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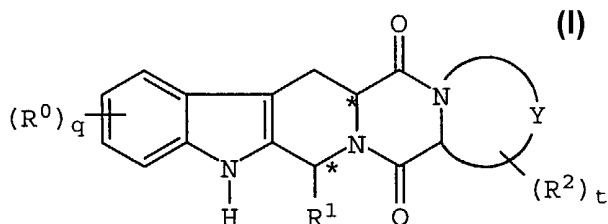
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- (74) Agent: **NAPOLI, James, J.**; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).
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- (71) Applicant (*for all designated States except US*): **LILLY ICOS LLC** [US/US]; 1209 Orange Street, Wilmington, DE 19801 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **ORME, Mark, W.** [US/US]; 4235 Francis Avenue, #203, Seattle, WA 98103 (US). **SAWYER, Jason, Scott** [US/US]; 5718 North Winthrop Avenue, Indianapolis, IN 46220 (US).
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(54) Title: CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS



(57) Abstract: Compounds of general structural formula (I) and use of the compounds and salts and solvates thereof, as therapeutic agents.

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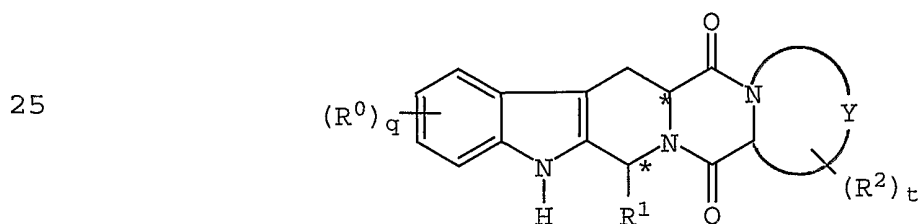
CYCLIC GMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS

FIELD AND BACKGROUND OF THE INVENTION

5 This invention relates to a series of compounds, to methods of preparing the compounds, to pharmaceutical compositions containing the compounds, and to their use as therapeutic agents. In particular, the invention relates to compounds that are potent and selective inhibitors of cyclic guanosine 3',5'-monophosphate specific phosphodiesterase (cGMP-specific PDE), in particular PDE5, and have utility in a variety of therapeutic areas wherein such inhibition is considered beneficial, including the treatment of cardiovascular disorders and erectile dysfunction.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention is directed to compounds having the general structural formula (I):



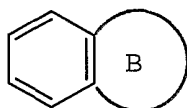
(I)

30

wherein R⁰, independently, is selected from the group consisting of halo and C₁₋₆alkyl;

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R¹ is selected from the group consisting of an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine, and an optionally substituted bicyclic ring



10

wherein the fused ring B is a 5- or 6-membered ring, saturated or partially or fully unsaturated, and comprises carbon atoms and optionally one or two heteroatoms selected from oxygen, sulfur, and nitrogen;

Y is a 3-, 4-, or 5-membered carbon chain of a 5-, 6-, or 7-membered substituted ring, or Y is a 3-, 4-, or 5-membered heteroatom chain of a 5-, 6-, or 7-membered unsubstituted or substituted ring, wherein said heteroatom chain contains one or two heteroatoms independently selected from the group consisting of oxygen, sulfur, and nitrogen;

R², independently, is selected from the group consisting of

nitro,
trifluoromethyl,
trifluoromethoxy,
halogen,
cyano,
C₁₋₆alkyl, optionally substituted with OR^a,
C(=O)R^a,
OC(=O)R^a,

30

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- $C(=O)OR^a$,
 C_{1-4} alkyleneHet,
 C_{1-4} alkyleneC(=O)OR^a,
 OC_{1-4} alkyleneC(=O)OR^a,
5 C_{1-4} alkyleneOC₁₋₄alkyleneC(=O)OR^a,
 $C(=O)NR^aSO_2R^c$,
 $C(=O)C_{1-4}$ alkyleneHet,
 $C(=O)C_{1-4}$ alkylenearyl, optionally substituted with one or more OR^a,
10 C_{1-4} alkyleneNR^aR^b,
 C_{2-6} alkenyleneNR^aR^b,
 $C(=O)NR^aR^b$,
 $C(=O)NR^aR^c$,
 $C(=O)NR^aC_{1-4}$ alkyleneOR^b,
15 $C(=O)NR^aC_{1-4}$ alkyleneHet,
OR^a,
 OC_{2-4} alkyleneNR^aR^b,
 OC_{1-4} alkyleneCH(OR^a)CH₂NR^aR^b,
 OC_{1-4} alkyleneHet,
20 OC_{2-4} alkyleneOR^a,
 OC_{2-4} alkyleneNR^aC(=O)OR^b,
NR^aR^b,
 NR^aC_{1-4} alkyleneNR^aR^b,
NR^aC(=O)R^b,
25 $NR^aC(=O)NR^aR^b$,
N(SO₂C₁₋₄alkyl)₂,
NR^a(SO₂C₁₋₄alkyl),
SO₂NR^aR^b,
and OSO₂trifluoromethyl;
30 R^a and R^b can be the same or different, and are independently selected from hydrogen and C₁₋₆-alkyl;

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R^c is phenyl or C₄₋₆cycloalkyl, wherein the phenyl or C₄₋₆cycloalkyl can be optionally substituted with one or more substituents selected from the group consisting of halogen atoms, C(=O)OR^a, and OR^a;

5

q is 0, 1, 2, 3; or 4;

t is 0, 1, or 2; and

pharmaceutically acceptable salts and hydrates thereof.

10

As used herein, the term "alkyl" includes straight chained and branched hydrocarbon groups containing the indicated number of carbon atoms, typically methyl, ethyl, and straight chain and branched propyl and butyl groups. The hydrocarbon group can contain up to 16 carbon atoms. The term "alkyl" includes "cycloalkyl," i.e., a cyclic C₃-C₈ hydrocarbon group, for example, cyclopropyl, cyclobutyl, cyclohexyl, and cyclopentyl, and "bridged alkyl," i.e., a C₆-C₁₆ bicyclic or polycyclic hydrocarbon group, for example, norbornyl, adamantyl, bicyclo[2.2.2]octyl, bicyclo[2.2.1]heptyl, bicyclo[3.2.1]octyl, or decahydronaphthyl.

15

20

The term "alkylene" refers to an alkyl group having a substituent. For example, the term "C₁₋₃alkylenearyl" refers to an alkyl group containing one to three carbon atoms, and substituted with an aryl group. The term "alkenylene" as used herein is similarly defined, and contains the indicated number of carbon atoms and a carbon-carbon double bond, and includes straight chained and branched alkenylene groups, like ethylenylene.

25

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- 5 -

The term "halo" or "halogen" is defined herein to include fluorine, bromine, chlorine, and iodine.

The term "haloalkyl" is defined herein as an alkyl group substituted with one or more halo substituents, either fluoro, chloro, bromo, iodo, or combinations thereof. Similarly, "halocycloalkyl" is defined as a cycloalkyl group having one or more halo substituents.

The term "aryl," alone or in combination, is defined herein as a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, that can be unsubstituted or substituted, for example, with one or more, and in particular one to three, halo, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methoxyphenyl, 3-trifluoromethylphenyl, 4-nitrophenyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing one or two aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring, and which can be unsubstituted or substituted, for example, with one or more, and in particular one to three, substituents, like halo, alkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkylsulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include thienyl, furyl, pyridyl, oxazolyl,

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quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidazolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

5 The term "Het" is defined as 5- or 6-membered heterocycloalkyl groups, e.g., morpholinyl, piperidyl, pyrrolidinyl, or piperazinyl, and heteroaryl groups, as exemplified above.

10 The terms "alkoxyalkyl" and "aryloxyalkyl" are defined as an alkyl group wherein a hydrogen has been replaced by an alkoxy group or an aryloxy group, respectively. The terms "(alkylthio)alkyl," "(arylthio)alkyl," and "(aralkylthio)alkyl" are defined similarly as the three above groups, except a sulfur atom, rather than an oxygen atom, is present.

15 The term "hydroxy" is defined as -OH.

The terms "alkoxy" and "aryloxy" are defined as -OR, wherein R is alkyl or aryl.

20 The term "hydroxyalkyl" is defined as a hydroxy group appended to an alkyl group.

The term "amino" is defined as -NH₂, and the term "alkylamino" is defined as -NR₂, wherein at least one R is alkyl and the second R is alkyl or hydrogen.

25 The term "acylamino" is defined as RC(=O)N, wherein R is alkyl or aryl.

The term "alkylthio" is defined as -SR, where R is alkyl.

30 The term "alkylsulfinyl" is defined as R-SO₂, where R is alkyl.

The term "alkylsulfonyl" is defined as R-SO₃, where R is alkyl.

The term "nitro" is defined as -NO₂.

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The term "trifluoromethyl" is defined as
-CF₃.

The term "trifluoromethoxy" is defined as
-OCF₃.

5 The term "cyano" is defined as -CN.

The term "Y" as used here and hereafter
refers to a 3-, 4-, or 5-membered component of a 5-,
6-, or 7-membered ring. The other two components of
the ring are the carbon atom and nitrogen atom of an
10 adjacent ring, as depicted in structural formula
(I). In one embodiment, Y is a 3-, 4-, or 5-mem-
bered carbon chain. In this embodiment, Y option-
ally is substituted with one or two R² substituents.

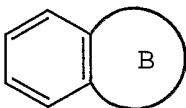
In another embodiment, Y is a 3-, 4-, or
15 5-membered heteroatom chain having the following
nonlimiting structures:

XCH₂CH₂, CH₂XCH₂, XCH₂X, XCH₂CH₂CH₂,
CH₂XCH₂CH₂, XCH₂CH₂X, XCH₂XCH₂, X(CH₂)₄,
20 CH₂X(CH₂)₃, CH₂CH₂XCH₂CH₂, CH₂XCH₂XCH₂,
XCH₂CH₂XCH₂, or XCH₂XCH₂CH₂, wherein X,
independently, is NR^a, S, or O.

The heteroatom chain can be unsubstituted, i.e., t
25 is 0, or substituted, i.e., one or two of the
hydrogen atoms are replaced by an R² substituent (t
is 1 or 2).

