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(54) Titre : PROCEDE DE TRAITEMENT DE LA MIGRAINE
(54) Title: METHOD FOR TREATMENT OF MIGRAINE USING PDE5 INHIBITORS

(57) Abrégé/Abstract:
A method of treating migraine using a PDE5 inhibitor, alone or in combination with a second PDE5 inhibitor and/or other antimigraine agents, is disclosed.

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(54) Title: METHOD FOR TREATMENT OF MIGRAINE USING PDE5 INHIBITORS

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METHOD FOR TREATMENT OF MIGRAINE

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of provisional U.S. application 60/229,083, filed August 30, 2000.

FIELD OF THE INVENTION

The present invention relates to a treatment for vascular headaches. In particular, the present invention relates to the use of Type 5 phosphodiesterase (PDE5) inhibitors to treat migraine.

BACKGROUND OF THE INVENTION

Cyclic nucleotide phosphodiesterases (PDEs) that catalyze the hydrolysis of 3'5'-cyclic nucleotides, such as cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), to the corresponding nucleoside 5'-monophosphates constitute a complex family of enzymes. By mediating the intracellular concentration of the cyclic nucleotides, the PDE isoenzymes function in signal transduction pathways involving cyclic nucleotide second messengers.

Multiple families of PDEs have been identified. The nomenclature system includes first a number that indicates the PDE family. To date, nine families (PDE1-9) are known which are classified by: (i) primary structure; (ii) substrate preference;

(iii) response to different modulators; (iv) sensitivity to specific inhibitors; and (v) modes of regulation (Loughney and Ferguson, in *Phosphodiesterase Inhibitors*, Schudt et al. (Eds.), Academic Press: New York, New York (1996) pp. 1-19). The number indicating the family is followed by a capital letter, indicating a distinct gene, and the capital letter followed by a second number, indicating a specific splice variant or a specific transcript that utilizes a unique transcription initiation site.

Members of the PDE1 family are activated by calcium-calmodulin. PDE1A and PDE1B preferentially hydrolyze cGMP, while PDE1C has been shown to exhibit a high affinity for both cAMP and cGMP. The sole member of the PDE2 family, i.e., PDE2A, is characterized as being specifically stimulated by cGMP (Loughney and Ferguson, supra). Enzymes in the PDE3 family, i.e., PDE3A and PDE3B, are specifically inhibited by cGMP. The PDE4 family effects cAMP hydrolysis and includes four genes, PDE4A, PDE4B, PDE4C, and PDE4D. The sole member of the PDE5 family, i.e., PDE5A, binds cGMP at noncatalytic sites and preferentially hydrolyze cGMP. The photoreceptor PDE6 enzymes specifically hydrolyze cGMP (Loughney and Ferguson, supra). Genes include PDE6A, PDE6B, and PDE6C. The PDE7 family effects cAMP hydrolysis but, in contrast to the PDE4 family, is not inhibited by rolipram (Loughney and Ferguson, supra). The PDE8 family has been shown to hydrolyze both cAMP and cGMP and is insensitive to inhibitors specific for PDEs 1-5. Depending on nomenclature used, PDE8 also is referred to as PDE10. The PDE9

family preferentially hydrolyzes cAMP and is not sensitive to inhibition by rolipram, a PDE4-specific inhibitor, or isobutyl methyl xanthine (IBMX), a nonspecific PDE inhibitor.

PDE5 inhibitors vary significantly in chemical structure. Limiting factors on the therapeutic value of various PDE5 inhibitory compounds include ease of administration, potency, and selectivity. It is generally preferred that an inhibitor is effective in low concentrations and can be administered orally. On the other hand, if adverse physiological side effects are to be avoided, it is important that the compound be highly selective in its inhibitory effect on PDE5.

Migraine is a common disease, and is the most common cause of severe, recurring headache. In any given year, about 15 to 20% of women and about 7 to 10% of men experience at least one migraine attack.

Migraine headaches can be subdivided into classic and common types. Eighty percent of all migraine headaches are the common type. Classic migraine headaches occur in three stages. The symptoms are related to an initial vasoconstriction and subsequent vasodilation of the blood vessels of the head. The initial vasoconstriction, called the prodrome, is quickly followed by vasodilation. A common migraine headache does not include a prodromal phase.

The first stage of a classic migraine headache consists chiefly of visual disturbances, including blurred or cloudy vision, scotomas, and/or flashes of light. Vertigo, chills, tremors, unilat-

eral numbness, aphasia, photophobia, or pallor also can occur. In the second stage, the patient experiences a severe, throbbing headache, which initially is unilateral. Nausea, vomiting, diarrhea, chills, tremors, and perspiration also can occur at this time. The third stage is a recovery phase. The pain decreases markedly, but the head is tender and exhaustion is present. A common migraine headache includes no prodromal stage (stage 1), but the actual headache can last longer (more than two hours) than a classic migraine.

The once widely held opinion that migraine is mainly a psychosomatic condition has been discredited. The actual attack has been attributed instead to a primarily neurogenic phenomenon with secondary consequences for the blood vessels of the brain and meninges. Migraine is intermittent by nature, and attack frequency can vary from one period to another. The symptoms and severity vary both between and within individuals from occasional mild attacks to frequent severe attacks. Migraine appears to be a hereditary disease.

Theories regarding pathophysiology of migraine have been dominated by the work of Graham et al., and Wolff, *Arch. Neurol. Psychiatry, 39,* pages 737-763 (1938). The authors proposed that the cause of migraine headache is vasodilation of extracranial vessels. This view is supported by knowledge that ergot alkaloids and sumatriptan, a 5-HT agonist, contract cephalic vascular smooth muscle and are effective in the treatment of migraine.

While this vascular mechanism for migraine has gained wide acceptance, total agreement as to

its validity does not exist. For example, Moskowitz et al., *Cephalalgia, 12*, pages 5-7 (1992), discloses that the occurrence of migraine headaches is independent of changes in vessel diameter. Furthermore, Moskowitz et al. proposed that a release of vaso-active neuropeptides from axons on the vasculature initiates a series of events leading to neurogenic inflammation, one consequence of which is pain. This neurogenic inflammation is blocked in humans by sumatriptan and ergot alkaloids at a dose similar to that required to treat acute migraine in humans.

A major obstacle in migraine therapy is a lack of clear guidelines for disease management. A limited number of effective antimigraine drugs have been identified, but their efficacy in a given individual is hard to predict accurately. Thus, migraine therapies often are individualized for each patient.

Treatment for migraine includes avoidance of factors that can trigger an attack, treatment of the acute headache, and the use of regular medication to prevent attacks. Many factors have been associated as triggers of individual migraine attacks: fasting, alcohol, oral contraceptives and hormone replacement therapy, caffeine and caffeine withdrawal, stress or release from stress, too little or too much sleep, menstruation, fatigue, change in weather, head trauma, exposure to bright lights, loud noises, smoke, strong scents, and foods, including chocolate, aged cheeses, cured and processed meats that contain nitrites, dairy products, foods containing monosodium glutamate or aspartame, citrus fruits, and others.

In general, however, identifying and avoiding migraine triggers is not an effective treatment plan. First, most migraine headaches are not caused by an identifiable trigger. Second, a patient's response to a trigger varies, e.g., fasting may or may not trigger a migraine, which decreases the patient's will to avoid all recognized triggers. Third, many migraine triggers are unavoidable.

A wide variety of drugs are available for both prevention of migraine and treatment of an acute attack. Preventative drugs currently available include β-blockers, such as propranolol, metoprolol, atenolol, timolol, and nadolol; calcium channel blockers, such as verapamil, nifedipine, nimopidine, and diltiazem; tricyclic antidepressants, such as amitriptyline and nortriptyline; anticonvulsants, such as divalproex sodium; 5-HT agonists, such as sumatriptan, naratriptan, rizatriptan, zolmitriptan, and pizotifen; and a monoamine oxidase inhibitor, such as phenelzine and isocarboxazid.

The adverse side effects of these drugs are varied. Adverse effects attributed to treatment with β-blockers include aggravation of asthma, brachycardia, hypotension, fatigue, depression, and masking of the symptoms of hypoglycemia. Calcium channel blockers can cause hypotension, constipation, and peripheral edema. Tricyclic antidepressants can cause sedation, dry mouth, weight gain, tremor, cardiac arrhythmias, aggravation of angle-closure glaucoma, and difficulty in urinating. Divalproex sodium can cause nausea, fatigue, weight

gain, hair loss, tremor, liver dysfunction, and neural tube defects in developing embryos. The 5-HT agonists can cause chest and neck pressure, and myocardial ischemia.

For most patients, early treatment of the migraine attack is the mainstay of therapy. Prompt treatment is intended to decrease the severity of pain, prevent or reduce associated symptoms such as nausea and vomiting, and shorten attack duration. Available drugs include over the counter analgesics, such as acetaminophen, aspirin, ibuprofen, indomethicin, and naproxen sodium; combinations of over the counter analgesics with caffeine; ergot alkaloids, like ergotamine and dihydroergotamine; and prescription analgesic/antimigraine preparations, including narcotics (e.g., butalbitol). Side effects of these drugs range from gastrointestinal upset and rebound headaches to liver toxicity, sedation, and dependence.

The efficacy of preventive treatments for migraine, therefore, needs improvement. For example, it has been reported that only about 55% of treated patients have a 50% or more reduction in the frequency of attacks. Furthermore, the above-described adverse side effects occur frequently, which makes these preventative treatments appropriate only for patients experiencing regular attacks.

In summary, although a wide variety of drugs are currently available for the treatment and prevention of migraine, additional compounds having improved efficacy and fewer adverse side effects are needed in the art.

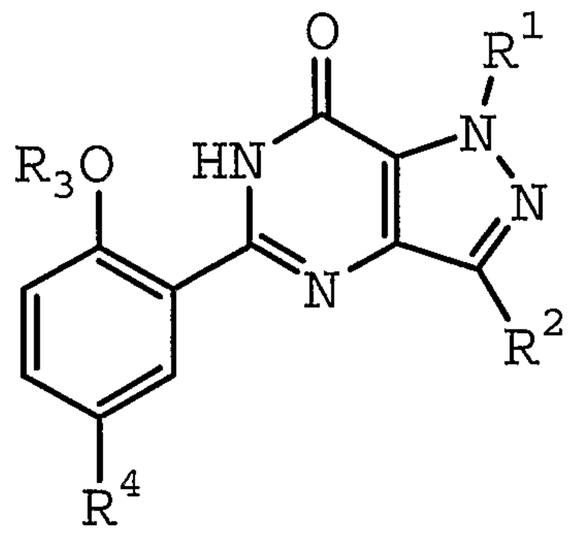
SUMMARY OF THE INVENTION

The present invention is directed to a method of treating migraine, both classic and common, and other vascular headaches in mammals. The method comprises administering a pharmaceutically effective amount of an agent that inhibits cyclic guanosine 3'5'-monophosphate specific PDE5, i.e., a PDE5 inhibitor, to an individual that suffers from migraine headaches. The PDE5 inhibitor can be administered prior to or after the onset of the migraine. The ability of a PDE5 inhibitor to treat migraine is unexpected in the art because a typical side effect of treatment with a PDE inhibitor, including PDE5 inhibitors, is headache.

