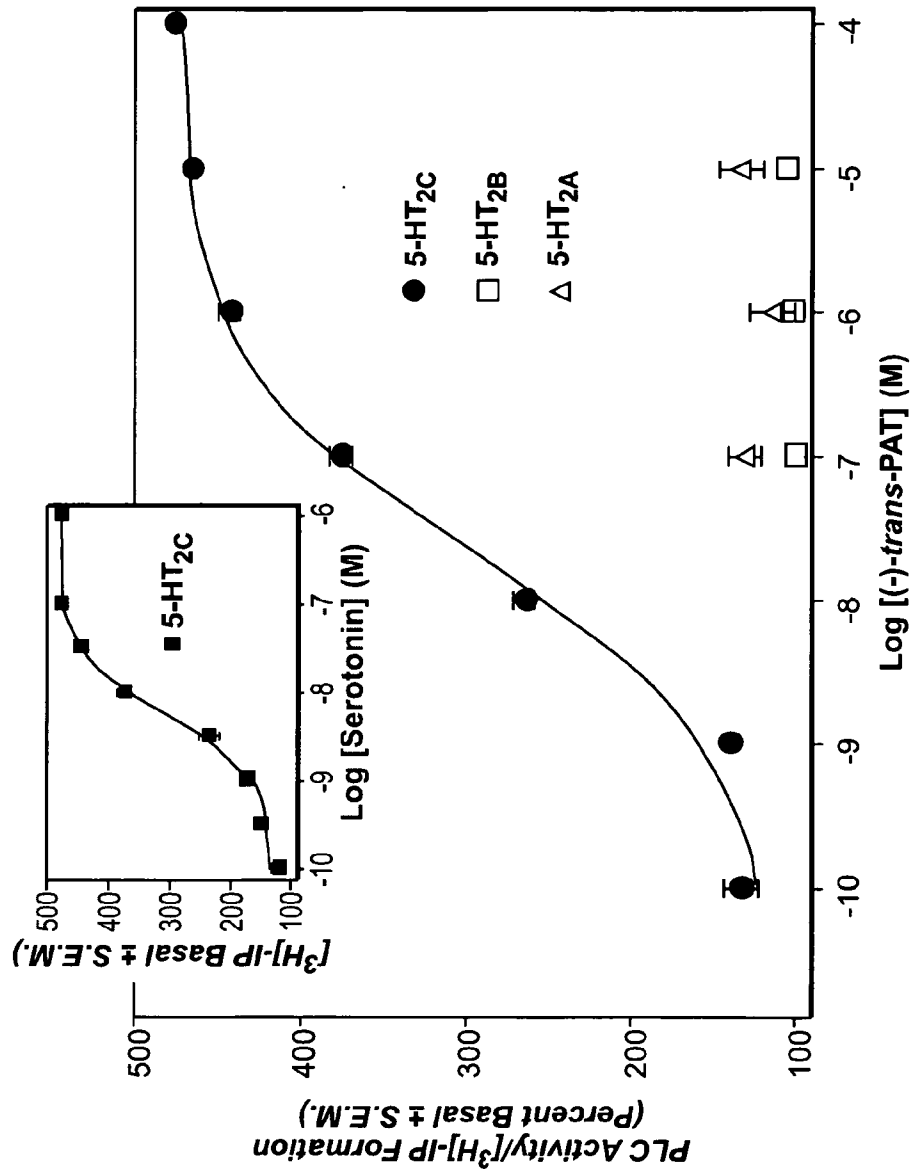
PLC Activity/[3H]-IP Formation
(Percent Basal ± S.E.M.)
Log [(-)-trans-PAT] (M)
5-HT2C
5-HT2B
5-HT2A
[3H]-IP Basal ± S.E.M.)
Log [Serotonin] (M)
5-HT2C