In a preferred embodiment, R¹ is the
optionally substituted bicyclic ring system

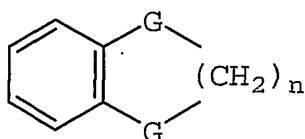
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wherein the bicyclic ring can represent, for example, naphthalene or indene, or a hetero-
 cycle, such as benzoxazole, benzothiazole, benzisox-
 azole, benzimidazole, quinoline, indole, benzothio-
 phene, or benzofuran, or

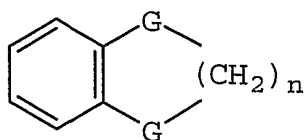
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wherein n is an integer 1 or 2, and G , indepen-
 dently, are $C(R^a)_2$, O , S , or NR^a . The bicyclic ring
 comprising the R^1 substituent typically is attached
 to the rest of the molecule by a phenyl ring carbon
 atom.

In a preferred group of compounds of
 formula (I), R^1 is represented by an optionally sub-
 stituted bicyclic ring

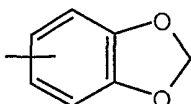
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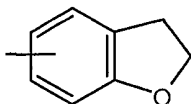
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wherein n is 1 or 2, and G, independently, are CH₂ or O. Especially preferred R¹ substituents include

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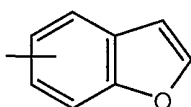


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, and

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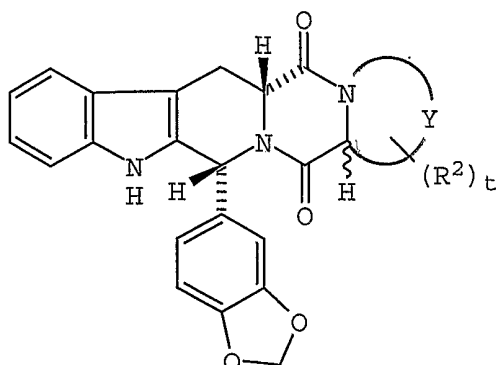
Within this particular group of compounds, nonlimiting examples of substituents for the bicyclic ring include halogen (e.g., chlorine), C₁₋₃alkyl (e.g., methyl, ethyl, or i-propyl), OR^a (e.g., methoxy, ethoxy, or hydroxy), CO₂R^a, halomethyl or halomethoxy (e.g., trifluoromethyl or trifluoromethoxy), cyano, nitro, and NR^aR^b.

25

An especially preferred subclass of compounds within the general scope of formula (I) is represented by compounds of formula (II)

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- 10 -



5

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(II)

wherein Y and the atoms to which Y is attached form a piperidine, piperazine, thiomorpholine, morpholine, pyrrolidine, imidazolidine, or thiazolidine ring system, and pharmaceutically acceptable salts and solvates (e.g., hydrates) thereof.

15

Compounds of formula (I) can contain one or more asymmetric center, and, therefore, can exist as stereoisomers. The present invention includes both mixtures and separate individual stereoisomers of the compounds of formula (I). Compounds of formula (I) also can exist in tautomeric forms, and the invention includes both mixtures and separate individual tautomers thereof.

20

25

Pharmaceutically acceptable salts of the compounds of formula (I) can be acid addition salts formed with pharmaceutically acceptable acids. Examples of suitable salts include, but are not limited to, the hydrochloride, hydrobromide, sulfate, bisulfate, phosphate, hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methanesulfonate, benzenesulfonate, and p-toluenesulfonate

30

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salts. The compounds of the formula (I) also can provide pharmaceutically acceptable metal salts, in particular alkali metal salts and alkaline earth metal salts, with bases. Examples include the sodium, potassium, magnesium, and calcium salts.

Compounds of the present invention are potent and selective inhibitors of cGMP-specific PDE5. Thus, compounds of formula (I) are of interest for use in therapy, specifically for the treatment of a variety of conditions where selective inhibition of PDE5 is considered beneficial.

Phosphodiesterases (PDEs) catalyze the hydrolysis of cyclic nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The PDEs have been classified into at least seven isoenzyme families and are present in many tissues (J.A. Beavo, *Physiol. Rev.*, 75, p. 725 (1995)).

PDE5 inhibition is a particularly attractive target. A potent and selective inhibitor of PDE5 provides vasodilating, relaxing, and diuretic effects, all of which are beneficial in the treatment of various disease states. Research in this area has led to several classes of inhibitors based on the cGMP basic structure (E. Sybertz et al., *Expert. Opin. Ther. Pat.*, 7, p. 631 (1997)).

The biochemical, physiological, and clinical effects of PDE5 inhibitors suggest their utility in a variety of disease states in which modulation of smooth muscle, renal, hemostatic, inflammatory, and/or endocrine function is desirable. The compounds of formula (I), therefore, have utility in the treatment of a number of disorders,

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stable, unstable, and variant (Prinzmetal) angina, hypertension, pulmonary hypertension, congestive heart failure, chronic obstructive pulmonary disease, malignant hypertension, pheochromocytoma, acute respiratory distress syndrome, acute and chronic renal failure, atherosclerosis, conditions of reduced blood vessel patency (e.g., postpercutaneous transluminal coronary or carotid angioplasty, or post-bypass surgery graft stenosis), peripheral vascular disease, vascular disorders, such as Raynaud's disease, thrombocythemia, inflammatory diseases, myocardial infarction, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, osteoporosis, preterm labor, benign prostatic hypertrophy, peptic ulcer, male erectile dysfunction, female sexual dysfunction, and diseases characterized by disorders of gut motility (e.g., irritable bowel syndrome).

An especially important use is the treatment of male erectile dysfunction, which is one form of impotence and is a common medical problem. Impotence can be defined as a lack of power, in the male, to copulate, and can involve an inability to achieve penile erection or ejaculation, or both. The incidence of erectile dysfunction increases with age, with about 50% of men over the age of 40 suffering from some degree of erectile dysfunction.

In addition, a further important use is the treatment of female arousal disorder, also termed female sexual arousal disorder. Female arousal disorders are defined as a recurrent inability to attain or maintain an adequate lubrication/swelling response of sexual excitement until

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completion of sexual activity. The arousal response consists of vasocongestion in the pelvis, vaginal lubrication, and expansion and swelling of external genitalia.

5 It is envisioned, therefore, that compounds of formula (I) are useful in the treatment of male erectile dysfunction and female sexual arousal disorder. Thus, the present invention concerns the use of compounds of formula (I), or a pharmaceuti-
10 cally acceptable salt thereof, or a pharmaceutical composition containing either entity, for the manufacture of a medicament for the curative or prophylactic treatment of erectile dysfunction in a male animal and arousal disorder in a female animal,
15 including humans.

 The term "treatment" includes preventing, lowering, stopping, or reversing the progression or severity of the condition or symptoms being treated. As such, the term "treatment" includes both medical
20 therapeutic and/or prophylactic administration, as appropriate.

 It also is understood that "a compound of formula (I)," or a physiologically acceptable salt or solvate thereof, can be administered as the neat
25 compound, or as a pharmaceutical composition containing either entity.

 Although the compounds of the invention are envisioned primarily for the treatment of sexual
30 dysfunction in humans, such as male erectile dysfunction and female arousal disorder, they also can be used for the treatment of other disease states.

 A further aspect of the present invention, therefore, is providing a compound of formula (I)

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for use in the treatment of stable, unstable, and
variant (Prinzmetal) angina, hypertension, pulmonary
hypertension, malignant hypertension, pheochromo-
cytoma, chronic obstructive pulmonary disease, con-
gestive heart failure, acute respiratory distress
5 syndrome, acute and chronic renal failure, athero-
sclerosis, conditions of reduced blood vessel paten-
cy (e.g., post-PTCA or post-bypass graft stenosis),
peripheral vascular disease, vascular disorders such
10 as Raynaud's disease, thrombocytopenia, inflammatory
diseases, prophylaxis of myocardial infarction,
prophylaxis of stroke, stroke, bronchitis, chronic
asthma, allergic asthma, allergic rhinitis, glau-
coma, osteoporosis, preterm labor, benign prostatic
15 hypertrophy, male and female erectile dysfunction,
or diseases characterized by disorders of gut
motility (e.g., IBS).

According to another aspect of the present
invention, there is provided the use of a compound
20 of formula (I) for the manufacture of a medicament
for the treatment of the above-noted conditions and
disorders.

In a further aspect, the present invention
provides a method of treating the above-noted con-
25 ditions and disorders in a human or nonhuman animal
body which comprises administering to said body a
therapeutically effective amount of a compound of
formula (I).

Compounds of the invention can be admin-
30 istered by any suitable route, for example by oral,
buccal, inhalation, sublingual, rectal, vaginal,
transurethral, nasal, topical, percutaneous, i.e.,
transdermal, or parenteral (including intravenous,

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intramuscular, subcutaneous, and intracoronary) administration. Parenteral administration can be accomplished using a needle and syringe, or using a high pressure technique, like POWDERJECT™.

5 Oral administration of a compound of the invention is the preferred route. Oral administration is the most convenient and avoids the disadvantages associated with other routes of administration. For patients suffering from a swallowing disorder or from impairment of drug absorption after
10 oral administration, the drug can be administered parenterally, e.g., sublingually or buccally.

 Compounds and pharmaceutical compositions suitable for use in the present invention include
15 those wherein the active ingredient is administered in an effective amount to achieve its intended purpose. More specifically, a "therapeutically effective amount" means an amount effective to prevent development of, or to alleviate the existing
20 symptoms of, the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

 A "therapeutically effective dose" refers
25 to that amount of the compound that results in achieving the desired effect. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining
30 the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic

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index, which is expressed as the ratio between LD₅₀ and ED₅₀. Compounds which exhibit high therapeutic indices are preferred. The data obtained from such data can be used in formulating a range of dosage for use in humans. The dosage of such compounds preferably lies within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed, and the route of administration utilized.

The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient's condition. Dosage amount and interval can be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the therapeutic effects.

