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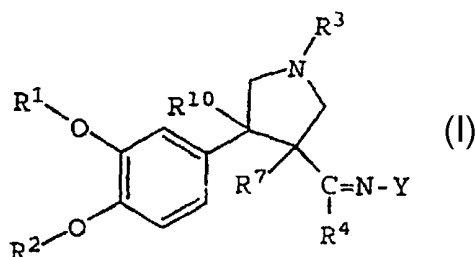
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(54) Title: HYDRAZONE AND OXIME DERIVATIVES OF PYRROLIDINE AS AMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS



(57) Abstract: Compounds having the formula (I) that are potent and selective inhibitors of PDE4, as well as methods of making the same, are disclosed. Use of the compounds in the treatment of inflammatory diseases and other diseases involving elevated levels of cytokines, as well as central nervous system (CNS) disorders, also is disclosed.



WO 01/46136 A1

HYDRAZONE AND OXIME DERIVATIVES OF PYRROLIDINE AS AMP-SPECIFIC
PHOSPHODIESTERASE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims benefit of provi-
sional application Serial No. 60/171,955, filed
December 23, 1999.

FIELD OF INVENTION

10

 The present invention relates to a series
of compounds that are potent and selective inhibi-
tors of cyclic adenosine 3',5'-monophosphate specif-
ic phosphodiesterase (cAMP specific PDE). In par-
15 ticular, the present invention relates to a series
of novel oxime and hydrazone compounds that are
useful for inhibiting the function of cAMP specific
PDE, in particular, PDE4, as well as methods of
making the same, pharmaceutical compositions con-
20 taining the same, and their use as therapeutic
agents, for example, in treating inflammatory di-
seases and other diseases involving elevated levels
of cytokines and proinflammatory mediators.

25

BACKGROUND OF THE INVENTION

 Chronic inflammation is a multi-factorial
disease complication characterized by activation of
multiple types of inflammatory cells, particularly
30 cells of lymphoid lineage (including T lymphocytes)
and myeloid lineage (including granulocytes, macro-
phages, and monocytes). Proinflammatory mediators,
including cytokines, such as tumor necrosis factor

- 2 -

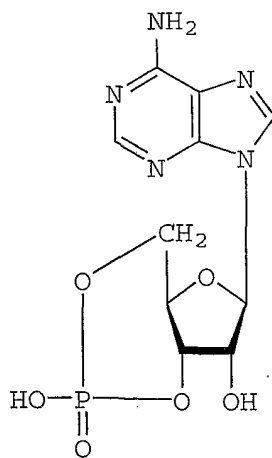
(TNF) and interleukin-1 (IL-1), are produced by these activated cells. Accordingly, an agent that suppresses the activation of these cells, or their production of proinflammatory cytokines, would be
5 useful in the therapeutic treatment of inflammatory diseases and other diseases involving elevated levels of cytokines.

Cyclic adenosine monophosphate (cAMP) is a second messenger that mediates the biologic responses of cells to a wide range of extracellular
10 stimuli. When the appropriate agonist binds to specific cell surface receptors, adenylate cyclase is activated to convert adenosine triphosphate (ATP) to cAMP. It is theorized that the agonist induced
15 actions of cAMP within the cell are mediated predominately by the action of cAMP-dependent protein kinases. The intracellular actions of cAMP are terminated by either a transport of the nucleotide to the outside of the cell, or by enzymatic cleavage
20 by cyclic nucleotide phosphodiesterases (PDEs), which hydrolyze the 3'-phosphodiester bond to form 5'-adenosine monophosphate (5'-AMP). 5'-AMP is an inactive metabolite. The structures of cAMP and 5'-AMP are illustrated below.

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- 3 -

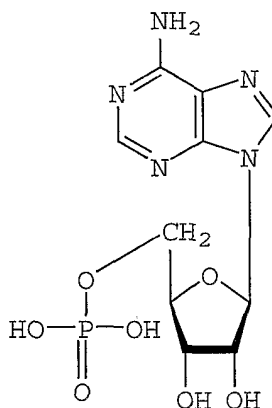
5



10

cAMP

15



20

25

5'-AMP

Elevated levels of cAMP in human myeloid and lymphoid lineage cells are associated with the suppression of cell activation. The intracellular enzyme family of PDEs, therefore, regulates the level of cAMP in cells. PDE4 is a predominant PDE isotype in these cells, and is a major contributor to cAMP degradation. Accordingly, the inhibition of

- 4 -

PDE function would prevent the conversion of cAMP to the inactive metabolite 5'-AMP and, consequently, maintain higher cAMP levels, and, accordingly, suppress cell activation (see Beavo et al., "Cyclic
5 Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action," Wiley and Sons, Chichester, pp. 3-14, (1990)); Torphy et al., *Drug News and Perspectives*, 6, pp. 203-214 (1993); Giembycz et al., *Clin. Exp. Allergy*, 22, pp. 337-344 (1992)).