FIG. 5

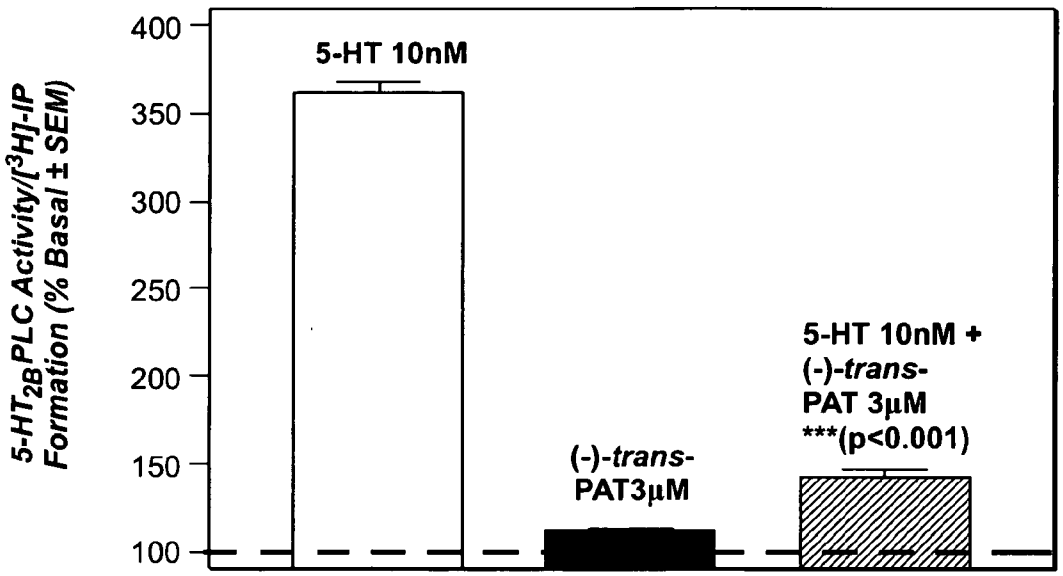
5-HT2B PLC Activity/[3H]-IP Formation (% Basal ± SEM)
400
350
300
250
200
150
100
5-HT 10nM
(-)-trans-PAT3μM
5-HT 10nM + (-)-trans-PAT 3μM ***(p<0.001)

FIG. 6A

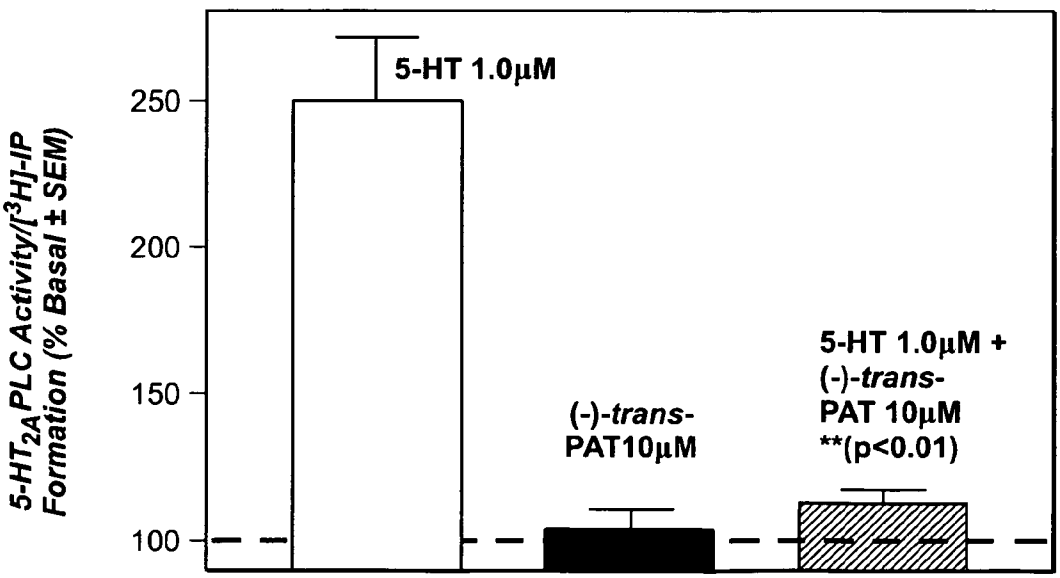
5-HT2A PLC Activity/[3H]-IP Formation (% Basal ± SEM)
250
200
150
100
5-HT 1.0μM
(-)-trans-PAT10μM
5-HT 1.0μM + (-)-trans-PAT 10μM **(p<0.01)