The amount of composition administered is dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

Specifically, for administration to a human in the curative or prophylactic treatment of the conditions and disorders identified above, oral dosages of a compound of formula (I) generally are about 0.5 to about 1000 mg daily for an average adult patient (70 kg). Thus, for a typical adult patient, individual tablets or capsules contain 0.2 to 500 mg of active compound, in a suitable pharmaceutically acceptable vehicle or carrier, for administration in single or multiple doses, once or several times per day. Dosages for intravenous,

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buccal, or sublingual administration typically are 0.1 to 500 mg per single dose as required. In practice, the physician determines the actual dosing regimen which is most suitable for an individual
5 patient, and the dosage varies with the age, weight, and response of the particular patient. The above dosages are exemplary of the average case, but individual instances exist wherein higher or lower dosages are merited, and such are within the scope
10 of this invention.

For human use, a compound of the formula (I) can be administered alone, but generally is administered in admixture with a pharmaceutical carrier selected with regard to the intended route
15 of administration and standard pharmaceutical practice. Pharmaceutical compositions for use in accordance with the present invention thus can be formulated in a conventional manner using one or more physiologically acceptable carriers comprising
20 excipients and auxiliaries that facilitate processing of compounds of formula (I) into preparations which can be used pharmaceutically.

These pharmaceutical compositions can be manufactured in a conventional manner, e.g., by
25 conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective
30 amount of a compound of the present invention is administered orally, the composition typically is in the form of a tablet, capsule, powder, solution, or elixir. When administered in tablet form, the comp-

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osition additionally can contain a solid carrier, such as a gelatin or an adjuvant. The tablet, capsule, and powder contain about 5 to about 95% compound of the present invention, and preferably from about 25 to about 90% compound of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, or oils of animal or plant origin can be added. The liquid form of the composition can further contain physiological saline solution, dextrose or other saccharide solutions, or glycols. When administered in liquid form, the composition contains about 0.5 to about 90% by weight of a compound of the present invention, and preferably about 1 to about 50% of a compound of the present invention.

When a therapeutically effective amount of a compound of the present invention is administered by intravenous, cutaneous, or subcutaneous injection, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred composition for intravenous, cutaneous, or subcutaneous injection typically contains, in addition to a compound of the present invention, an isotonic vehicle.

For oral administration, the compounds can be formulated readily by combining a compound of formula (I) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the present compounds to be formulated as tablets, pills, dragees, capsules, liquids, gels,

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5 syrups, slurries, suspensions and the like, for oral
ingestion by a patient to be treated. Pharmaceuti-
cal preparations for oral use can be obtained by
adding a compound of formula (I) with a solid
10 excipient, optionally grinding a resulting mixture,
and processing the mixture of granules, after adding
suitable auxiliaries, if desired, to obtain tablets
or dragee cores. Suitable excipients include, for
example, fillers and cellulose preparations. If
15 desired, disintegrating agents can be added.

For administration by inhalation, com-
pounds of the present invention are conveniently
delivered in the form of an aerosol spray presen-
tation from pressurized packs or a nebulizer, with
15 the use of a suitable propellant. In the case of a
pressurized aerosol, the dosage unit can be deter-
mined by providing a valve to deliver a metered
amount. Capsules and cartridges of, e.g., gelatin,
for use in an inhaler or insufflator can be formu-
20 lated containing a powder mix of the compound and a
suitable powder base such as lactose or starch.

The compounds can be formulated for
parenteral administration by injection, e.g., by
bolus injection or continuous infusion. Formula-
25 tions for injection can be presented in unit dosage
form, e.g., in ampules or in multidose containers,
with an added preservative. The compositions can
take such forms as suspensions, solutions, or emul-
sions in oily or aqueous vehicles, and can contain
30 formulatory agents such as suspending, stabilizing,
and/or dispersing agents.

Pharmaceutical formulations for parenteral
administration include aqueous solutions of the

- 20 -

active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include
5 fatty oils or synthetic fatty acid esters. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility
10 of the compounds and allow for the preparation of highly concentrated solutions. Alternatively, a present composition can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

15 Compounds of the present invention also can be formulated in rectal compositions, such as suppositories or retention enemas, e.g., containing conventional suppository bases. In addition to the formulations described previously, the compounds
20 also can be formulated as a depot preparation. Such long-acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with
25 suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

30 Many of the compounds of the present invention can be provided as salts with pharmaceutically compatible counterions. Such pharmaceutically acceptable base addition salts are those salts that retain the biological effectiveness and

- 21 -

properties of the free acids, and that are obtained by reaction with suitable inorganic or organic bases.

In particular, a compound of formula (I) can be administered orally, buccally, or sublingually in the form of tablets containing excipients, such as starch or lactose, or in capsules or ovules, either alone or in admixture with excipients, or in the form of elixirs or suspensions containing
5
10
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20
flavoring or coloring agents. Such liquid preparations can be prepared with pharmaceutically acceptable additives, such as suspending agents. A compound also can be injected parenterally, for example, intravenously, intramuscularly, subcutaneously, or intracoronarily. For parenteral administration, the compound is best used in the form of a sterile aqueous solution which can contain other substances, for example, salts, or monosaccharides, such as mannitol or glucose, to make the solution isotonic with blood.

For veterinary use, a compound of formula (I) or a nontoxic salt thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can
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readily determine the dosing regimen and route of administration that is most appropriate for a particular animal.

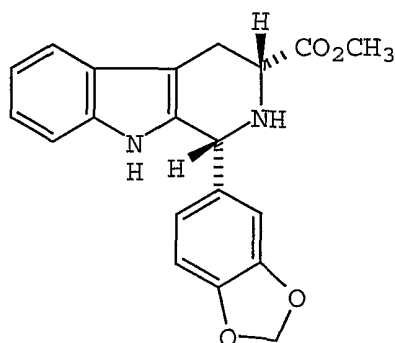
Thus, the invention provides in a further aspect a pharmaceutical composition comprising a
30
compound of the formula (I), together with a pharmaceutically acceptable diluent or carrier therefor. There is further provided by the present invention a process of preparing a pharmaceutical composition

- 22 -

comprising a compound of formula (I), which process comprises mixing a compound of formula (I), together with a pharmaceutically acceptable diluent or carrier therefor.

5 In a particular embodiment, the invention includes a pharmaceutical composition for the curative or prophylactic treatment of erectile dysfunction in a male animal, or arousal disorder in a female animal, including humans, comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

10 Compounds of formula (I) can be prepared by any suitable method known in the art, or by the following processes which form part of the present invention. In the methods below, R⁰, R¹, R², and Y are as defined in structural formula (I) above. In particular, Daugan U.S. Patent No. 5,859,006, incorporated herein by reference, discloses preparation of a compound of structural formula (III).



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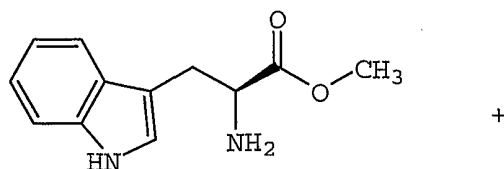
(III)

In short, the compound of structural formula (III), i.e., the *cis*-isomer of Intermediates 1 and 2 of

- 23 -

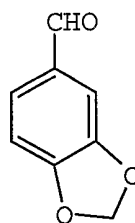
Daugan U.S. Patent No. 5,859,006 was prepared according to the following reaction scheme:

5



D-Tryptophan methyl ester

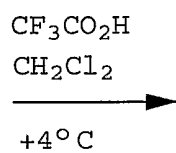
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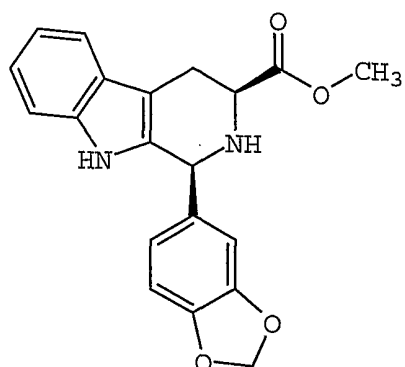
Piperonal

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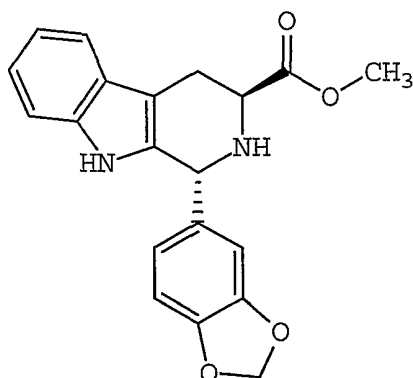
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(42%)
(III) (cis-isomer)

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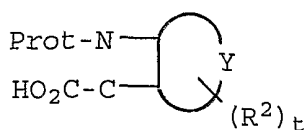
20

(28%)
(trans-isomer).

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A compound of structural formula (I) is prepared by reacting the compound of structural formula (III) with a compound of structural formula (IV):

- 25 -

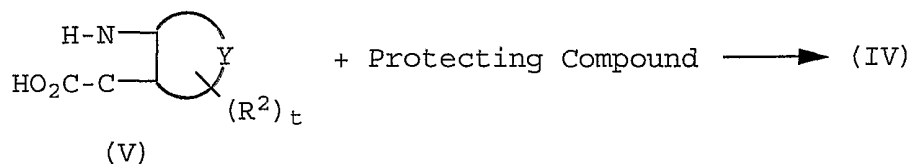


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(IV)

A compound of structural formula (IV) is prepared by reacting a compound of structural formula (V) with a protecting compound, i.e.,

10



15

Protecting compounds and protecting groups, like benzyl chloroformate and trichloroethyl chloroformate, are well known to persons skilled in the art, for example, see T.W. Greene et al. "Protective Groups in Organic Synthesis, Third Edition," John Wiley and Sons, Inc., NY, NY (1999).