10 In particular, PDE4 inhibitors, such as rolipram, have been shown to inhibit production of TNF α and partially inhibit IL-1 β release by monocytes (see Semmler et al., *Int. J. Immunopharmacol.*, 15, pp. 409-413, (1993); Molnar-Kimber et al.,
15 *Mediators of Inflammation*, 1, pp. 411-417, (1992)). PDE4 inhibitors also have been shown to inhibit the production of superoxide radicals from human polymorphonuclear leukocytes (see Verghese et al., *J. Mol. Cell. Cardiol.*, 21 (Suppl. 2), S61 (1989);
20 Nielson et al., *J. Allergy Immunol.*, 86, pp. 801-808, (1990)); to inhibit the release of vasoactive amines and prostanoids from human basophils (see Peachell et al., *J. Immunol.*, 148, pp. 2503-2510, (1992)); to inhibit respiratory bursts in eosino-
25 phils (see Dent et al., *J. Pharmacol.*, 103, pp. 1339-1346, (1991)); and to inhibit the activation of human T-lymphocytes (see Robicsek et al., *Biochem. Pharmacol.*, 42, pp. 869-877, (1991)).

Inflammatory cell activation and excessive
30 or unregulated cytokine (e.g., TNF α and IL-1 β) production are implicated in allergic, autoimmune, and inflammatory diseases and disorders, such as

- 5 -

rheumatoid arthritis, osteoarthritis, gouty arthritis, spondylitis, thyroid associated ophthalmopathy, Behcet's disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive
5 sepsis, toxic shock syndrome, asthma, chronic bronchitis, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, such as chronic obstructive pulmonary disease, silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium,
10 brain, and extremities, fibrosis, cystic fibrosis, keloid formation, scar formation, atherosclerosis, transplant rejection disorders, such as graft vs. host reaction and allograft rejection, chronic glomerulonephritis, lupus, inflammatory bowel
15 disease, such as Crohn's disease and ulcerative colitis, proliferative lymphocyte diseases, such as leukemia, and inflammatory dermatoses, such as atopic dermatitis, psoriasis, and urticaria.

Other conditions characterized by elevated
20 cytokine levels include brain injury due to moderate trauma (see Dhillon et al., *J. Neurotrauma*, 12, pp. 1035-1043 (1995); Suttorp et al., *J. Clin. Invest.*, 91, pp. 1421-1428 (1993)), cardiomyopathies, such as congestive heart failure (see Bristow et al., *Circulation*, 97, pp. 1340-1341 (1998)), cachexia, cachexia
25 secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS related complex), fever myalgias due to infection, cerebral malaria, osteoporosis and
30 bone resorption diseases, keloid formation, scar tissue formation, and pyrexia.

In particular, TNF α has been identified as having a role with respect to human acquired immune

- 6 -

deficiency syndrome (AIDS). AIDS results from the infection of T-lymphocytes with Human Immunodeficiency Virus (HIV). Although HIV also infects and is maintained in myeloid lineage cells, TNF has been shown to upregulate HIV infection in T-lymphocytic and monocytic cells (see Poli et al., *Proc. Natl. Acad. Sci. USA*, 87, pp. 782-785, (1990)).

Several properties of TNF α , such as stimulation of collagenases, stimulation of angiogenesis *in vivo*, stimulation of bone resorption, and an ability to increase the adherence of tumor cells to endothelium, are consistent with a role for TNF in the development and metastatic spread of cancer in the host. TNF α recently has been directly implicated in the promotion of growth and metastasis of tumor cells (see Orosz et al., *J. Exp. Med.*, 177, pp. 1391-1398, (1993)).

PDE4 has a wide tissue distribution. There are at least four genes for PDE4 of which multiple transcripts from any given gene can yield several different proteins that share identical catalytic sites. The amino acid identity between the four possible catalytic sites is greater than 85%. Their shared sensitivity to inhibitors and their kinetic similarity reflect the functional aspect of this level of amino acid identity. It is theorized that the role of these alternatively expressed PDE4 proteins allows a mechanism by which a cell can differentially localize these enzymes intracellularly and/or regulate the catalytic efficiency via post translational modification. Any given cell type that expresses the PDE4 enzyme typ-

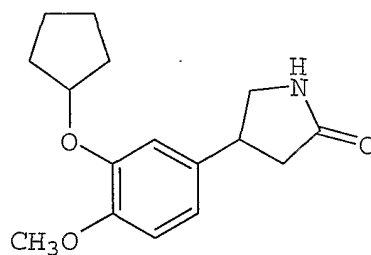
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ically expresses more than one of the four possible genes encoding these proteins.