In a preferred embodiment, the amount of PDE5 inhibitor administered to the individual is about 0.1 to about 1000 mg daily. More preferably, the amount of PDE5 inhibitor administered is about 1 to about 100 mg daily. Most preferably, the amount of PDE5 inhibitor administered is about 2 to about 20 mg daily.

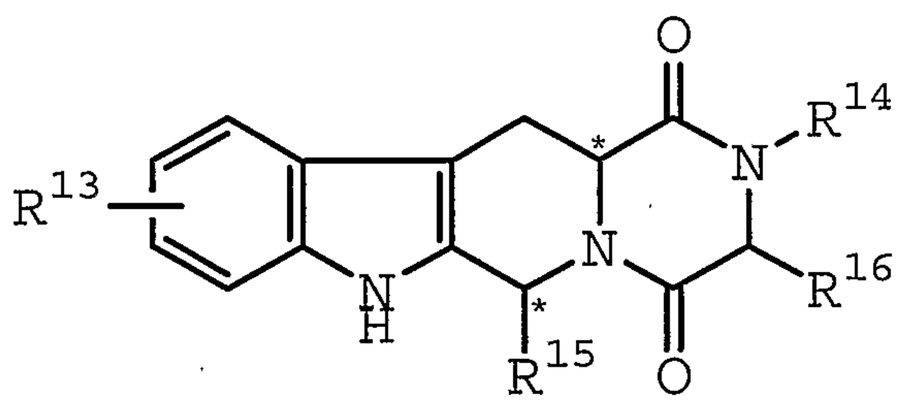
According to another aspect of the present invention, the method comprises administering an effective amount of a first PDE5 inhibitor in combination with a second PDE5 inhibitor or with another known antimigraine agent, preferably an analgesic.

Another aspect of the present invention is to administer a pharmaceutically effective amount of a compound having a structure (I), (II), or (III) to an individual to treat a migraine or other vascular headaches



(I) ,

wherein R^1 is methyl or ethyl; R^2 is n-propyl; R^3 is ethyl, n-propyl, or allyl; R^4 is $COCH_2NR^5R^6$, $CONR^5R^6$, $SO_2NR^9R^{10}$, or 1-methyl-2-imidazolyl; R^5 and R^6 together represent, with the nitrogen atom to which they are attached, a morpholino or 4-N(R^{11})-piperazinyl group; R^9 and R^{10} together represent, with the nitrogen atom to which they are attached, a 4-N(R^{12})-piperazinyl group; R^{11} is methyl or acetyl; and R^{12} is hydrogen, methyl, 2-propyl, or 2-hydroxyethyl;

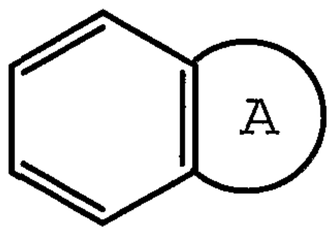


(II) ,

wherein R^{13} is selected from the group consisting of hydrogen, halogen, and C_{1-6}alkyl;

R^{14} is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halo-C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{3-8}cycloalkylC_{1-3}alkyl, and arylC_{1-3}alkyl, wherein aryl is phenyl or phenyl substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, C_{1-6}alkoxy, and methylenedioxy, and heteroarylC_{1-3}alkyl, wherein heteroaryl is thienyl, furyl, or pyridyl, each optionally substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, and C_{1-6}alkoxy.

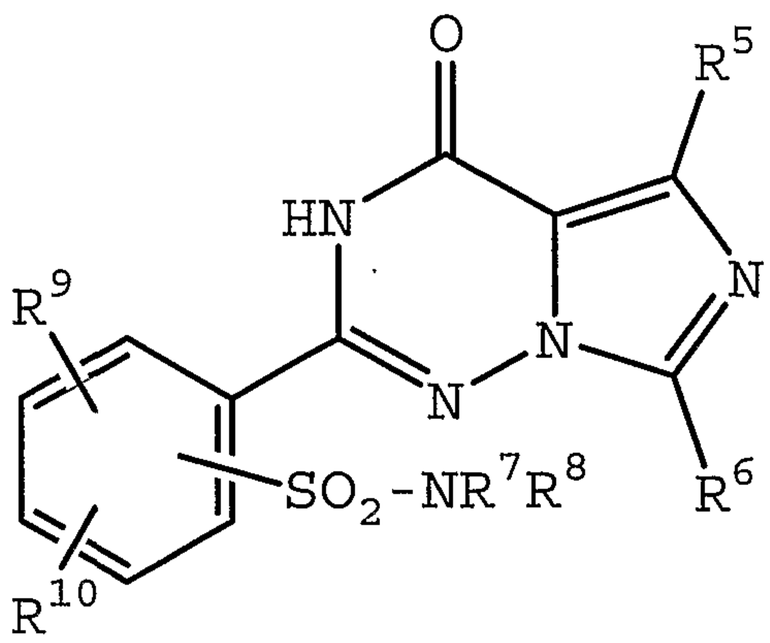
R^{15} represents an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine, or an optionally substituted bicyclic ring



attached to the rest of the molecule via one of the benzene ring carbon atoms and wherein the fused ring A 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, sulphur, and nitrogen; and

R^{16} represents hydrogen or C_{1-3}alkyl, or R^1 and R^3 together represent a 3- or 4-membered alkyl or alkenyl chain component of a 5- or 6-membered ring;

and salts and solvates thereof,



(III)

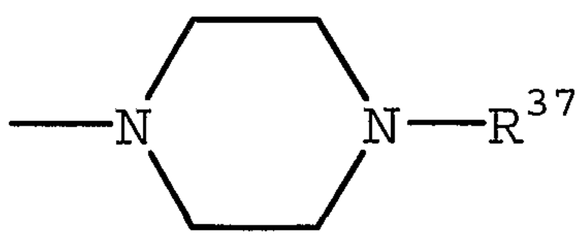
wherein

R^5 is methyl or ethyl,

R^6 is ethyl or propyl,

R^7 and R^8, independently, are a straight-chain or branched alkyl chain having up to five carbon atoms, optionally substituted, independently, with up to two hydroxy or methoxy, or

R^7 and R^8 together with the nitrogen form piperidinyl, morpholinyl, thiomorpholinyl, or a residue of the formula



wherein R^{37} is hydrogen, formyl, or straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms,

or is a straight-chain or branched alkyl having up to three carbon atoms, optionally substituted, independently, with one or two hydroxy,

carboxyl, or straight-chain or branched alkoxy or alkoxycarbonyl, each having up to three carbon atoms, or by groups of the formula $-(D)_fNR^{38}R^{39}$ or $-P(O)(OR^{42})(OR^{43})$,

wherein f is 0 or 1,

D is a group of the formula -CO,

R^{38} and R^{39}, independently, are hydrogen or methyl,

R^{42} and R^{43}, independently are hydrogen, methyl, or ethyl, or

R^{37} is cyclopentyl,

and the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted, independently, with one or two, optionally geminally, hydroxy, formyl, carboxyl, and straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms, or a group of the formula $-P(O)(OR^{46})(OR^{47})$ or $-(CO)_jNR^{49}R^{50}$,

wherein R^{46} and R^{47}, independently, are hydrogen, methyl, or ethyl,

j is 0 or 1, and

R^{49} and R^{50}, independently, are hydrogen or methyl and/or the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted by straight-chain or branched with up to three carbon atoms, which is optionally substituted, independently, with one or two hydroxy, carboxyl, or a residue of the formula $P(O)OR^{53}OR^{54}$,

wherein R^{53} and R^{54}, independently, are hydrogen, methyl, or ethyl, and/or the heterocycles mentioned under R^3 and R^4 that are formed together

with the nitrogen atom are optionally substituted via N-linked piperidinyl or pyrrolidinyl,

R^9 is hydrogen, and

R^{10} is ethoxy or propoxy, and salts, hydrates, N-oxides, and isometric forms thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method for treating migraine and other vascular headaches in mammals. The method comprises administering a pharmaceutically effective amount of an agent that inhibits cyclic guanosine 3'5'-monophosphate specific PDE5 to an individual in need thereof. It is contemplated that a PDE5 inhibitor can be administered alone, in combination with a second PDE5 inhibitor, or in combination with other known antimigraine treatments.

As used herein, "treating" is defined as reducing or eliminating a clinical symptom of migraine and/or increasing the time between migraine attacks and/or preventing the recurrence of migraine. A "clinical symptom" of migraine includes headache, nausea, vomiting, extreme sensitivity to light and/or sound, vertigo, chills, tremors, pallor, aphasia, unilateral numbness, diarrhea, and visual disturbances.

The term "IC_{50}" is defined as the concentration of a compound that results in 50% enzyme inhibition, in a single dose response experiment. The IC_{50} value therefore is a measure of the potency of a compound to inhibit PDEs, including PDE5. Determining the IC_{50} value of a compound is readily

carried out by a known *in vitro* methodology generally described in Cheng et al., *Biochem. Pharmacology, 22*, pages 3099-3108 (1973).

The term "inhibiting" or "inhibits" refers to blocking the enzymatic activity of PDE5 to a sufficient degree to reduce or eliminate a clinical symptom of migraine and/or increase the time between migraine attacks, or to prevent the recurrence of a clinical symptom of migraine.

The term "a pharmaceutically effective amount" represents an amount of a compound that is capable of inhibiting PDE5, and causes an improvement in a clinical symptom of migraine and/or prevents or reduces recurrence of migraine or a clinical symptom thereof.

The term "agent" or "drug" refers to a chemical compound suitable for pharmaceutical use.