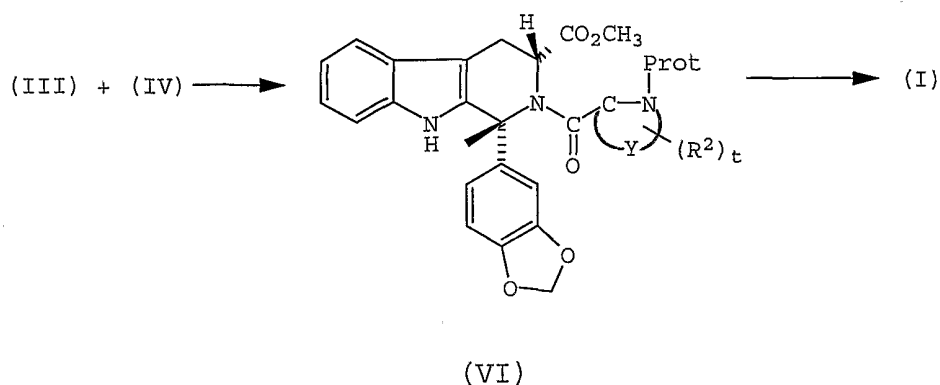
20

The compounds of structural formulae (III) and (IV) then are reacted to form a compound of structural formula (VI), which in turn is cyclized with the removal of protecting groups to form a compound of structural formula (I). The structure of a compound of structural formula (I) can be varied, for example, by using an aldehyde different from piperonal to change the identity of R^1 , by using a halo or alkyl phenyl-substituted tryptophan ester,

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- 26 -

and by using an appropriately substituted compound of structural formula (V).



5 Compounds of formula (I) can be converted to other compounds of formula (I). Thus, for example, when R^1 is a substituted benzene ring, it is possible to prepare another suitably substituted compound of formula (I). Examples of appropriate interconversions include, but are not limited to, nitro to amino, OR^a to hydroxy by suitable means (e.g., using a reducing agent, such as $SnCl_2$ or a palladium catalyst, such as palladium-on-carbon), or

10 amino to substituted amino, such as acylamino or sulphonylamino, using standard acylating or sulfonylating conditions. In cases wherein R^1 represents a substituted bicyclic system, suitable interconversion can involve removal of a substituent, such as by treatment with a palladium catalyst whereby, for

15 example, a benzyl substituent is removed from a suitable bicyclic system. Similar interconversions can be made on R^2 substituents, i.e., conversion of a carboxylic acid to an ester or amide, or conversion of amino to substituted amino.

20

- 27 -

Compounds of formula (I) can be prepared by the method above as individual stereoisomers from the appropriate stereoisomer of formula (III) or as a racemic mixture from the appropriate racemic compound of formula (III). Individual stereoisomers of the compounds of the invention can be prepared from racemates by resolution using methods known in the art for the separation of racemic mixtures into their constituent stereoisomers, for example, using HPLC on a chiral column, such as Hypersil naphthyl urea, or using separation of salts of stereoisomers. Compounds of the invention can be isolated in association with solvent molecules by crystallization from, or evaporation of, an appropriate solvent.

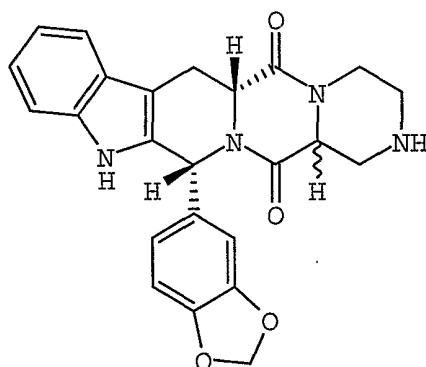
The pharmaceutically acceptable acid addition salts of the compounds of formula (I) that contain a basic center can be prepared in a conventional manner. For example, a solution of the free base can be treated with a suitable acid, either neat or in a suitable solution, and the resulting salt isolated either by filtration or by evaporation under vacuum of the reaction solvent. Pharmaceutically acceptable base addition salts can be obtained in an analogous manner by treating a solution of a compound of formula (I) with a suitable base. Both types of salt can be formed or interconverted using ion-exchange resin techniques. Thus, according to a further aspect of the invention, a method for preparing a compound of formula (I) or a salt or solvate (e.g., hydrate) is provided, followed by (i) salt formation, or (ii) solvate (e.g., hydrate) formation.

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The following abbreviations are used hereafter in the accompanying examples: rt (room temperature), min (minute), h (hour), g (gram), mmol (millimole), m.p. (melting point), eq (equivalents),
5 L (liter), mL (milliliter), μ L (microliters), DMSO (dimethyl sulfoxide), CH_2Cl_2 (dichloromethane), CHCl_3 (chloroform), IPA (isopropyl alcohol), TFA (trifluoroacetic acid), EtOH (ethanol), MeOH (methanol), Et_3N (triethylamine), and THF (tetra-
10 hydrofuran).

Preparation of Example 1

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(Mixture of Diastereomers)

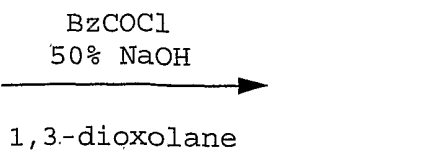
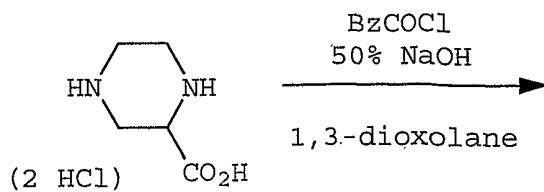
Example 1

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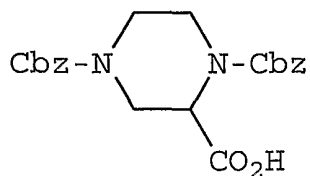
Example 1 was prepared from Intermediates 1 and 2 as depicted in the following synthetic scheme. Intermediate 1 was prepared from 2-carboxy-piperazine dihydrochloride, a commercially available
30 compound, from Aldrich Chemical Co., Milwaukee, WI.

- 29 -

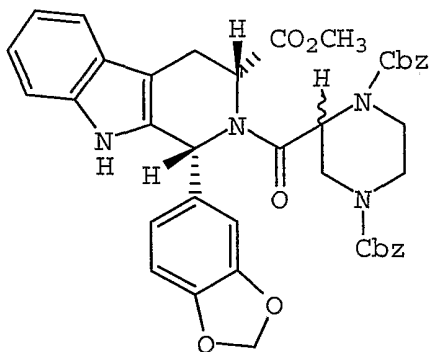
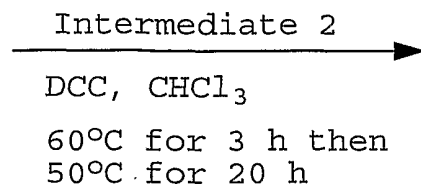
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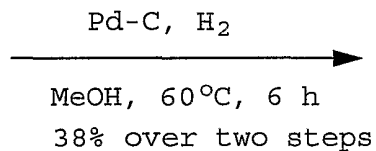
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Intermediate 1



Intermediate 3



15

Example 1

- 30 -

Intermediate 1Preparation of N,N-Bis-Cbz-2-carboxypiperazine

To a mixture of 2-carboxypiperazine
5 dihydrochloride (5.08 g, 25 mmol), 1,3-dioxolane (45
mL), and water (30 mL) at 0°C was added 50% aqueous
sodium hydroxide (NaOH) (6 mL) dropwise. Benzyl
chloroformate (BzCOCl) (7.8 mL, 55 mmol) then was
added to the resulting mixture dropwise in alternat-
10 ing portions with 50% aqueous sodium hydroxide (4
mL) in order to maintain a pH between 8-11. The
temperature of the reaction was maintained below
15°C during a 30-minute addition period. The re-
sulting biphasic mixture was stirred at 0°C for an
15 additional 30 minutes, after which the reaction was
acidified to pH 2 with 2 N hydrochloric acid (HCl),
then diluted with ethyl acetate (100 mL). The
organic layer was washed with brine (2 x 10 mL), and
the aqueous phase was reextracted with ethyl acetate
20 (20 mL). The combined organic extracts then were
dried over sodium sulfate (Na₂SO₄), filtered, and
concentrated under reduced pressure. The residue
was purified by flash column chromatography, eluting
with methylene chloride/methanol (40:1 to 10:1), to
25 provide the bis-protected piperazine as a white
solid (7.0 g, 70%): TLC R_f (10:1 methylene chlor-
ide/methanol)=0.55; ¹H NMR (300 MHz, DMSO-d₆) δ 7.40-
7.20 (m, 10H), 5.01-5.27 (m, 4H), 4.92-4.60 (m, 2H),
4.20-3.81 (m, 2H), 3.40-2.82 (m, 3H).

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- 31 -

Intermediate 2Preparation of Methyl 1,2,3,4-Tetrahydro-1-(3,4-methylenedioxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxylate

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Intermediate 2, i.e., the compound of structural formula (III), was prepared as set forth in Daugan, U.S. Patent No. 5,859,006, incorporated herein by reference.

10

Intermediate 3Preparation of the cis-Amide

15

To a solution of Intermediate 1 (4.5 g, 11.3 mmol) in chloroform (CHCl₃) (50 mL) at 0°C was added dicyclohexylcarbodiimide (DCC) (2.3 g, 11.2 mmol) in one portion. The resulting white slurry was stirred at 0°C for 40 min., after which Intermediate 2 (3.9 g, 11.0 mmol) was added. The resulting yellow slurry was heated at 60°C for 3 hours, after which the mixture was heated at 50°C for an additional 20 hours. The slurry then was cooled to room temperature, vacuum filtered, and the solid was washed with methylene chloride. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate (1:0 to 20:1), to provide the *cis*-amide of Intermediate 3 as a yellow solid (6.1 g): TLC R_f (20:1 methylene chloride/ethyl acetate)=0.29.