FIG. 6B

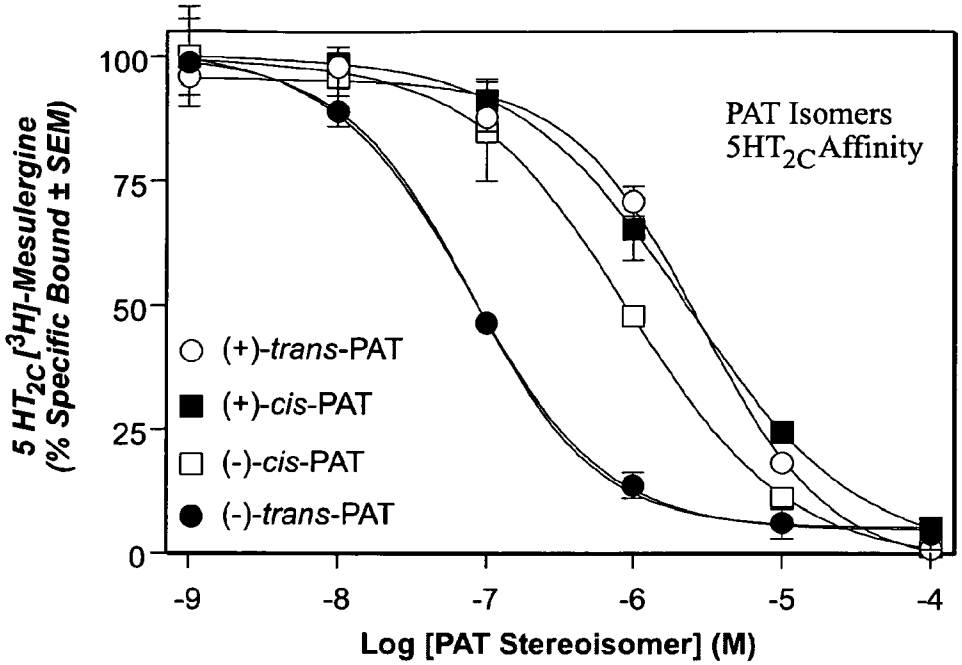
5 HT2C [3H]-Mesulergine
(% Specific Bound ± SEM)
100
75
50
25
0
-9
-8
-7
-6
-5
-4
Log [PAT Stereoisomer] (M)
PAT Isomers
5HT2C Affinity
(+)-trans-PAT
(+)-cis-PAT
(-)-cis-PAT
(-)-trans-PAT