The term "PDE5 inhibitor" refers to an agent that inhibits cGMP-specific PDE5 activity. A PDE5 inhibitor useful in the present invention is an agent that inhibits cyclic guanosine 3'5'-monophosphate specific PDE5 and has an IC_{50} value against human recombinant PDE5 of about 10 nM or less. Preferably, the IC_{50} value of the PDE5 inhibitor is about 5 nM or less, more preferably about 3 nM or less, and most preferably about 1 nM or less. Most preferred PDE5 inhibitors are selective PDE5 inhibitors, i.e., compounds that inhibit PDE5, but do not significantly inhibit other PDE enzymes, i.e., PDE1 through PDE4 and PDE6 through PDE9, and particularly PDE6 and PDE1c. Therefore, for preferred inhibitors, the IC_{50} value for PDE5 inhibition is about 100 times less than the IC_{50} value for PDE1-PDE4 and

PDE6-PDE9, particularly, PDE6 or PDE1c inhibition, more preferably about 500 times less, and most preferably about 1000 times less.

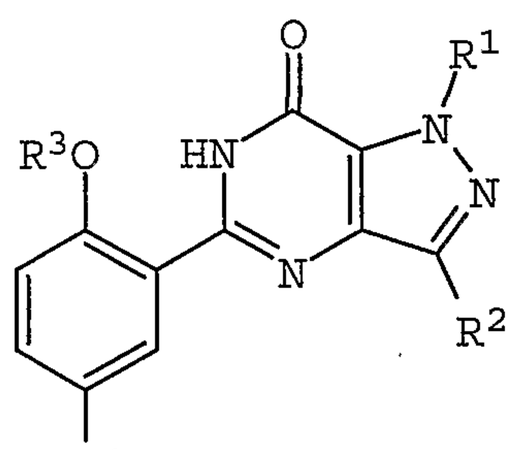
PDE5 inhibitors can be administered systemically, e.g., by oral, intravenous, intramuscular, or subcutaneous routes. The PDE5 inhibitor can be administered as an aerosol for pulmonary administration, as a spray for nasal administration, administered intraventricularly or intrathecally into the cerebrospinal fluid, or administered intravenously via continuous infusion pump. The PDE inhibitor also can be administered topically, e.g., via drops (particularly ophthalmic drops), ointment, patch, or per rectum or vagina, for example, by suppositories or enemas. For combination treatments, a first PDE5 inhibitor and a second PDE5 inhibitor and/or another antimigraine treatment can be administered simultaneously or sequentially.

PDE5 inhibitors can be administered at doses of about 0.1 to about 1000 mg, preferably about 1 to about 100 mg daily, and most preferably about 2 to about 20 mg, over a 24-hour period. The dosage can be administered as needed, once daily, or in equivalent doses at longer or shorter intervals. Those of ordinary skill in the art can readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient.

For example, appropriate dosages can be ascertained through use of established assays for determining blood level dosages in conjunction with appropriate dose-response data. The final dosage regimen is determined by the attending physician,

considering various factors, for example, the specific activity of the drug, the severity of the condition and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of the clinical symptoms, time of administration, and other clinical factors. When used in combination, the doses of each agent required to exert a therapeutic effect may be less than the dose necessary when a single PDE5 inhibitor is used alone.

The invention is based on the discovery that inhibition of PDE5 in a patient suffering from migraine provides effective therapy. Accordingly, PDE5 inhibitors useful in the present invention vary significantly in chemical structure and the use of a PDE5 inhibitor in the present method is not dependent on a particular chemical structure. However, preferred compounds having the ability to inhibit PDE5 include compounds having a structural formula (I):



(I)

wherein R^1 is methyl or ethyl; R^2 is n-propyl; R^3 is ethyl, n-propyl, or allyl; R^4 is $COCH_2NR^5R^6$, $CONR^5R^6$, $SO_2NR^9R^{10}$, or 1-methyl-2-imidazol-

yl; R^5 and R^6 together represent, with the nitrogen atom to which they are attached, a morpholino or 4-N(R^{11})-piperazinyl group; R^9 and R^{10} together with the nitrogen atom to which they are attached represent a 4-N(R^{12})-piperazinyl group; R^{11} is methyl or acetyl; and R^{12} is hydrogen, methyl, 2-propyl, or 2-hydroxy-ethyl.

Compounds of structural formula (I), and their preparation, are disclosed in EP 0 702 555, the disclosure of which is incorporated herein by reference. Preferred compounds of formula (I) include:

5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-(5-morpholinoacetyl-2-n-propoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-(2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)-phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo-[4,3-d]pyrimidin-7-one (sildenafil);

5-(2-alkyloxy-5-(4-methyl-1-piperazinylsulphonyl)-phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo-(4,3-d]pyrimidin-7-one;

5-(2-ethoxy-5-[4-(2-propyl)-1-piperazinylsulphonyl]-phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo-[4,3-d]pyrimidin-7-one;

5-(2-ethoxy-5-[4-(2-hydroxyethyl)-1-piperazinylsul-phonyl]phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

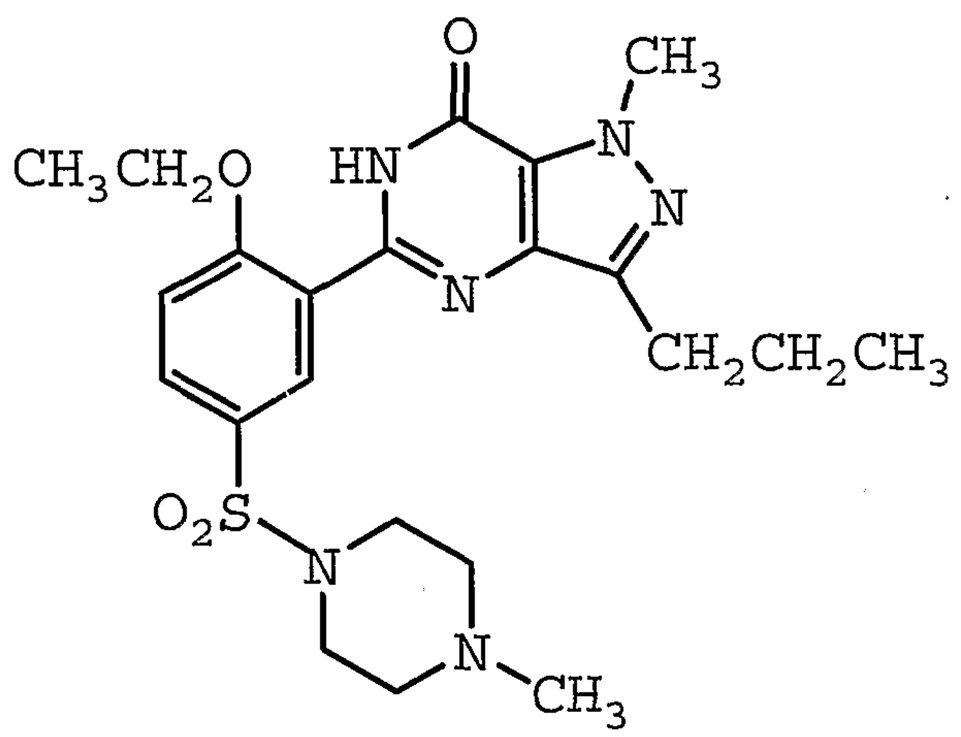
5-(5-[4-(2-hydroxyethyl)-1-piperazinylsulphonyl]-2-n-propoxyphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-(2-ethoxy-5-(4-methyl-1-piperazinylcarbonyl)-phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo-[4,3-d]pyrimidin-7-one;

5-(2-ethoxy-5-(1-methyl-2-imidazolyl)phenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]-pyrimidin-7-one;

and mixtures thereof.

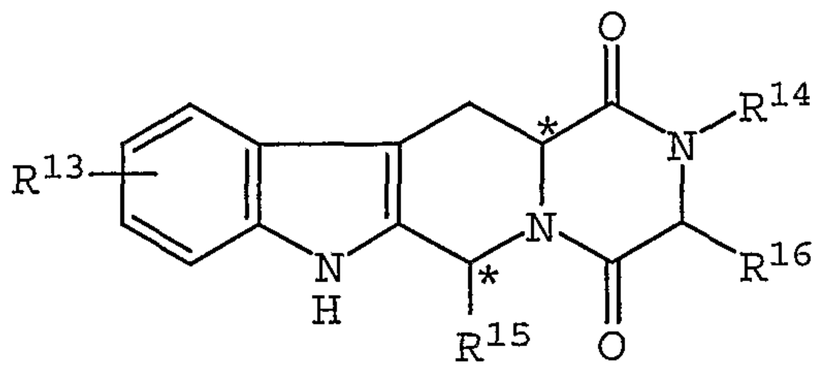
An especially preferred compound of structural formula (I) is sildenafil having the following structural formula:



(Ia)

(sildenafil)

Another class of preferred compounds is disclosed in Daugan U.S. Patent No. 5,859,006 and Daugan et al. U.S. Patent No. 5,981,527, each of which is incorporated herein by reference. This class of compounds, which contains potent and selective PDE5 inhibitors, is useful in treating migraine and has the following structural formula (II):



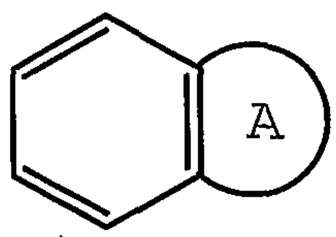
(II)

and salts or solvates thereof,

wherein R^{13} is selected from the group consisting of hydrogen, halogen, and C_{1-6}alkyl;

R^{14} is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, haloC_{1-6}alkyl, C_{3-8}cycloalkyl, C_{3-8}cycloalkylC_{1-3}alkyl, and arylC_{1-3}alkyl, wherein aryl is phenyl or phenyl substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, C_{1-6}alkoxy, and methylenedioxy, and heteroarylC_{1-3}alkyl, wherein heteroaryl is thienyl, furyl, or pyridyl, each optionally substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, and C_{1-6}alkoxy;

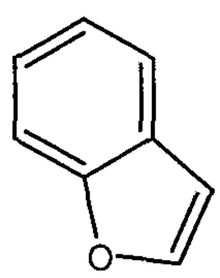
R^{15} represents an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine, or an optionally substituted bicyclic ring



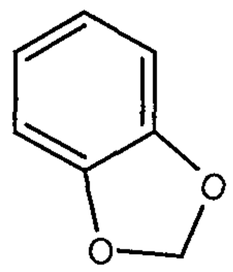
attached to the rest of the molecule via one of the benzene ring carbon atoms, wherein the fused ring A is a 5- or 6-membered ring, saturated or partially or fully unsaturated, and comprises carbon atoms and optionally one or two heteroatoms selected from oxygen, sulphur and nitrogen; and

R^{16} represents hydrogen or C_{1-3}alkyl, or R^{14} and R^{16} together represent a 3- or 4-membered alkyl or alkenyl chain component of a 5- or 6-membered ring.