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- 32 -

Example 1Preparation of (5aR,12R)-12-Benzo(1,3)dioxol-5-yl-5,5a,8,9,10,10a,12,13-octahydro-7H-6a,9,11a,13-tetraza-indeno[1,2-b]anthracene-6,11-dione

5

The Cbz-protected Intermediate 3 in methanol (200 mL) was treated with a catalytic amount of 5% palladium on carbon (0.55 g, about 55% wet). The mixture was stirred under a hydrogen atmosphere at 60°C for 6 hours, after which the palladium catalyst was removed by vacuum filtration through a plug of Celite, eluting with methanol. The filtrate was concentrated under reduced pressure, and the residue was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate/methanol/acetonitrile (4:1:0.2:0.1), to provide the compound of Example 1 (1.79 g, 38% over two steps, 1:1 mixture of diastereomers): mp 226-237°C; TLC R_f (4:1:0.2 methylene chloride/ethyl acetate/methanol)=0.50; ^1H NMR (300 MHz, CDCl_3): δ 8.04 (s, 0.5H), 7.97 (s, 0.5H), 7.60-7.50 (m, 2H), 7.25-7.02 (m, 3H), 6.87-6.74 (m, 1H), 6.70-6.60 (m, 2H), 6.00 (s, 0.5H), 5.94 (s, 0.5H), 5.85 (s, 1H), 5.82 (s, 1H), 4.70-4.59 (m, 0.5H), 4.55-4.42 (m, 0.5H), 4.40-4.24 (m, 1H), 4.08-3.79 (m, 2H), 3.56-3.42 (m, 1H), 3.20-2.89 (m, 2H), 2.87-2.48 (m, 3H), 1.92 (bs, 2H); ^{13}C NMR (125 MHz, CDCl_3): δ 167.3, 166.3, 166.1, 163.9, 147.9, 147.0, 136.7, 136.1, 135.8, 132.8, 126.2, 122.5, 120.4, 120.1, 119.9, 118.6, 111.1, 108.4, 108.3, 107.1, 106.7, 106.5, 101.1, 59.6, 57.8, 57.6, 57.2, 56.7, 56.4, 49.9, 49.3, 44.9, 44.7, 43.0, 42.3, 25.5, 25.0 ppm; API MS m/z 431 ($\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_4+\text{H}$) $^+$; $[\alpha]_D^{25^\circ\text{C}}=+86.5^\circ$ ($c=0.5$, DMSO).

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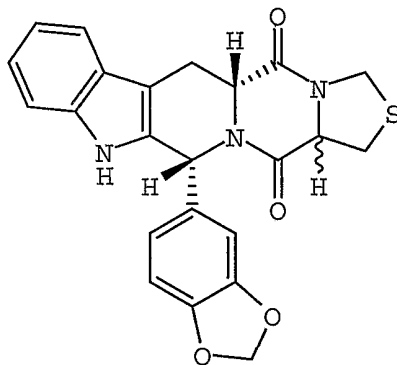
- 33 -

Anal. Calcd. for $C_{24}H_{22}N_4O_4 \cdot H_2O$: C, 64.28; H, 5.39; N, 12.49. Found: C, 64.36; H, 5.21; N, 12.46.

Preparation of Example 2

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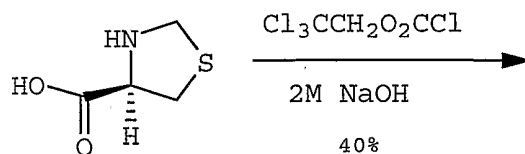
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2:1 Mixture of Diastereomers

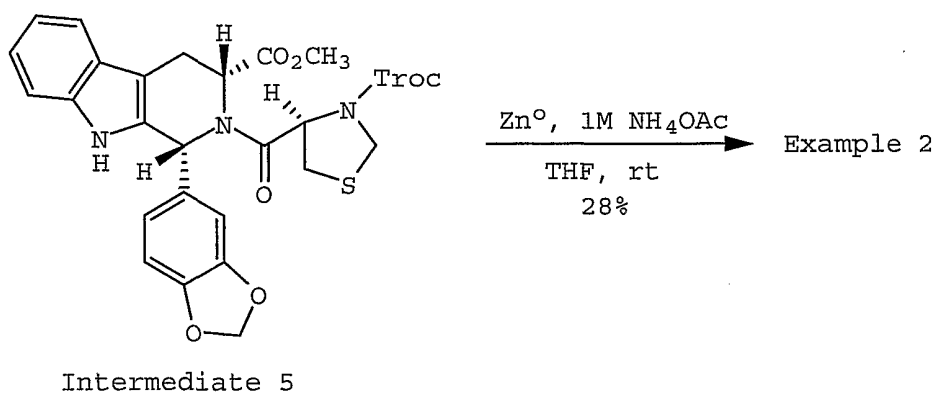
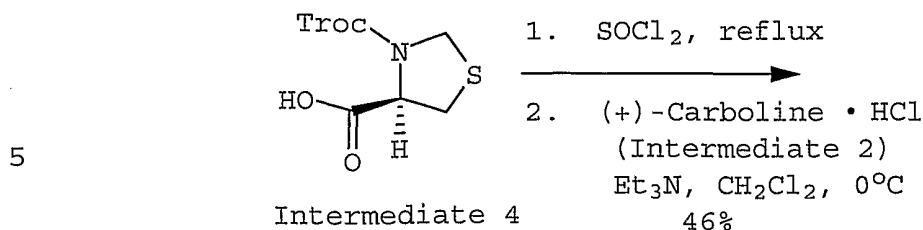
Example 2

Example 2 was prepared from Intermediates 2 and 4 as depicted in the following synthetic scheme. L-thiaproline is commercially available from Aldrich Chemical Co.

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10 Intermediate 4

Preparation of Troc-Protected Thiaproline

L-Thiaproline (3.0 g, 22.5 mmol) was dissolved in 2M sodium hydroxide (11.25 mL), and the resulting solution was cooled to 0°C . To this mixture was added trichloroethyl chloroformate (3.4 mL, 24.6 mmol) and 2M sodium hydroxide (15.5 mL) dropwise and simultaneously over 1 hour, while maintaining the reaction temperature at 0°C . After the addition was complete, the resulting mixture was allowed to warm slowly to room temperature, with stirring, for a total of 12 hours. The mixture was extracted with diethyl ether (3 x 50 mL), and the aqueous layer was acidified to pH 1 with 2M HCl.

15

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- 35 -

The aqueous layer was further extracted with ethyl acetate (3 x 50 mL), and the combined organic extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to provide the Troc-protected thiaproline Intermediate 4 as a colorless oil (2.78 g, 40%): ¹H NMR (300 MHz, CDCl₃): δ 9.61 (br s, 1H), 4.99-4.90 (m, 1H), 4.85-4.71 (m, 3H), 4.60 (d, J=8.8 Hz, 1H), 3.48-3.30 (m, 2H).

10

Intermediate 5

Preparation of cis-β-Carboline Amide

A solution of Troc-protected thiaproline Intermediate 4 (2.0 g, 6.5 mmol) in thionyl chloride (7 mL) was heated at reflux for 4 hours, after which the excess thionyl chloride was removed under reduced pressure. The residue was diluted with methylene chloride (10 mL). This residual solution was added dropwise to a mixture of Intermediate 2 (2.34 g, 6.10 mmol) and triethylamine (Et₃N) (2.60 mL, 18.7 mmol) in methylene chloride (CH₂Cl₂) (50 mL) at 0°C under an argon atmosphere. The resulting mixture was warmed slowly to room temperature over 12 hours, diluted with methylene chloride (100 mL), then washed with water (100 mL), saturated sodium bicarbonate (NaHCO₃) solution, and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to provide an orange foam residue. This residue was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate (97:3), to provide cis-β-carboline amide Intermediate 5 as a yellow foam (1.8

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- 36 -

g, 46%): TLC R_f (95:5 methylene chloride/ethyl acetate)=0.53.

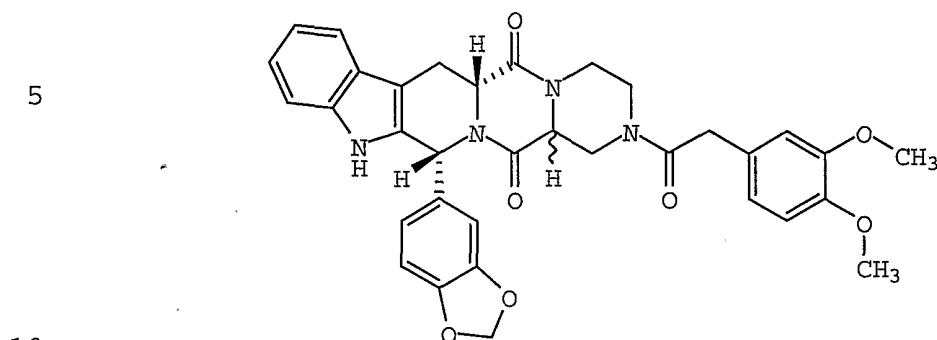
Preparation of Example 2

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A solution of 1M ammonium acetate (NH_4OAc) (40 mL) was added to a solution of Intermediate 5 (2.00 g, 3.13 mmol) in THF (200 mL) followed by the addition of freshly activated zinc granules (12.95 g, 0.20 mol). The activated zinc was prepared by washing zinc granules with 2M HCl followed by rinsing with water until the washings were neutral to pH paper. The activated zinc then was washed with acetone and diethyl ether followed by quickly drying at room temperature under reduced pressure. The resulting mixture was stirred at room temperature under an argon atmosphere for 3 days, after which the zinc was removed by vacuum filtration, and washed with THF (3 x 10 mL). The resulting filtrate was concentrated under reduced pressure to provide a yellow foam, which was partitioned between ethyl acetate (300 mL) and 0.1M HCl (100 mL). The ethyl acetate layer was washed with water (200 mL), saturated NaHCO_3 solution (200 mL), and brine (200 mL), dried over Na_2SO_4 , and the solvents were removed under reduced pressure to provide a yellow foam. The residue was purified by flash column chromatography, eluting with methylene chloride/ethyl acetate (8:1), to provide a yellow foam. This residue was further purified by a slurry followed by vacuum filtration to produce a yellow powder which was dried overnight under vacuum at 70°C to provide Example 2 as a pale yellow powder (0.38 g, 28%, 2:1