FIG. 7

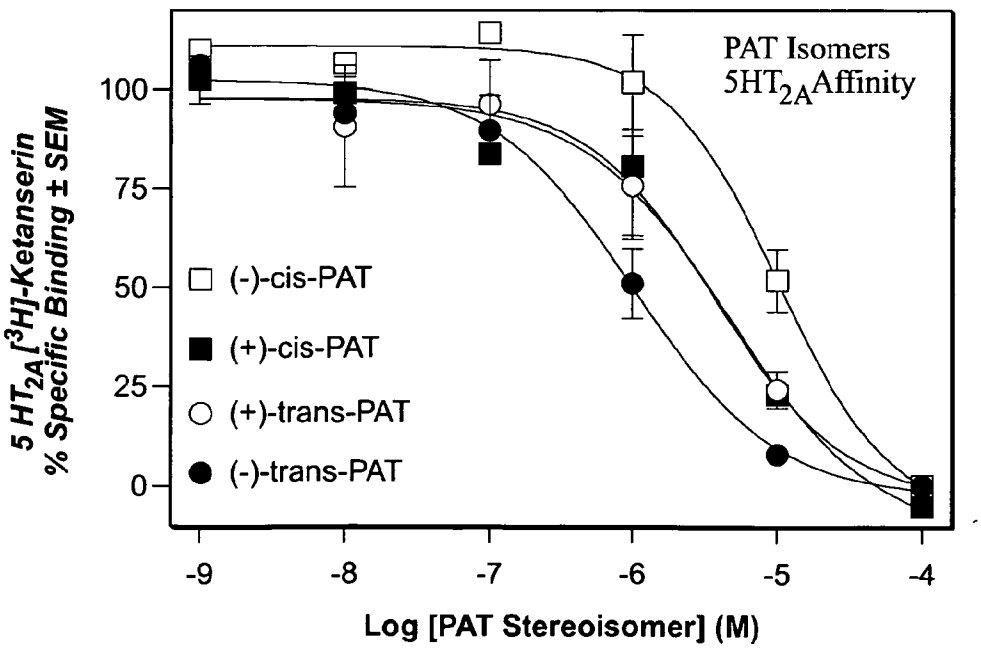
5 HT2A [3H]-Ketanserin
% Specific Binding ± SEM
100
75
50
25
0
-9
-8
-7
-6
-5
-4
Log [PAT Stereoisomer] (M)
PAT Isomers
5HT2A Affinity
(-)-cis-PAT
(+)-cis-PAT
(+)-trans-PAT
(-)-trans-PAT