Preferred compounds of structural formula (II) are those wherein R^{13} is hydrogen, halogen, or C_{1-6}alkyl; R^{14} is hydrogen or C_{1-6}alkyl; R^{15} is the bicyclic ring



or



which can be optionally substituted by one or more groups independently selected from halogen and C_{1-3}alkyl; and R^{16} is hydrogen or C_{1-3}alkyl.

Preferred compounds of structural formula (II) include:

Cis-2,3,6,7,12,12a-hexahydro-2-(4-pyridylmethyl)-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]pyrido-[3,4-b]indole-1,4-dione;

Cis-2,3,6,7,12,12a-hexahydro-6-(2,3-dihydrobenzo[b]-furan-5-yl)-2-methylpyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

Cis-2,3,6,7,12,12a-hexahydro-6-(5-bromo-2-thienyl)-2-methylpyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

Cis-2,3,6,7,12,12a-hexahydro-2-butyl-6-(4-methyl-phenyl)pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-isopropyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-cyclopentyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-cyclopropyl-methyl-6-(4-methoxyphenyl)pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(3-chloro-4-methoxyphenyl)-2-methylpyrazino[2',1':6,1]pyrido-[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(3,4-methyl-enedioxyphenyl)pyrazino[2',1':6,1]pyrido[3,4-b]-indole-1,4-dione;

(5aR,12R,14aS)-1,2,3,5,6,11,12,14a-octahydro-12-(3,4-methylenedioxyphenyl)pyrrolo[1",2":4',5']-pyrazino[2',1':6,1]pyrido[3,4-b]indole-5-1,4-dione;

Cis-2,3,6,7,12,12a-hexahydro-2-cyclopropyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(3S,6R,12aR)-2,3,6,7,12,12a-hexahydro-3-methyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzo-furanyl)-2-methylpyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzo-furanyl)pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(3S,6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzo-furanyl)-3-methylpyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(3S,6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzo-furanyl)-2,3-dimethylpyrazino[2'1':6,1]pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzo-furanyl)-2-isopropyl-pyrazino[2',1':6,1]pyrido [3,4-b]indole-1,4-dione;

physiologically acceptable salts and solvates thereof;

and mixtures thereof. The compounds of structural formula (II) are particularly advantageous due to their selectivity in inhibiting PDE5 over other PDE enzymes.

Three preferred compounds of structural formula (II) are:

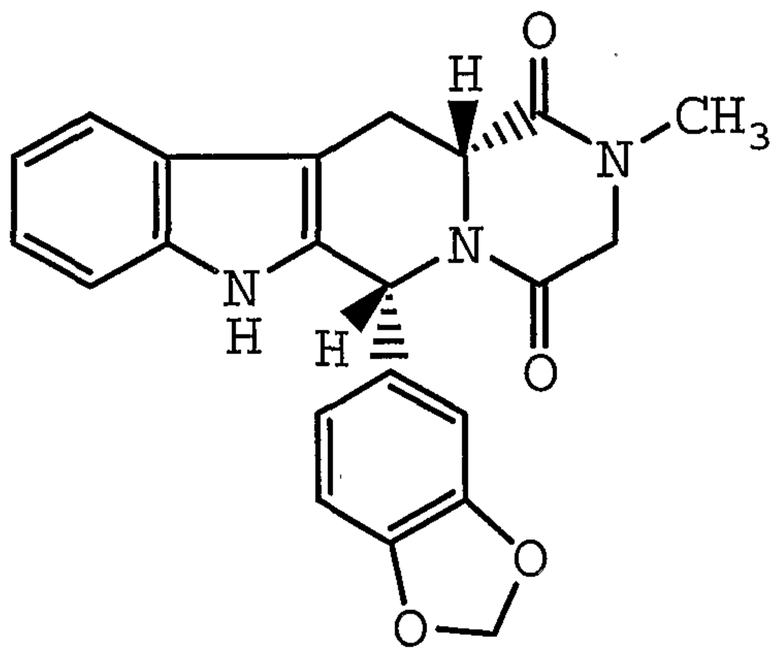
(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-6-(5-benzofuranyl)-2-methylpyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione; and

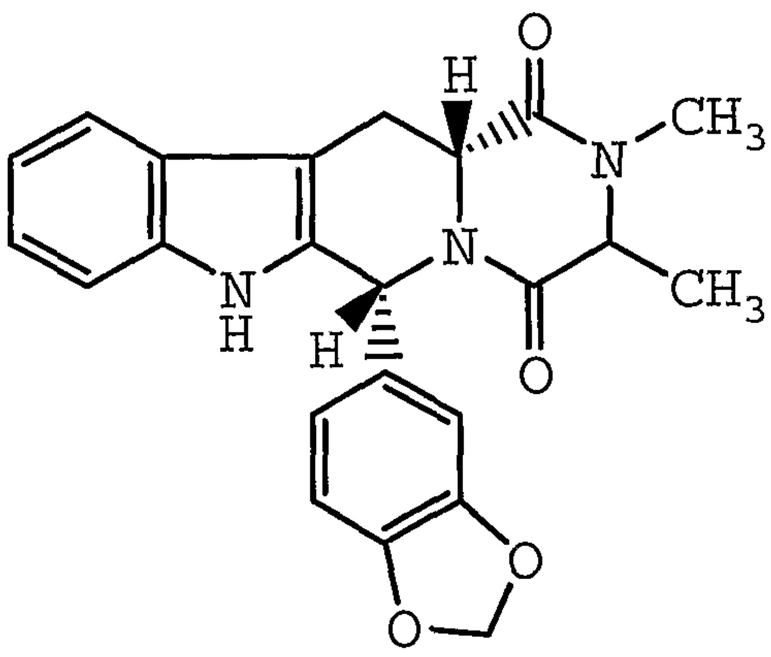
(3S,6R,12aR)-2,3,6,7,12,12a-hexahydro-2,3-dimethyl-6-(3,4-methylenedioxyphenyl)pyrazino[2',1':6,1]-pyrido[3,4-b]indole-1,4-dione;

and physiologically acceptable salts and solvates (e.g., hydrates) thereof.

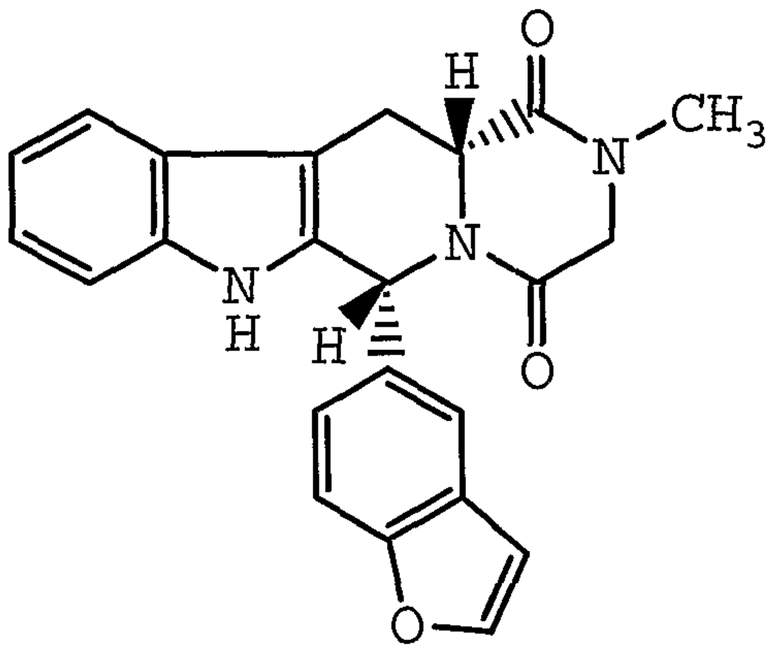
These especially preferred compounds of structural formula (II) have the following structural formulae:



(IIa)

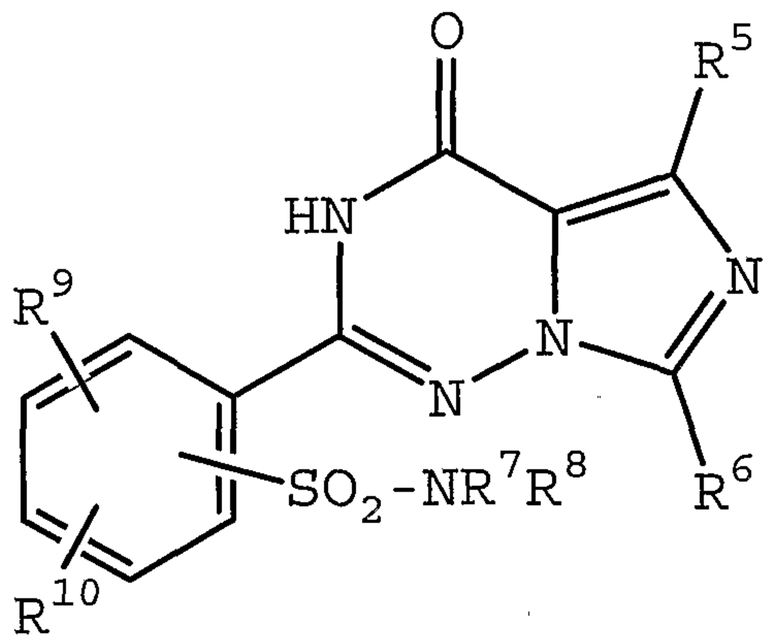


(IIb)



(IIc)

Yet another class of preferred compounds is disclosed in WO 99/24433, incorporated herein by reference. The compounds have a structural formula (III):



(III) ,

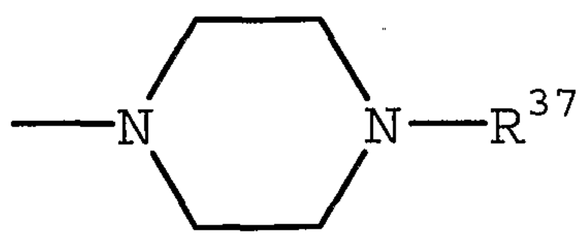
wherein

R^5 is methyl or ethyl,

R^6 is ethyl or propyl,

R^7 and R^8, independently, are a straight-chain or branched alkyl chain having up to five carbon atoms, optionally substituted, independently, with up to two hydroxy or methoxy, or