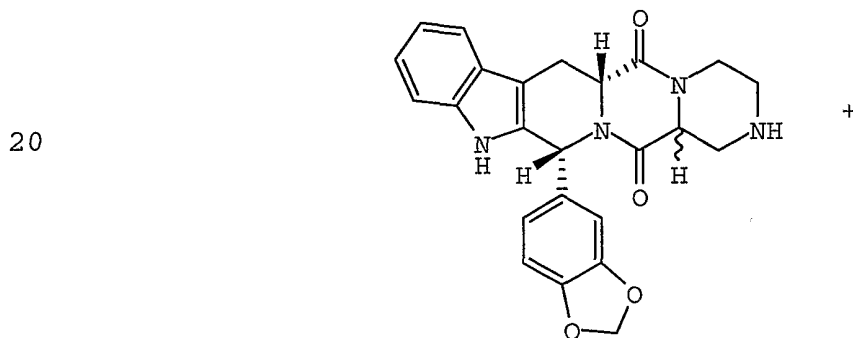
- 37 -

mixture of diastereomers at C3): mp 188-192°C; TLC
 R_f (8:1 methylene chloride/ethyl acetate)=0.41; ^1H
NMR (300 MHz, DMSO- d_6): δ 11.10 (s, 2H), 10.8 (s,
1H), 7.55 (d, $J=7.8$ Hz, 2H), 7.52 (d, $J=8.1$ Hz, 1H),
5 7.32 (d, $j=7.9$ Hz, 2H), 7.25 (d, $J=8.0$ Hz, 1H),
7.08-6.97 (m, 12H), 6.90-6.87 (m, 2H), 6.82 (s, 2H),
6.78-6.75 (m, 3H), 6.72-6.70 (m, 2H), 6.24 (s, 2H),
5.96-5.90 (m, 7H), 5.24 (d, $J=9.2$ Hz, 1H), 4.86 (d,
 $J=9.8$ Hz, 2H), 4.64 (t, $J=6.9$ Hz, 2H), 4.61-4.58
10 (dd, $j=5.1, 11.6$ Hz, 2H), 4.48-4.46 (m, 3H), 4.42-
4.38 (m, 1H), 4.23 (d, $J=9.2$ Hz, 1H), 3.56-3.52 (dd,
 $J=3.4, 15.6$ Hz, 1H), 3.48-3.44 (dd, $J=5.0, 16.0$ Hz,
2H), 3.41-3.20 (m, 5H), 3.12-3.08 (m, 2H), 3.02-2.97
(dd, $J=11.7, 15.7$ Hz, 2H) ppm; CI MS m/z 434
15 ($\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_4\text{S}+\text{H}$) $^+$; $[\alpha]_D^{25^\circ\text{C}}=+53.3^\circ$ ($c=0.5$, chloroform).
Anal. Calcd. for $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_4\text{S}\cdot 0.25\text{H}_2\text{O}$: C, 63.07; H,
4.44; N, 9.59. Found: C, 63.03; H, 4.43; N, 9.39.
Chiral HPLC analysis (Chiralcel OD Column, 250 x 4.6
mm, Retention Times=49.2 and 58.3 minutes; 1:1
20 isopropanol/hexanes; flow=0.5 mL/min; detector at
254 nm; 25°C) showed two major peaks, with a ratio
of 67:29, respectively, and with a total purity of
96.2%. The relative stereochemistry of Example 2
was confirmed to be the *cis* isomer by a series of
25 NOE difference experiments: positive NOE enhance-
ments from the C12a protons at 4.60 ppm and 4.40 ppm
to the C6 protons at 6.24 ppm and 5.96 ppm; a posi-
tive NOE enhancement from the C6 protons at 6.24 ppm
and 5.96 ppm to the C12a protons at 4.60 ppm and
30 4.40 ppm.

Preparation of Example 3

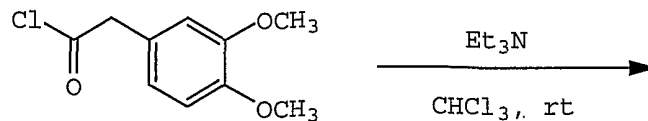
Example 3

15 Example 3 was prepared from Example 1 by
the following synthetic reaction.



Example 1
(1:1 Mixture of Diastereomers)

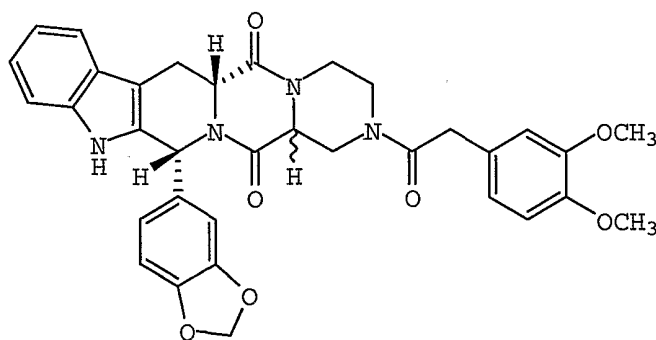
- 39 -



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Intermediate 6

10



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Example 3

(1:1 Mixture of Diastereomers)

20

Preparation of (5aR,12R)-12-Benzo(1,3)dioxol-5-yl-9-[2-(3,4-dimethoxyphenyl)ethanoyl]-5,5a,8,9,10,10a,12,13-octahydro-7H-6a,9,11a,13-tetraza-indeno[1,2-b]anthracene-6,11-dione

25

(3,4-Dimethoxyphenyl)acetyl chloride

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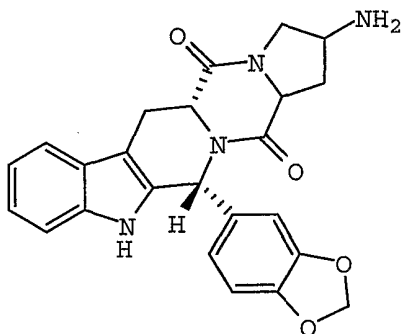
(Intermediate 6, 139 mg, 0.65 mmol) was added in one portion to a mixture of Example 1 (138 mg, 0.32 mmol) and triethylamine (0.058 mL, 0.42 mmol) in chloroform (4.0 mL) at room temperature under a nitrogen blanket, and the resulting mixture was stirred for 5 hours. The solution was diluted with methylene chloride (60 mL), washed successively with saturated NaHCO₃ solution (10 mL), and water (10 mL), dried over Na₂SO₄, then the solvent was removed under

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reduced pressure. The isolated solid was washed with methylene chloride (2 x 1 mL), then dried in a vacuum oven at room temperature for 18 hours to provide Example 3 as an off-white solid (98 mg, 50%):
5 mp 284-289°C; TLC R_f (5:1 methylene chloride/ethyl acetate)=0.33; ¹H NMR (500 MHz, DMSO-d₆): δ 11.00-10.80 (m, 1H), 7.55-7.50 (m, 1H), 7.31-7.24 (m, 1H), 7.10-6.68 (m, 8H), 6.09-5.88 (m, 3H), 4.72-3.87 (m, 4H), 3.87-3.40 (m, 8H), 3.30-2.55 (m, 4H); API MS
10 m/z 609 [C₃₄H₃₂N₄O₇+H]⁺; [α]_D^{25°C}=+49.7 (c=1.0, DMSO). HPLC analysis (Chiralcel OD Column, 250 x 4.6 mm, Retention Time=34.4 minutes; 1:1 isopropanol/-hexanes; flow=0.5 mL/min; detector @ 254 nm; ambient temperature) showed a single peak with a purity of
15 96.7%.

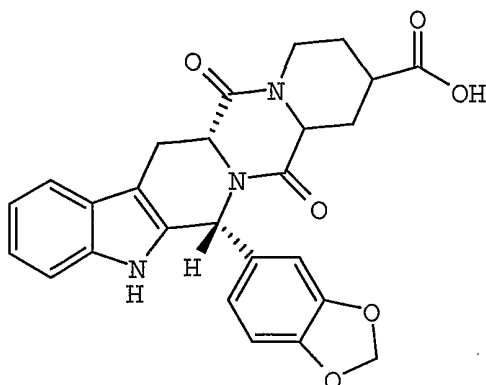
The following compounds are two additional examples of compounds of structural formula (I) that can be prepared by methods analogous to the preparation of Examples 1-3.



Example 4

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Example 5

Compounds of the present invention can be formulated into tablets for oral administration. For example, a compound of formula (I) can be formed into a dispersion with a polymeric carrier by the coprecipitation method set forth in WO 96/38131, incorporated herein by reference. The coprecipitated dispersion can be blended with excipients, then pressed into tablets, which optionally are film-coated.

The compounds of structural formula (I) were tested for an ability to inhibit PDE5. The ability of a compound to inhibit PDE5 activity is related to the IC_{50} value for the compound, i.e., the concentration of inhibitor required for 50% inhibition of enzyme activity. The IC_{50} value for compounds of structural formula (I) were determined using recombinant human PDE5.

The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE5 of less than about 50 μM , and preferably less than about 25 μM , and more preferably less than

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about 15 μM . The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE5 of less than about 1 μM , and often less than about 0.05 μM . To achieve the full advantage of the present invention, a present PDE5 inhibitor has an IC_{50} of about 0.1 nM to about 15 μM .

The production of recombinant human PDEs and the IC_{50} determinations can be accomplished by well-known methods in the art. Exemplary methods are described as follows:

EXPRESSION OF HUMAN PDEs

Expression in *Saccharomyces cerevisiae* (Yeast)

Recombinant production of human PDE1B, PDE2, PDE4A, PDE4B, PDE4C, PDE4D, PDE5, and PDE7 was carried out similarly to that described in Example 7 of U.S. Patent No. 5,702,936, incorporated herein by reference, except that the yeast transformation vector employed, which is derived from the basic ADH2 plasmid described in Price et al., *Methods in Enzymology*, 185, pp. 308-318 (1990), incorporated yeast ADH2 promoter and terminator sequences and the *Saccharomyces cerevisiae* host was the protease-deficient strain BJ2-54 deposited on August 31, 1998 with the American Type Culture Collection, Manassas, Virginia, under accession number ATCC 74465. Transformed host cells were grown in 2X SC-leu medium, pH 6.2, with trace metals, and vitamins. After 24 hours, YEP medium-containing glycerol was added to a final concentration of 2X YET/3% glycerol. Approxi-

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mately 24 hr later, cells were harvested, washed, and stored at -70°C .