FIG. 8

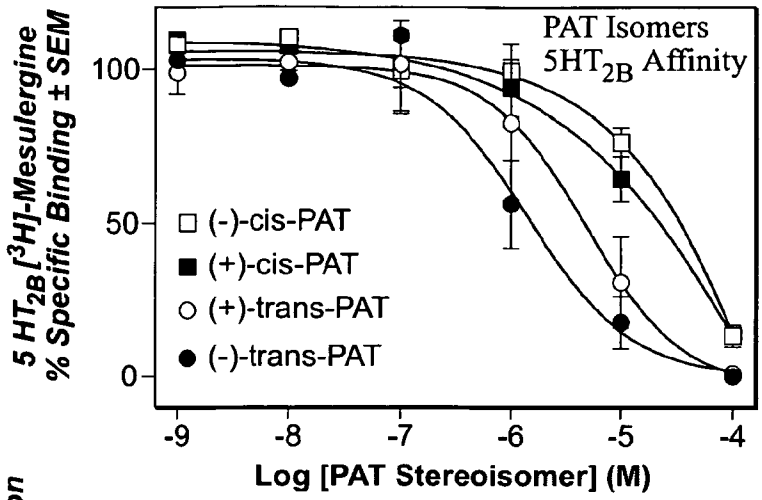

FIG. 9

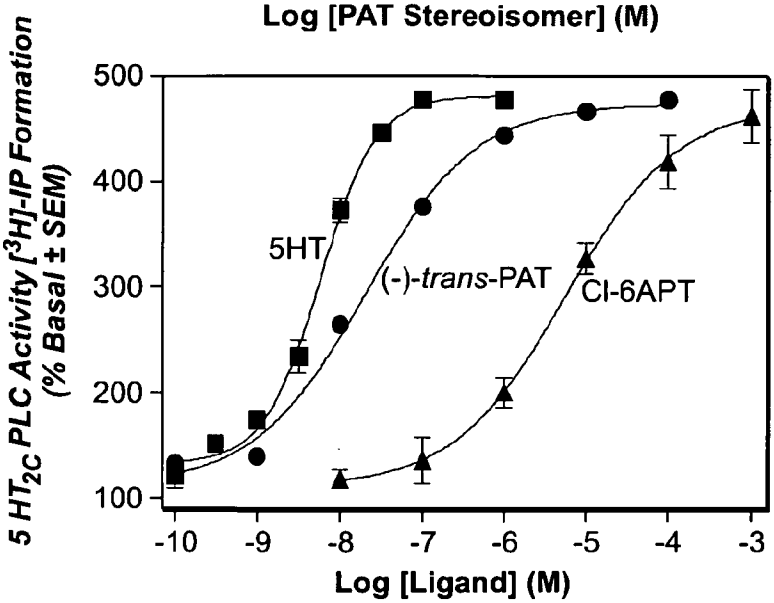

FIG. 10A

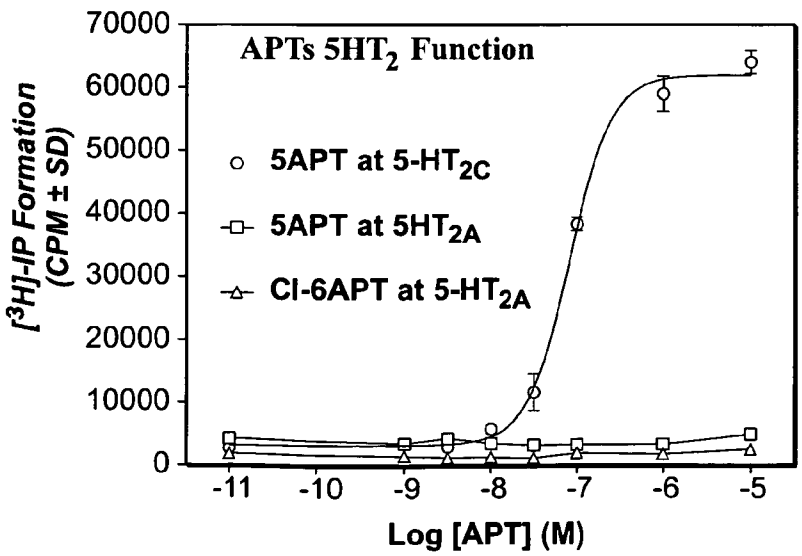

FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/12523

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/165, A61K 31/415 (2009.01)
USPC - 564/222, 564/428, 514/406
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 564/222, 564/428, 514/406
IPC(8) - A61K 31/165, A61K 31/415 (2009.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 564/222, 564/428, 514/406
IPC(8) - A61K 31/165, A61K 31/415 (2009.01) (text delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPTO, Google Scholar, DialogPro, PubWEST. Search Terms: (IR,3S)-(-)trans-1-phenyl-3-dimethylamino-1,2,3,4 - tetrahydronaphthalene , PAT compounds, obesity, amphetamine, phentermine, 5-HT2a, 5-HT2b, 5-HT2c tetrahydronaphthlenyl moiety substituted with an optionally substituted-aryl (e.g. phenyl) group and a substituted amino

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,545,755 A (Lin et al.) 13 August 1996 (12.08.1996), Abstract; col 4, ln 45-67; col 5, ln 1-53; col 6, ln 24-35; and claim 1	1-4, 5, 6, 8
Y	US 6,207,699 B1 (Rothman) 27 March 2001 (27.03.2001), col 1, ln 41-67; ln 1-17; col 5, ln 41-47; and col 5, ln 52-62	1-4, 5, 6, 7, 8, 9, 10, 12-18
Y	Wyrick et al., "Synthesis and pharmacological evaluation of 1-phenyl-3-amino-1,2,3,4 - tetrahydronaphthalenes as ligands for a novel receptor with sigma-like neuromodulatory activity" J. Med. Chem., 1993, 36 (17), 2542-2551, August 1993 (08.1993), Table 1, compound 35; pg 2549, col 2, Section:trans-(1R,3S)-(-)-1-Phenyl-3-(N,N-dimethylamino)-1,2,3,4-tetrahydronaphthaalene	5, 7, 11
Y	Miller, "Serotonin 5-HT Receptor Agonists: Potential for the Treatment of Obesity" Molecular Interventions 2005, vol 5, issue 5, 282-291, 01 October 2005 (01.10.2005), pg 287, col 1, para 2 and 3; and pg 288, col 1, Section: Serotonin Receptor Modulators: Clinical Data and Perspective	8, 9, 10, 11,12- 18

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2009 (14.01.2009)	29 JAN 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)