R^7 and R^8 together with the nitrogen form piperidinyl, morpholinyl, thiomorpholinyl, or a residue of the formula



wherein R^{37} is hydrogen, formyl, or straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms,

or is a straight-chain or branched alkyl having up to three carbon atoms, optionally sub-

stituted, independently, with one or two hydroxy, carboxyl, or straight-chain or branched alkoxy or alkoxycarbonyl, each having up to three carbon atoms, or by groups of the formula $-(D)_fNR^{38}R^{39}$ or $-P(O)(OR^{42})(OR^{43})$,

wherein f is 0 or 1,

D is a group of the formula -CO,

R^{38} and R^{39}, independently, are hydrogen or methyl,

R^{42} and R^{43}, independently are hydrogen, methyl, or ethyl, or

R^{37} is cyclopentyl,

and the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted, independently, with one or two, optionally geminally, hydroxy, formyl, carboxyl, and straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms, or a group of the formula $-P(O)(OR^{46})(OR^{47})$ or $-(CO)_jNR^{49}R^{50}$,

wherein R^{46} and R^{47}, independently, are hydrogen, methyl, or ethyl,

j is 0 or 1, and

R^{49} and R^{50}, independently, are hydrogen or methyl and/or the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted by straight-chain or branched with up to three carbon atoms, which is optionally substituted, independently, with one or two hydroxy, carboxyl, or a residue of the formula $P(O)OR^{53}OR^{54}$,

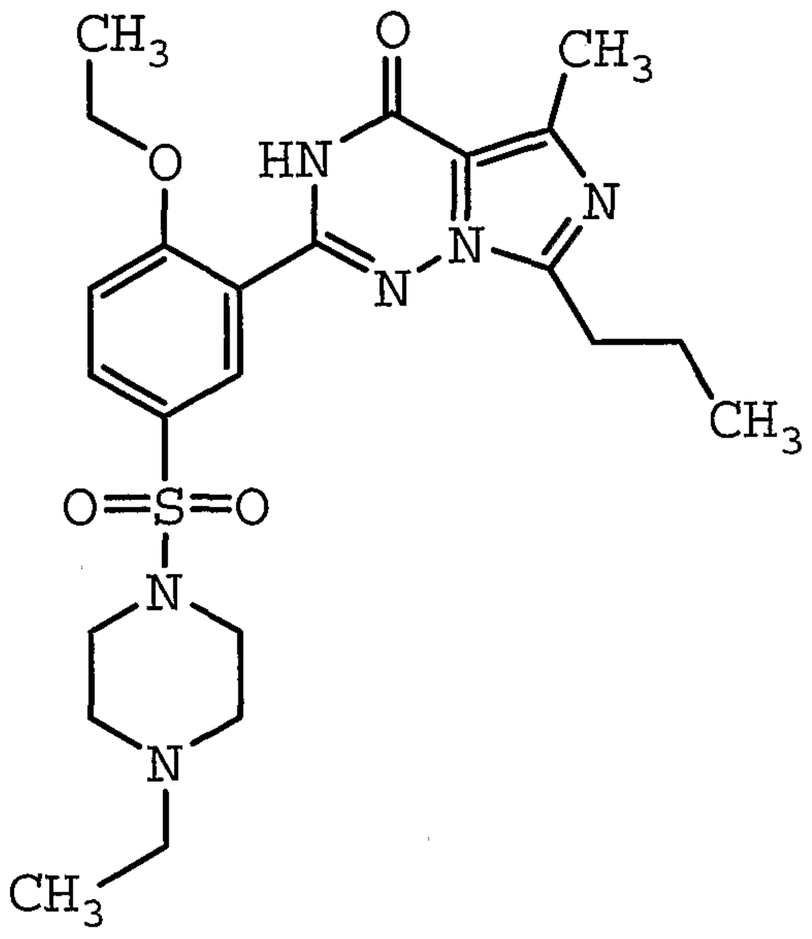
wherein R^{53} and R^{54}, independently, are hydrogen, methyl, or ethyl, and/or the heterocycles

mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted via N-linked piperidinyl or pyrrolidinyl,

R^9 is hydrogen, and

R^{10} is ethoxy or propoxy, and salts, hydrates, N-oxides, and isometric forms thereof.

A preferred compound of structural formula (III) is 2-[2-ethoxy-5-(4-ethylpiperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one, also known as 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-triazin-2-yl)-4-ethoxyphenyl]sulphonyl]-4-ethylpiperazine and vardenafil, having a formula:



(IIIa)

(vardenafil)

Still other exemplary PDE5 inhibitors useful in the present method are those disclosed in Daugan et al. U.S. Patent No. 6,001,847; WO 93/07124, WO 93/07149, WO 93/12095, WO 94/05661,

WO 94/00453, WO 96/26940, WO 97/43287, WO 98/49166, WO 98/53819, WO 99/21831, WO 99/26946, WO 99/28319, WO 99/28325, WO 99/42452, WO 99/54284, WO 99/54333, WO 00/15639, WO 00/27745, EP 0 463 756, EP 0 526 004, and EP 0 995 750, each incorporated herein by reference.

Examples of other PDE5 inhibitors useful in the treatment of migraine headache include, but are not limited to:

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidilin-7-one;

(+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl)-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 3-ethyl-5-(5-[4-ethylpiperazin-1-ylsulphonyl]2-([(1R)-2-methoxy-1-methylethyl]oxy)pyridin-3-yl)-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 1-(6-ethoxy-5-[3-ethyl-6,8-dihydro-2-(2-methoxyethyl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-5-yl]3-pyridylsulphonyl)-4-ethylpiperazine;

5-[2-*iso*-butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl)-3-ethyl-2-(1-

methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl)-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2H)pyridazinone;

1-[4-[(1,3-benzodioxol-5-ylmethyl)amiono]-6-chloro-2-quinozolinyl]-4-piperidine-carboxylic acid, monosodium salt;

(+)-cis-5,6a,7,9,9,9a-hexahydro-2-[4-trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-4,5]imidazo[2,1-b]purin-4(3H)one furazlocillin;

cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a-octahydrocyclopent[4,5]-imidazo[2,1-b]purin-4-one;

3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate;

4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl)propoxy)-3-(2H)pyridazinone;

1-methyl-5-(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro-7H-pyrazole(4,3-d)pyrimidin-7-one;

1-[4-[(1,3-benzodioxol-5-ylmethyl)amino]-6-chloro-2-quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt;

E-8010 and E-4010 (Eisai); and

Bay-38-3045 and 38-9456 (Bayer).

The most preferred PDE5 inhibitors for use in the present invention are (a) potent, i.e., have an IC_{50} value vs. PDE5 less than 10 nM, (b) selective, i.e., have a IC_{50} against human recombinant PDE5 at least 100-fold less than PDE6 or PDE1c, and (c) possess desirable physical and biological prop-

erties, e.g., a sufficient water solubility, bioavailability, and metabolic stability, for therapeutic use in the treatment of migraine and other vascular headaches. The ability of a PDE5 inhibitor to treat migraine is unexpected because PDE inhibitors are known cause headaches as an adverse side effect. For example, 16% of the patients treated with sildenafil reported headache as an adverse side effect (vs. 4% for a placebo).

With respect to selectivity, a preferred PDE5 inhibitor exhibits a PDE6/PDE5 and a PDE1c/PDE5 IC_{50} inhibition quotient of at least 200, and can range to 1,000 or greater. The PDE6/PDE5 IC_{50} inhibition quotient is the ratio of the IC_{50} value of a compound vs. PDE6 to the IC_{50} value of the same compound vs. PDE5. The PDE1c/PDE5 inhibition quotient is identically defined for PDE1c and PDE5. To achieve the full advantage of the present invention, the compound has a PDE6/PDE5 and a PDE1c/PDE5 IC_{50} inhibition quotient of at least 100 and an IC_{50} for human recombinant PDE5 of about 5 nM or less, e.g., about 0.1 to about 5 nM.

While it is possible to administer a compound employed in the methods of this invention directly without any formulation, the PDE5 inhibitors typically are administered in the form of pharmaceutical compositions comprising a pharmaceutically acceptable excipient and at least one active ingredient. These compositions can be administered by a variety of routes including oral, buccal, sublingual, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Many of the compounds employed in the methods of

this invention are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

In making a composition employed in the present invention, the PDE5 inhibitor usually is mixed with an excipient, diluted by an excipient, or enclosed within a carrier, which can be in the form of a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier, or medium for the PDE5 inhibitor. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the PDE5 inhibitor, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, the PDE5 inhibitor can be milled to provide the appropriate particle size prior to combining with the other ingredients. If the PDE5 inhibitor is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the PDE5 inhibitor is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g., about 40 mesh.

The compositions used in the invention can be formulated to provide a rapid, sustained, or delayed release of the PDE5 inhibitor after adminis-

tration to the patient by employing procedures known in the art.

The compositions preferably are formulated in a unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of PDE5 inhibitor, and an optional second active agent, calculated to produce the desired therapeutic effect, and, typically, a suitable pharmaceutical excipient. The PDE5 inhibitors generally are effective over a wide dosage range. However, it will be understood that the amount of the PDE5 inhibitor and optional second active agent actually administered is determined by a physician, in the light of the relevant circumstances.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following nonlimiting embodiments thereof.

EXAMPLE 1

Human PDE5 Preparation

Recombinant production of human PDE5 was carried out essentially as 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 V. Price et al., *Methods in Enzymology*, 185, pages 308-318 (1990) incorporated yeast ADH2 promoter and terminator sequences rather than ADH1 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 YEP/3% glycerol. Approximately 24 hours later, cells were harvested, washed, and stored at -70°C.

Cell pellets (29 g) were thawed on ice with an equal volume of lysis buffer (25 mM Tris-Cl, pH 8, 5 mM $MgCl_2$, 0.25 mM dithiothreitol, 1 mM benzamidine, and 10 μM $ZnSO_4$). Cells were lysed in a microfluidizer with N_2 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 $MgCl_2$, 0.25 mM dithiothreitol, 10 μM $ZnSO_4$) 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 hydroxylapatite column in Buffer B (20 mM Bis-Tris Propane (pH 6.8), 1 mM $MgCl_2$, 0.25 mM dithiothreitol, 10 μM $ZnSO_4$, 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

dithiothreitol, and 10 μM $ZnSO_4$). The pool was applied to a 140 ml column of Sephacryl S-300 HR and eluted with Buffer C. Active fractions were diluted to 50% glycerol and stored at -20°C. The resultant preparations were about 85% pure by SDS-PAGE.