HUMAN PHOSPHODIESTERASE PREPARATIONS

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Phosphodiesterase Activity Determinations

Phosphodiesterase activity of the preparations was determined as follows. PDE assays utilizing a charcoal separation technique were performed essentially as described in Loughney et al. (1996). In this assay, PDE activity converts $[^{32}\text{P}]\text{cAMP}$ or $[^{32}\text{P}]\text{cGMP}$ to the corresponding $[^{32}\text{P}]\text{5'-AMP}$ or $[^{32}\text{P}]\text{5'-GMP}$ in proportion to the amount of PDE activity present. The $[^{32}\text{P}]\text{5'-AMP}$ or $[^{32}\text{P}]\text{5'-GMP}$ then was quantitatively converted to free $[^{32}\text{P}]\text{phosphate}$ and unlabeled adenosine or guanosine by the action of snake venom 5'-nucleotidase. Hence, the amount of $[^{32}\text{P}]\text{phosphate}$ liberated is proportional to enzyme activity. The assay was performed at 30°C in a $100\ \mu\text{L}$ reaction mixture containing (final concentrations) $40\ \text{mM}$ Tris HCl (pH 8.0), $1\ \mu\text{M}$ ZnSO_4 , $5\ \text{mM}$ MgCl_2 , and $0.1\ \text{mg/mL}$ bovine serum albumin (BSA). PDE enzyme was present in quantities that yield <30% total hydrolysis of substrate (linear assay conditions). The assay was initiated by addition of substrate ($1\ \text{mM}$ $[^{32}\text{P}]\text{cAMP}$ or cGMP), and the mixture was incubated for 12 minutes. Seventy-five (75) μg of *Crotalus atrox* venom then was added, and the incubation was continued for 3 minutes (15 minutes total). The reaction was stopped by addition of $200\ \mu\text{L}$ of activated charcoal ($25\ \text{mg/mL}$ suspension in $0.1\ \text{M}$ NaH_2PO_4 , pH 4). After centrifugation ($750\ \text{X g}$ for

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3 minutes) to sediment the charcoal, a sample of the supernatant was taken for radioactivity determination in a scintillation counter and the PDE activity was calculated.

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Purification of PDE5 from *S. cerevisiae*

Cell pellets (29 g) were thawed on ice with an equal volume of Lysis Buffer (25 mM Tris HCl, pH 8, 5 mM MgCl₂, 0.25 mM DTT, 1 mM benzamidine, and 10 μM ZnSO₄). Cells were lysed in a Microfluidizer[®] (Microfluidics Corp.) using nitrogen at 20,000 psi. The lysate was centrifuged and filtered through 0.45 μm disposable filters. The filtrate was applied to a 150 mL column of Q SEPHAROSE[®] Fast-Flow (Pharmacia). The column was washed with 1.5 volumes of Buffer A (20 mM Bis-Tris Propane, pH 6.8, 1 mM MgCl₂, 0.25 mM DTT, 10 μM ZnSO₄) and eluted with a step gradient of 125 mM NaCl in Buffer A followed by a linear gradient of 125-1000 mM NaCl in Buffer A. Active fractions from the linear gradient were applied to a 180 mL hydroxyapatite column in Buffer B (20 mM Bis-Tris Propane (pH 6.8), 1 mM MgCl₂, 0.25 mM DTT, 10 μM ZnSO₄, and 250 mM KCl). After loading, the column was washed with 2 volumes of Buffer B and eluted with a linear gradient of 0-125 mM potassium phosphate in Buffer B. Active fractions were pooled, precipitated with 60% ammonium sulfate, and resuspended in Buffer C (20 mM Bis-Tris Propane, pH 6.8, 125 mM NaCl, 0.5 mM DTT, and 10 μM ZnSO₄). The pool was applied to a 140 mL column of SEPHACRYL[®] S-300 HR and eluted with Buffer C.

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Active fractions were diluted to 50% glycerol and stored at -20°C.

The resultant preparations were about 85% pure by SDS-PAGE. These preparations had specific activities of about 3 μmol cGMP hydrolyzed per minute per milligram protein.

Inhibitory Effect on cGMP-PDE

cGMP-PDE activity of compounds of the present invention was measured using a one-step assay adapted from Wells et al., *Biochim. Biophys. Acta*, 384, 430 (1975). The reaction medium contained 50 mM Tris-HCl, pH 7.5, 5 mM magnesium acetate, 250 $\mu\text{g/ml}$ 5'-Nucleotidase, 1 mM EGTA, and 0.15 μM 8-[H³]-cGMP. Unless otherwise indicated, the enzyme used was a human recombinant PDE5 (ICOS Corp., Bothell, Washington).

Compounds of the invention were dissolved in DMSO finally present at 2% in the assay. The incubation time was 30 minutes during which the total substrate conversion did not exceed 30%.

The IC₅₀ values for the compounds examined were determined from concentration-response curves typically using concentrations ranging from 10 nM to 10 μM . Tests against other PDE enzymes using standard methodology showed that compounds of the invention are selective for the cGMP-specific PDE enzyme.

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Biological Data

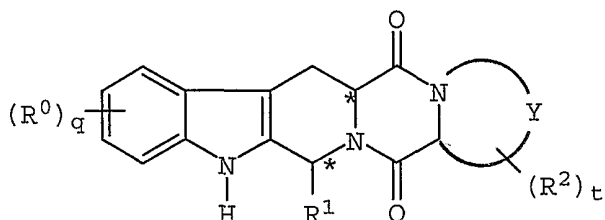
The compounds according to the present invention were typically found to exhibit an IC₅₀ value of less than 500 nM. An *in vitro* test using Examples 1 and 3, representative compounds of the invention, gave IC₅₀ values versus PDE5 of 1.7 nM and 3.9 nM, respectively.

Obviously, many modifications and variations of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof, and, therefore, only such limitations should be imposed as are indicated by the appended claims.

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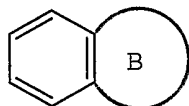
WHAT IS CLAIMED IS:

1. A compound having a formula



wherein R^0 , independently, is selected from the group consisting of halo and C_{1-6} alkyl;

R^1 is selected from the group consisting of an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine, and an optionally substituted bicyclic ring



wherein the fused ring B is a 5- or 6-membered ring, saturated or partially or fully unsaturated, and comprises carbon atoms and optionally one or two heteroatoms selected from oxygen, sulfur, and nitrogen;

Y is a 3-, 4-, or 5-membered carbon chain of a 5-, 6-, or 7-membered substituted ring, or Y is a 3-, 4-, or 5-membered heteroatom chain of a 5-, 6-, or 7-membered unsubstituted or substituted ring, wherein said heteroatom chain contains one or two

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heteroatoms independently selected from the group consisting of oxygen, sulfur, and nitrogen;

R^2 , independently, is selected from the group consisting of

nitro,
 trifluoromethyl,
 trifluoromethoxy,
 halogen,
 cyano,
 C_{1-6} alkyl, optionally substituted with OR^a ,
 $C(=O)R^a$,
 $OC(=O)R^a$,
 $C(=O)OR^a$,
 C_{1-4} alkyleneHet,
 C_{1-4} alkylene $C(=O)OR^a$,
 OC_{1-4} alkylene $C(=O)OR^a$,
 C_{1-4} alkylene OC_{1-4} alkylene $C(=O)OR^a$,
 $C(=O)NR^aSO_2R^c$,
 $C(=O)C_{1-4}$ alkyleneHet,
 $C(=O)C_{1-4}$ alkylenearyl, optionally substituted with one or more OR^a ,
 C_{1-4} alkylene NR^aR^b ,
 C_{2-6} alkenylene NR^aR^b ,
 $C(=O)NR^aR^b$,
 $C(=O)NR^aR^c$,
 $C(=O)NR^aC_{1-4}$ alkylene OR^b ,
 $C(=O)NR^aC_{1-4}$ alkyleneHet,
 OR^a ,
 OC_{2-4} alkylene NR^aR^b ,
 OC_{1-4} alkylene $CH(OR^a)CH_2NR^aR^b$,
 OC_{1-4} alkyleneHet,
 OC_{2-4} alkylene OR^a ,
 OC_{2-4} alkylene $NR^aC(=O)OR^b$,

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NR^aR^b,
NR^aC₁₋₄alkyleneNR^aR^b,
NR^aC(=O)R^b,
NR^aC(=O)NR^aR^b,
N(SO₂C₁₋₄alkyl)₂,
NR^a(SO₂C₁₋₄alkyl),
SO₂NR^aR^b,
and OSO₂trifluoromethyl;

Het is a 5- or 6-membered heterocyclic group containing at least one heteroatom selected from the group consisting of oxygen, nitrogen, and sulfur, and is optionally substituted with C₁₋₄alkyl;

R^a and R^b can be the same or different, and are independently selected from hydrogen and C₁₋₆alkyl;

R^c is phenyl or C₄₋₆cycloalkyl, wherein the phenyl or C₄₋₆cycloalkyl can be optionally substituted with one or more substituents selected from the group consisting of halogen atoms, C(=O)OR^a, and OR^a;

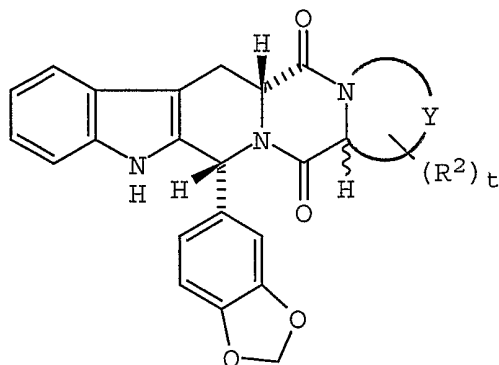
q is 0, 1, 2, 3, or 4;

t is 0, 1, or 2; and

pharmaceutically acceptable salts and solvates thereof.

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2. The compound of claim 1 represented by the formula



and pharmaceutically acceptable salts and solvates thereof.

3. The compound of claim 1 wherein Y is a 3-, 4-, or 5-membered carbon chain of a 5-, 6-, or 7-membered substituted ring.

4. The compound of claim 1 wherein Y is a 3-, 4-, or 5-membered heteroatom chain of a 5-, 6-, or 7-membered unsubstituted or substituted ring, wherein Y contains one or two heteroatoms selected from the group consisting of oxygen, sulfur, and nitrogen.