EXAMPLE 2

Assay for PDE5 Activity

Activity of PDE5 can be measured by standard assays in the art. For example, specific activity of any PDE can be determined as follows. PDE assays utilizing a charcoal separation technique are performed essentially as described in Loughney, et al., *J. Biol. Chem.*, *271*, pages 796-806 (1996). In this assay, PDE5 activity converts [^{32}P]cGMP to [^{32}P]5'GMP in proportion to the amount of PDE5 activity present. The [^{32}P]5'GMP is then quantitatively converted to free [^{32}P] phosphate and unlabeled adenosine by the action of snake venom 5'-nucleotidase. Hence, the amount of [^{32}P] phosphate liberated is proportional to enzyme activity. The assay is performed at 30°C in a 100 μL reaction mixture containing (final concentrations) 40 mM Tris-Cl (pH 8.0), 1 μM $ZnSO_4$, 5 mM $MgCl_2$, and 0.1 mg/ml bovine serum albumin. PDE5 is present in quantities that yield <30% total hydrolysis of substrate (linear assay conditions). The assay is initiated by addition of substrate (1 mM [^{32}P]cGMP), and the mixture is incubated for twelve minutes. Seventy-five (75) μg of *Crotalus atrox* venom is then added, and the incubation is continued for three

more minutes (fifteen minutes total). The reaction is stopped by addition of 200 μL of activated charcoal (25 mg/ml suspension in 0.1 M NaH_2PO_4, pH 4). After centrifugation (750 X g for three minutes) to sediment the charcoal, a sample of the supernatant is taken for radioactivity determination in a scintillation counter and the PDE5 activity is calculated.

EXAMPLE 3

Assays to Measure the Effects of PDE5 Inhibitors

Effects of inhibitors of the present invention on enzymatic activity of PDE5 preparations can be assessed in either of two assays which differ from each other principally on the basis of scale and provide essentially the same results in terms of IC_{50} values. Both assays involve modification of the procedure of Wells et al., *Biochim. Biophys. Acta*, *384*, page 430 (1975). The first of the assays is performed in a total volume of 200 μl containing 50 mM Tris pH 7.5, 3 mM Mg acetate, 1 mM EGTA, 50 μg/ml snake venom nucleotidase and 50 nM [^{3}H]-cGMP (Amersham). Compounds of the invention are dissolved in DMSO finally present at 2% in the assay. The assays are incubated for 30 minutes at 30°C and stopped by addition of 800 μl of 10 mM Tris pH 7.5, 10 mM EDTA, 10 mM theophylline, 0.1 mM adenosine, and 0.1 mM guanosine. The mixtures are loaded on 0.5 ml QAE Sephadex columns, and eluted by 2 ml of 0.1 M formate (pH 7.4). The eluted radioactivity is measured by scintillation counting in Optiphase Hisafe 3.

A second, microplate PDE assay uses Multiscreen plates and a vacuum manifold. The assay (100 μl) contains 50 mM Tris pH 7.5, 5 mM Mg acetate, 1 mM EGTA and 250 μg/ml snake venom nucleotidase. The other components of the reaction mixture are as described above. At the end of the incubation, the total volume of the assay is loaded on a QAE Sephadex microcolumn plate by filtration. Free radioactivity is eluted with 200 μl of water from which 50 μl aliquots are analyzed by scintillation counting as described above.

EXAMPLE 4

Assessing the Physiological Effect of PDE5 Inhibitors in Migraine

The use of a compound that inhibits PDE5 for the treatment of migraine is demonstrated in a clinical study assessing the physiological effect of the compound in reducing a clinical symptom of migraine and/or reducing or preventing the recurrence of a clinical symptom of migraine. This study is a double-blinded placebo controlled crossover study in otherwise normal, healthy patients. Patients receive either the test compound, at doses from 1 to 20 mg, or a placebo. Endpoints of the study are measured using a questionnaire specifically developed to assess the reduction of clinical symptoms. An additional endpoint is the measurement of elapsed time before the next migraine attack.

EXAMPLE 5

Animal Model of Dural Protein Extravasation

A number of preclinical laboratory animal models for migraine have been described. A commonly used model is the dural extravasation model that has been described in Phebus et al., *Life Sci., 61(21)*, pages 2117-2126 (1997), which can be used to evaluate the present compounds.

In this model, Harlan Sprague-Dawley rats (250 to 350 g) were anesthetized with sodium pentobarbital intraperitoneally (65 mg/kg) and placed in a stereotaxic frame (David Kopf Instruments) with the incisor bar set at -3.5 mm. Following a midline sagital scalp incision, two pairs of bilateral holes were drilled through the skull (6 mm posterially, 2.0 and 4.0 mm laterally, all coordinates referenced to bregma). Pairs of stainless steel stimulating electrodes, insulated except at the tips (Rhodes Medical Systems, Inc.), were lowered through the holes in both hemispheres to a depth of 9 mm.

The femoral vein was exposed and a dose of the test compound was injected intravenously (i.v.) at a dosing volume of 1 mL/kg or, in the alternative, test compound was administered orally (p.o.) via gavage at a volume of 2 mL/kg. Approximately 8 minutes post i.v. injection, a 50 mg/kg dose of Evans Blue, a fluorescent dye, also was injected intravenously. The Evans Blue complexed with proteins in the blood and functioned as a marker for protein extravasation. Exactly 10 minutes post-injection of the test compound, the left trigeminal ganglion was stimulated for 3 minutes at a current

intensity of 1.0 mA (5 Hz, 4 msec duration) with a Model 273 potentiostat/galvanostat (EG&G Princeton Applied Research).

Fifteen minutes following stimulation, the animals were killed and exsanguinated with 40 mL of saline. The top of the skull was removed to facilitate the collection of the dural membranes. The membrane samples were removed from both hemispheres, rinsed with water, and spread flat on microscopic slides. Once dried, the tissues were cover-slipped with a 70% glycerol/water solution.

A fluorescence microscope (Zeiss) equipped with a grating monochromator and a spectrophotometer was used to quantify the amount of Evans Blue dye in each sample. An excitation wavelength of approximately 535 nm was utilized and the emission intensity at 600 nm was determined. The microscope was equipped with a motorized stage and also interfaced with a personal computer. This facilitated the computer-controlled movement of the stage with fluorescence measurements at 25 points (500 mm steps) on each dural sample. The mean and standard deviation of the measurements were determined by the computer.

The extravasation induced by the electrical stimulation of the trigeminal ganglion was an ipsilateral effect (i.e., occurs only on the side of the dura in which the trigeminal ganglion was stimulated). This allows the other (unstimulated) half of the dura to be used as a control. The ratio of the amount of extravasation in the dura from the stimulated side, over the amount of extravasation in the unstimulated side, was calculated. Control

animals dosed only with saline, yielded a ratio of approximately 1.9. In contrast, a compound which effectively prevented the extravasation in the dura from the stimulated side would yield a ratio of approximately 1.0.

Compound (IIc) was tested in rats using the dural extravasation model described above. Compound (IIc) has an IC_{50} versus human recombinant PDE5 of 2.4 nM. The minimum selectivity of compound (IIc) for PDE5 vs. PDEs 1-4 and 7-10 is 2800-fold. An intravenous 20 mg/kg dose of compound (IIc) in rats provides a total plasma level of about 1500 ng/mL of compound (IIc) with a half-life ($t^{1/2}$) of 3.8 hours. This corresponds to a free concentration of compound (IIc) of about 375 nM at maximum concentration (C_{max}). This amount of compound (IIc) inhibits of PDE5, but does not inhibit PDEs 1-4 and 7-10.

In a particular rat plasma protein extravasation (PPE) model for migraine, compound (IIc) was administered intravenously. Doses of 3 and 10 ng/kg of compound (IIc) maximally inhibited PPE in anaesthetized rats ten minutes after administration, i.e., an extravasation ratio of 1.2 and 1.0, respectively. A extravasation ratio of about one indicates a complete inhibition of extravasation. A dose of 0.3 ng/kg of compound (IIc) exhibited an extravasation ratio of about 2, wherein a control sample (i.e., vehicle only) exhibited an extravasation ratio of about 1.8. For comparison, a control compound, naratriptan, inhibits PPE at 0.1 ng/kg in an identical test. This example illustrates that

administration of a PDE5 inhibitor is an effective treatment for migraine.

EXAMPLE 6

Clinical Example

A 54-year-old male suffering for over 30 years from three to four severe migraine attacks per month, each lasting 12 to 24 hours and accompanied by nausea and occasional vomiting, was the subject of the study. In years prior to the study, the subject required permanent medication during the migraine attacks, in particular a sumatriptan-containing medication. The patient also suffered from mild diabetes and erectile dysfunction. The patient was treated with VIAGRA® (i.e., active ingredient is sildenafil) taking the drug about 2 to 3 times a week at dosages of 50 or 100 mg. The patient was satisfied with respect to the positive effects on his migraine, and began taking VIAGRA® 3 to 4 times weekly at dosages of 25 to 50 mg. Starting with the onset of VIAGRA® regimen, the patient remained completely free of migraine attacks for more than one and one-half years. When the patient ceased the VIAGRA® regimen, the severe migraines returned almost immediately. After a short time, the patient resumed the VIAGRA® regimen, and the migraine attacks stopped completely.

EXAMPLE 7

Exemplary formulations for use in the method of the invention include, but are not limited to the following:

TABLETS FOR ORAL ADMINISTRATION

A. Direct Compression

	mg/tablet
Active Ingredient	50.0
Colloidal Silicon Dioxide	0.5
Crospovidone	8.0
Sodium Lauryl Sulfate	1.0
Magnesium Stearate Ph Eur	1.0
Microcrystalline Cellulose USNF	139.5

The active ingredient was sieved and blended with the excipients. The resultant mix was compressed into tablets.

B. Wet Granulation

	mg/tablet
Active ingredient	50.0
Polyvinylpyrrolidone	150.0
Polyethylene glycol	50.0
Polysorbate 80	10.0
Magnesium Stearate Ph Eur	2.5
Croscarmellose Sodium	25.0
Colloidal Silicon Dioxide	2.5
Microcrystalline Cellulose USNF	210.0

The polyvinylpyrrolidone, polyethylene glycol, and polysorbate 80 were dissolved in water. The resultant solution was used to granulate the active ingredient. After drying, the granules were screened then extruded at elevated temperatures and pressures. The extrudate was milled and/or screened, then was blended with the microcrystalline cellulose, croscarmellose sodium, colloidal silicon dioxide, and magnesium stearate. The resultant mix was compressed into tablets.

Tablets of other strengths may be prepared by altering the ratio of active ingredient to the other excipients.

FILM COATED TABLETS

The aforementioned tablet formulations were film coated.

Coating Suspension	% w/w
Opadry white †	13.2
Purified water Ph Eur	to 100.0*

* The water did not appear in the final product. The maximum theoretical weight of solids applied during coating was 20 mg/tablet.

† Opadry white is a proprietary material obtainable from Colorcon Limited, UK, which contains hydroxypropyl methylcellulose, titanium dioxide, and triacetin.

The tablets were film coated using the coating suspension in conventional film coating equipment.

CAPSULES

	mg/capsule
Active Ingredient	50.0
Lactose	148.5
Polyvinylpyrrolidone	100.0
Magnesium Stearate	1.5

The active ingredient was sieved and blended with the excipients. The mix was filled

into size No. 1 hard gelatin capsules using suitable equipment.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating a migraine comprising administering a PDE5 inhibitor to an individual in need thereof in an amount effective to reduce or alleviate a clinical symptom of the migraine.

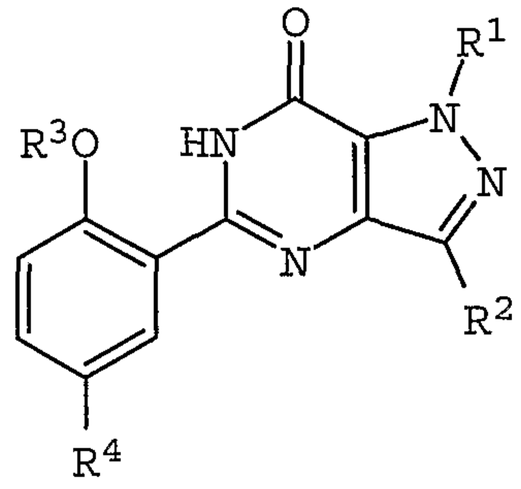
2. A method of treating a migraine comprising administering (a) a first PDE5 inhibitor and (b) a second PDE inhibitor, an antimigraine agent, or a mixture thereof, to an individual in need thereof in an amount effective to reduce or alleviate a clinical symptom of the migraine.

3. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 about 100 times less than its IC_{50} value vs. PDE1 through PDE4 and PDE6 through PDE9.

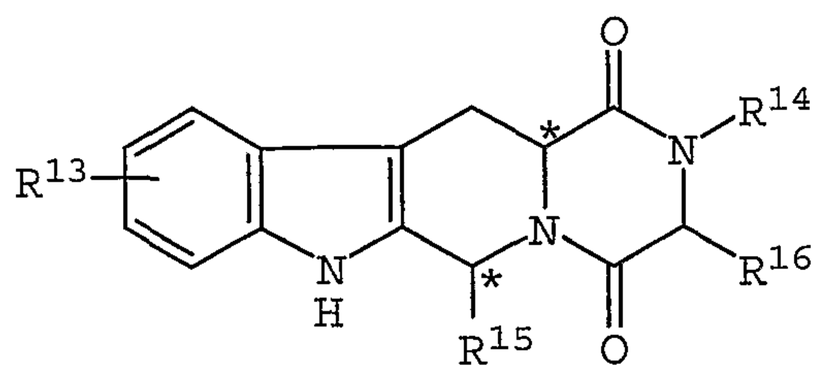
4. The method of claim 1 or 2 wherein the PDE5 inhibitor is administered at onset of the migraine.

5. The method of claim 1 or 2 wherein the PDE5 inhibitor is administered after onset of the migraine.

6. The method of claim 1 or 2 wherein the PDE5 inhibitor is selected from the group consisting of:



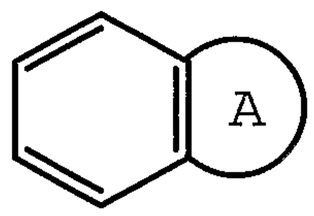
wherein R^1 is methyl or ethyl; R^2 is n-propyl; R^3 is ethyl, n-propyl, or allyl; R^4 is $COCH_2NR^5R^6$, $CONR^5R^6$, $SO_2NR^9R^{10}$, or 1-methyl-2-imidazolyl; R^5 and R^6 together represent, with the nitrogen atom to which they are attached, a morpholino or 4-N(R^{11})-piperazinyl group; R^9 and R^{10} together represent, with the nitrogen atom to which they are attached, a 4-N(R^{12})-piperazinyl group; R^{11} is methyl or acetyl; and R^{12} is H, methyl, 2-propyl, or 2-hydroxyethyl;



wherein R^{13} is selected from the group consisting of hydrogen, halogen, and C_{1-6}alkyl;

R^{14} is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, haloC_{1-6}alkyl, C_{3-8}cycloalkyl, C_{3-8}cycloalkylC_{1-3}alkyl, and arylC_{1-3}alkyl, wherein aryl is phenyl or phenyl substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, C_{1-6}alkoxy, and methylenedioxy, and heteroarylC_{1-3}alkyl, wherein heteroaryl is thienyl, furyl, or pyridyl, each optionally substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-6}alkyl, and C_{1-6}alkoxy;

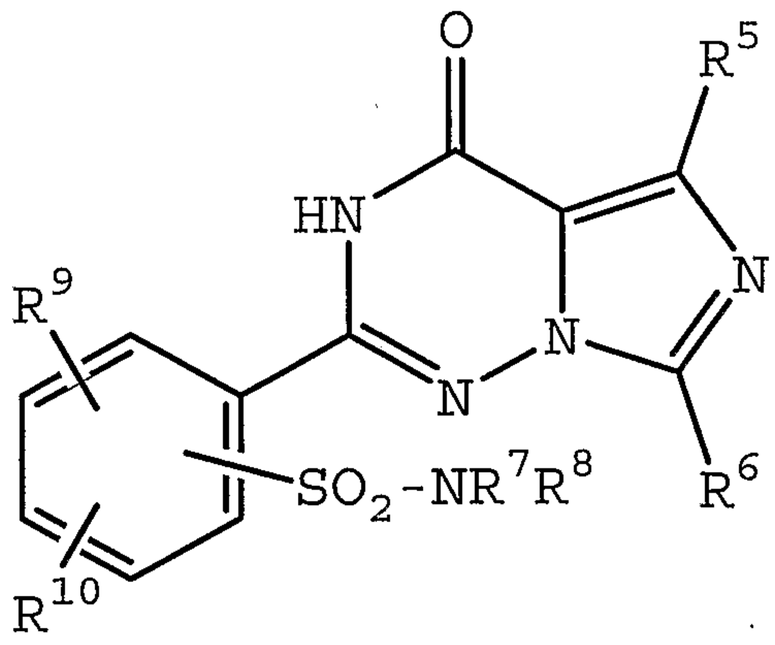
R^{15} represents an optionally substituted monocyclic aromatic ring selected from the group consisting of benzene, thiophene, furan, and pyridine, or an optionally substituted bicyclic ring



attached to the rest of the molecule via one of the benzene ring carbon atoms and wherein the fused ring A 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, sulphur and nitrogen; and

R^{16} represents hydrogen or C_{1-3}alkyl, or R^1 and R^3 together represent a 3- or 4-membered alkyl or alkenyl chain component of a 5- or 6-membered ring;

and salts and solvates thereof;



(III)

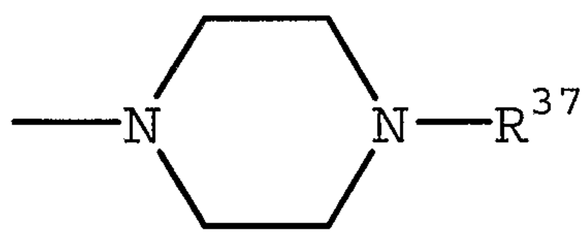
wherein

R^5 is methyl or ethyl,

R^6 is ethyl or propyl,

R^7 and R^8, independently, are a straight-chain or branched alkyl chain having up to five carbon atoms, optionally substituted, independently, with up to two hydroxy or methoxy, or

R^7 and R^8 together with the nitrogen form piperidinyl, morpholinyl, thiomorpholinyl, or a residue of the formula



wherein R^{37} is hydrogen, formyl, or straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms,

or is a straight-chain or branched alkyl having up to three carbon atoms, optionally

substituted, independently, with one or two hydroxy, carboxyl, or straight-chain or branched alkoxy or alkoxycarbonyl, each having up to three carbon atoms, or by groups of the formula $-(D)_fNR^{38}R^{39}$ or $-P(O)(OR^{42})(OR^{43})$,

wherein f is 0 or 1,

D is a group of the formula -CO,

R^{38} and R^{39}, independently, are hydrogen or methyl,

R^{42} and R^{43}, independently are hydrogen, methyl, or ethyl, or

R^{37} is cyclopentyl,

and the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted, independently, with one or two, optionally geminally, hydroxy, formyl, carboxyl, and straight-chain or branched acyl or alkoxycarbonyl each having up to three carbon atoms, or a group of the formula $-P(O)(OR^{46})(OR^{47})$ or $-(CO)_jNR^{49}R^{50}$,

wherein R^{46} and R^{47}, independently, are hydrogen, methyl, or ethyl,

j is 0 or 1, and

R^{49} and R^{50}, independently, are hydrogen or methyl and/or the heterocycles mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted by straight-chain or branched with up to three carbon atoms, which is optionally substituted, independently, with one or two hydroxy, carboxyl, or a residue of the formula $P(O)OR^{53}OR^{54}$,

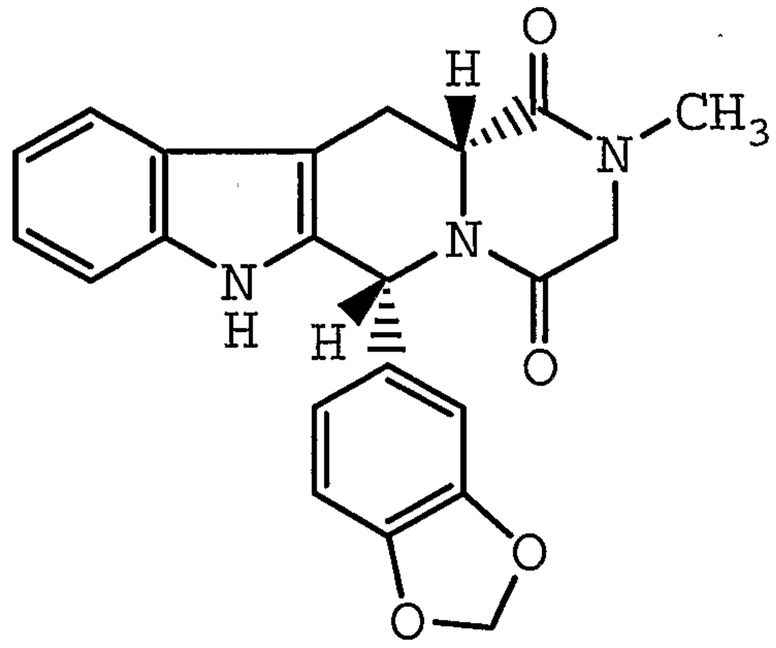
wherein R^{53} and R^{54}, independently, are hydrogen, methyl, or ethyl, and/or the heterocycles