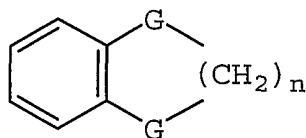
- 51 -

5. The compound of claim 1 wherein Y, substituted or unsubstituted, is selected from the group consisting of XCH_2CH_2 , CH_2XCH_2 , XCH_2X , $XCH_2CH_2CH_2$, $CH_2XCH_2CH_2$, XCH_2CH_2X , XCH_2XCH_2 , $X(CH_2)_4$, $CH_2X(CH_2)_3$, $CH_2CH_2XCH_2CH_2$, $CH_2XCH_2XCH_2$, $XCH_2CH_2XCH_2$, and $XCH_2XCH_2CH_2$, wherein X, independently, is NR^a , S, or O.

6. The compound of claim 1 wherein R^1 is an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine.

7. The compound of claim 1 wherein R^1 is an optionally substituted bicyclic ring selected from the group consisting of naphthalene, indene, benzoxazole, benzothiazole, benzisoxazole, benzimidazole, quinoline, indole, benzothiophene, and benzofuran.

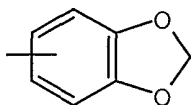
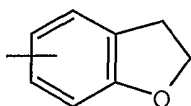
8. The compound of claim 1 wherein R^1 is



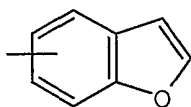
and wherein n is an integer 1 or 2, and G, independently, are $C(R^a)_2$, O, S, or NR^a .

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9. The compound of claim 1 wherein R^1 is selected from the group consisting of substituted or unsubstituted



, and



10. The compound of claim 9 wherein R^1 is substituted with a substituent selected from the group consisting of halogen, C_{1-3} alkyl, OR^a , cyano, nitro, and NR^aR^b .

11. The compound of claim 1 wherein R^2 is selected from the group consisting of trifluoromethyl, C_{1-6} alkyl, $C(=O)R^a$, $C(=O)OR^a$, $C(=O)NR^aR^c$, OR^a , C_{1-4} alkylene NR^aR^b , OC_{2-4} alkylene NR^aR^b , $SO_2NR^aR^b$, and $C(=O)C_{1-4}$ alkylenearyl optionally substituted with one or more OR^a .

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12. The compound of claim 11 wherein R^2 is selected from the group consisting of $CH_2NR^aR^b$, $C(=O)OR^a$, and $C(=O)CH_2$ aryl optionally substituted with one or two OR^a .

13. The compound of claim 11 wherein R^2 is selected from the group consisting of CH_2NH_2 , CO_2H , $C(=O)CH_2C_6H_5$ substituted with one or two OCH_3 .

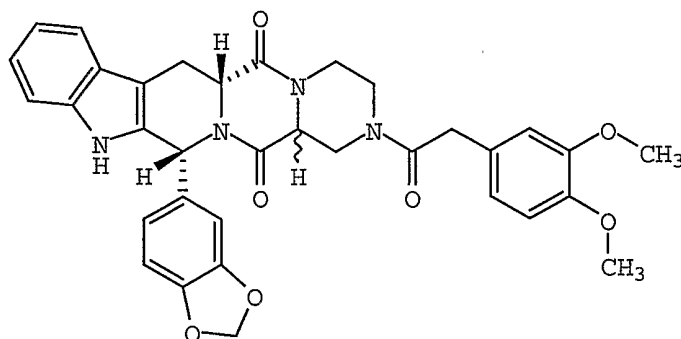
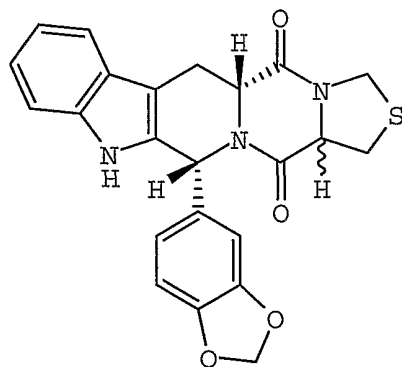
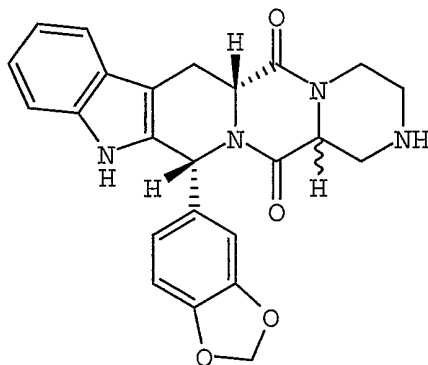
14. The compound of claim 1 wherein Y, substituted and unsubstituted, is selected from the group consisting of CH_2XCH_2 , CH_2CH_2X , and $CH_2CH_2XCH_2$, wherein X is selected from the group consisting of NR^a , S, and O.

15. The compound of claim 14 wherein X is sulfur or nitrogen and R^2 is selected from the group consisting of trifluoromethyl, C_{1-6} alkyl, $C(=O)R^a$, $C(=O)OR^a$, $C(=O)NR^aR^c$, OR^a , C_{1-4} alkylene NR^aR^b , OC_{2-4} alkylene NR^aR^b , $SO_2NR^aR^b$, and $C(=O)C_{1-4}$ alkylenearyl optionally substituted with one or more OR^a .

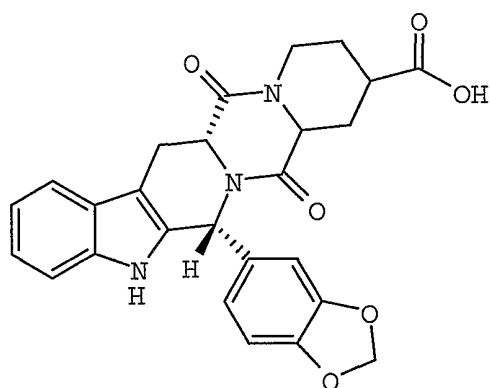
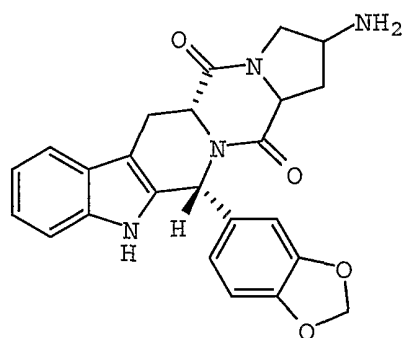
16. The compound of claim 1 wherein Y is a 3- or 4-membered carbon chain, and t is 1 or 2.

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17. A compound selected from the group consisting of



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and pharmaceutically acceptable salts and solvates thereof.

18. A pharmaceutical composition comprising a compound of claim 1, together with a pharmaceutically acceptable diluent or carrier.

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19. A method of treating a male or female animal for a condition where inhibition of a cGMP-specific PDE is of a therapeutic benefit comprising administering to said animal an effective amount of a pharmaceutical composition comprising a compound of claim 1, together with a pharmaceutically acceptable diluent or carrier.

20. The method of claim 19 wherein the condition is male erectile dysfunction.

21. The method of claim 20 wherein the treatment is an oral treatment.

22. The method of claim 19 wherein the condition is female arousal disorder.

23. The method of claim 22 wherein the treatment is an oral treatment.

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24. The method of claim 19 wherein the condition is selected from the group consisting of stable angina, unstable angina, variant angina, hypertension, pulmonary hypertension, chronic obstructive pulmonary disease, malignant hypertension, pheochromocytoma, acute respiratory distress syndrome, congestive heart failure, acute renal failure, chronic renal failure, atherosclerosis, a condition of reduced blood vessel patency, a peripheral vascular disease, a vascular disorder, thrombocythemia, an inflammatory disease, myocardial infarction, stroke, bronchitis, chronic asthma, allergic asthma, allergic rhinitis, glaucoma, peptic ulcer, a gut motility disorder, postpercutaneous transluminal coronary angioplasty, carotid angioplasty, post-bypass surgery graft stenosis, osteoporosis, preterm labor, benign prostatic hypertrophy, and irritable bowel syndrome.

25. A method of treating a condition where inhibition of a cGMP-specific PDE is of therapeutic benefit, in a human or a nonhuman animal body, comprising administering to said body a therapeutically effective amount of a compound of claim 1.

26. A method for the curative or prophylactic treatment of male erectile dysfunction or female arousal disorder, comprising administration of an effective dose of a compound of claim 1, and pharmaceutically acceptable salts and solvates thereof, to an animal.

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27. Use of a compound of claim 1 for the manufacture of a medicament for the curative or prophylactic treatment of a condition where inhibition of a cGMP-specific PDE is of a therapeutic benefit.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/16164

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D471/22 C07D513/22 A61K31/437 A61K31/4375 A61K31/4365
A61P15/10 A61P9/00 A61P19/10 A61P37/00

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)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 97 43287 A (ICOS CORPORATION) 20 November 1997 (1997-11-20) page 1 -page 3, column 2 page 113 -page 114 ---	1-27
Y	WO 97 03985 A (LABORATOIRE GLAXO WELLCOME S.A) 6 February 1997 (1997-02-06) page 1 -page 3, line 18 page 15, line 12 -page 17 ---	1-27
	-/--	

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* 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 document but published on or after the international filing date
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- *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
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- *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.
- *Z* document member of the same patent family

Date of the actual completion of the international search 28 August 2001	Date of mailing of the international search report 03/09/2001
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Kyriakakou, G
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INTERNATIONAL SEARCH REPORT

Int lional Application No
PCT/US 01/16164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	MIGUEL F. BRAÑA ET AL.: "Synthesis and reactivity of beta-Carboline-Hydantoin systems" SYNTHETIC COMMUNICATIONS, vol. 20, no. 12, 1990, pages 1793-1810, XP000196528 page 1794 -page 1795 ---	1-17
A	MIGUEL F. BRAÑA ET AL.: "Synthesis of new Derivatives of beta-Carboline-Hydantoin" J. HETEROCYCLIC CHEM., vol. 27, 1990, pages 703-706, XP000608321 the whole document ---	1-17
A	PILAR DE MIGUEL ET AL.: "Synthesis of Tetrahydroimidazo'1,5-b!-beta-carboline Derivatives with Complex Basic Substituents" J. HETEROCYCLIC CHEM., vol. 31, 1994, pages 1235-1239, XP000608322 the whole document -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No
PCT/US 01/16164

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