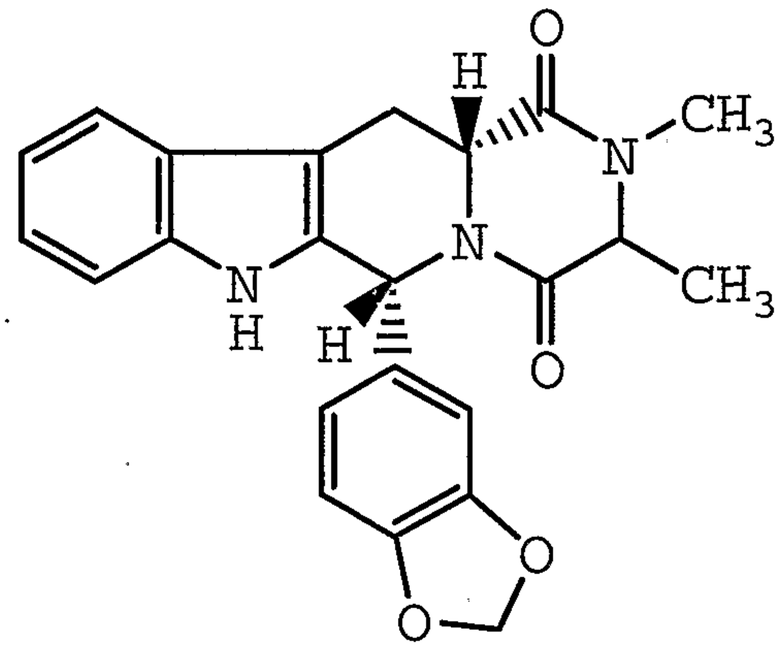
mentioned under R^3 and R^4 that are formed together with the nitrogen atom are optionally substituted via N-linked piperidinyl or pyrrolidinyl,

R^9 is hydrogen, and

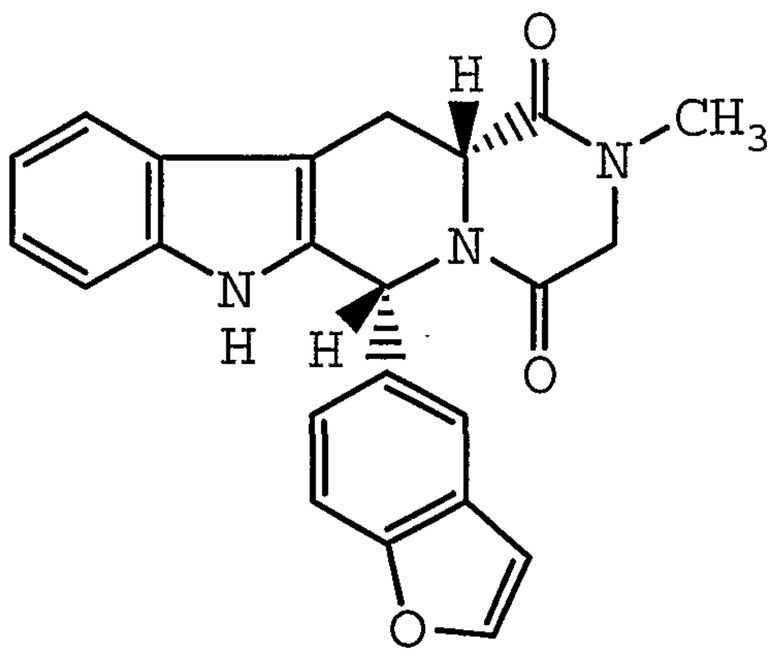
R^{10} is ethoxy or propoxy, and salts, hydrates, N-oxides, and isometric forms thereof.

7. The method of claim 1 or 2 wherein the PDE5 inhibitor is selected from the group consisting of

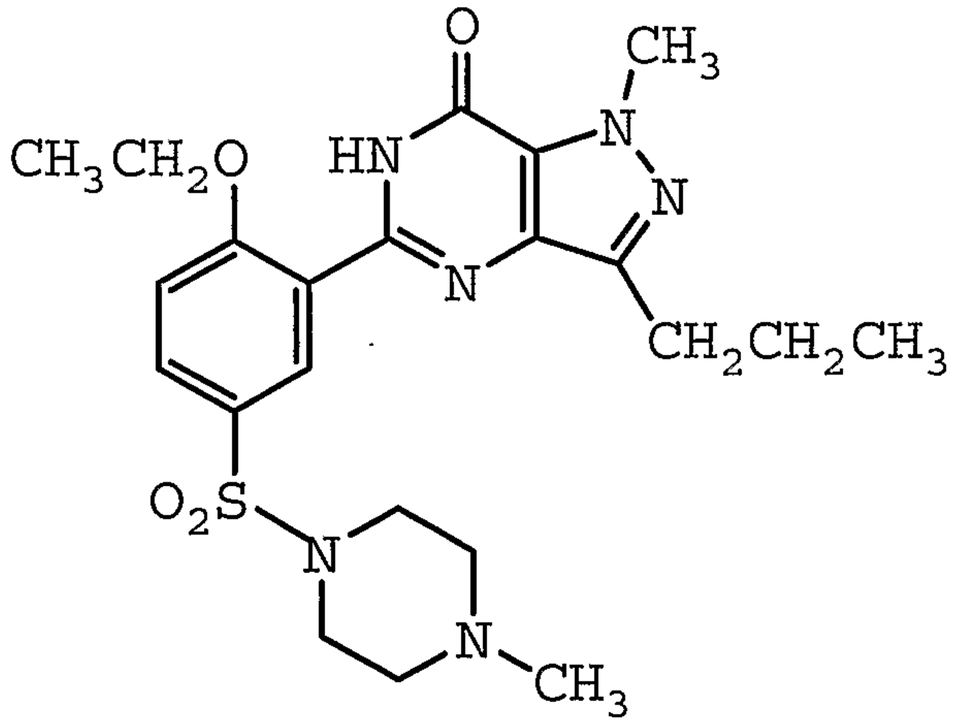




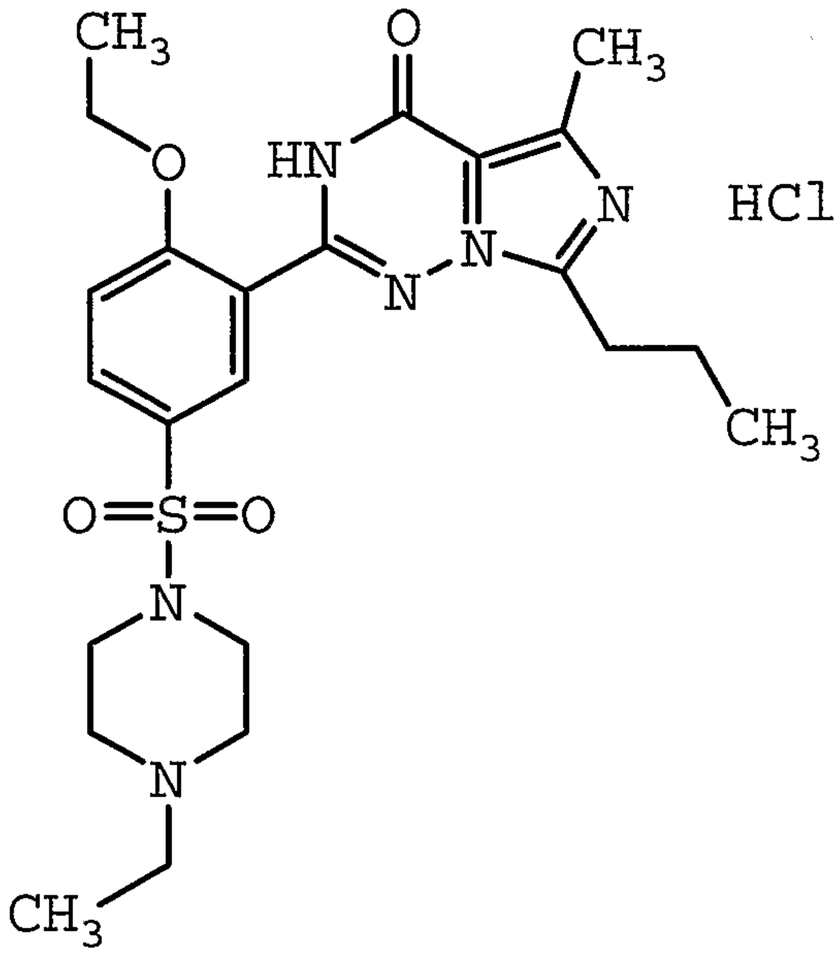
, and



8. The method of claim 1 or 2 wherein the PDE5 inhibitor is selected from the group consisting of



and



9. The method of claim 1 or 2 wherein the PDE5 inhibitor is administered in an amount of about 1 mg to about 100 mg over a 24-hour period.

10. The method of claim 2 wherein (a) the first PDE5 inhibitor and (b) second PDE5 inhibitor, antimigraine agent, or mixture thereof are administered simultaneously.

11. The method of claim 2 wherein (a) the first PDE5 inhibitor and (b) second PDE5 inhibitor, antimigraine agent, or mixture thereof are administered sequentially.

12. The method of claim 2 wherein the antimigraine agent is selected from the group consisting of an ergot alkaloid, an analgesic, a narcotic, a β-blocker, a calcium channel blocker, a tricyclic antidepressant, an anticonvulsant, a monoamine oxidase inhibitor, and a 5-HT agonist.

13. The method of claim 2 wherein the antimigraine agent is selected from the group consisting of propranolol, metoprolol, atenolol, tinolol, nadolol, verapamil, diltiazem, nifedipine, nimopidine, amitriptyline, nortriptyline, divalproex sodium, sumatriptan, naratriptan, rizatriptan, zolmitriptan, pizotifen, acetaminophen, aspirin, ibuprofen, indomethacin, ergotamine, dihydroergotamine, butalbital, phenelzine, and isocarboxazid.

14. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 of about 10 nM or less.

15. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 of about 5 nM or less.

16. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 of about 3 nM or less.

17. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 about 100 times less than its IC_{50} value vs. PDE6 and PDE1c.

18. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 about 500 times less than its IC_{50} value vs. PDE6 and PDE1c.

19. The method of claim 1 or 2 wherein the PDE5 inhibitor has an IC_{50} value vs. human recombinant PDE5 about 1000 times less than its IC_{50} value vs. PDE6 and PDE1c.

20. A method of treating migraine comprising administering a PDE5 inhibitor to an individual in need thereof in an amount effective to reduce or alleviate a clinical symptom of the migraine, said PDE5 inhibitor administered at onset of a clinical symptom of the migraine headache.

21. Use of PDE5 inhibitor in the preparation of a medicament for the treatment of migraine.