Investigators have shown considerable interest in the use of PDE4 inhibitors as anti-inflammatory agents. Early evidence indicates that PDE4 inhibition has beneficial effects on a variety of inflammatory cells such as monocytes, macrophages, T-cells of the Th-1 lineage, and granulocytes. The synthesis and/or release of many pro-inflammatory mediators, such as cytokines, lipid mediators, superoxide, and biogenic amines, such as histamine, have been attenuated in these cells by the action of PDE4 inhibitors. The PDE4 inhibitors also affect other cellular functions including T-cell proliferation, granulocyte transmigration in response to chemotoxic substances, and integrity of endothelial cell junctions within the vasculature.

The design, synthesis, and screening of various PDE4 inhibitors have been reported. Methylxanthines, such as caffeine and theophylline, were the first PDE inhibitors discovered, but these compounds are nonselective with respect to which PDE is inhibited. The drug rolipram, an antidepressant agent, was one of the first reported specific PDE4 inhibitors. Rolipram, having the following structural formula, has a reported 50% Inhibitory Concentration (IC_{50}) of about 200 nM (nanomolar) with respect to inhibiting recombinant human PDE4.

- 8 -



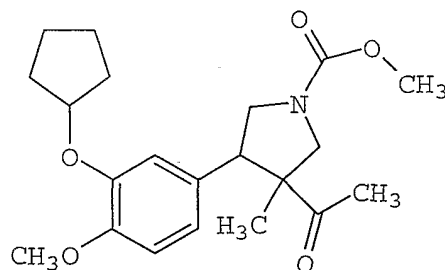
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Rolipram

10 Investigators have continued to search for
PDE4 inhibitors that are more selective with respect
to inhibiting PDE4, that have a lower IC₅₀ than roli-
pram, and that avoid the undesirable central nervous
system (CNS) side effects, such as retching, vomit-
15 ing, and sedation, associated with the administra-
tion of rolipram. One class of compounds is dis-
closed in Feldman et al. U.S. Patent No. 5,665,754.
The compounds disclosed therein are substituted
pyrrolidines having a structure similar to rolipram.
20 One particular compound, having structural formula
(I), has an IC₅₀ with respect to human recombinant
PDE4 of about 2 nM. Inasmuch as a favorable separa-
tion of emetic side effect from efficacy was ob-
served, these compounds did not exhibit a reduction
25 in undesirable CNS effects.

- 9 -

5



(I)

10 In addition, several companies are now undertaking clinical trials of other PDE4 inhibitors. However, problems relating to efficacy and adverse side effects, such as emesis and central nervous system disturbances, remain unsolved.

15 Accordingly, compounds that selectively inhibit PDE4, and that reduce or eliminate the adverse CNS side effects associated with prior PDE4 inhibitors, would be useful in the treatment of allergic and inflammatory diseases, and other
20 diseases associated with excessive or unregulated production of cytokines, such as TNF. In addition, selective PDE4 inhibitors would be useful in the treatment of diseases that are associated with elevated cAMP levels or PDE4 function in a particu-
25 lar target tissue.

SUMMARY OF THE INVENTION

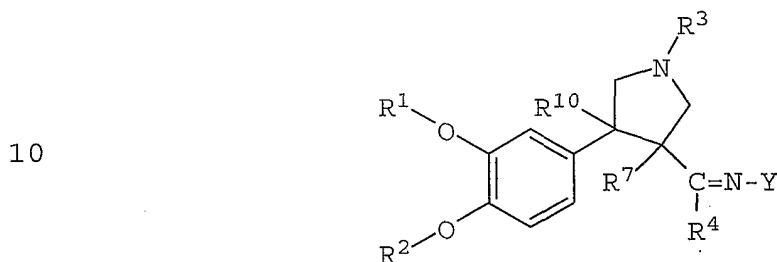
The present invention is directed to
30 potent and selective PDE4 inhibitors useful in treatment of diseases and conditions where inhibition of PDE4 activity is considered beneficial. The present PDE4 inhibitors unexpectedly reduce or

- 10 -

eliminate the adverse CNS side effects associated with prior PDE4 inhibitors.

In particular, the present invention is directed to compounds having the structural formula

5 (II):



15

(II)

wherein Y is OR⁵ or NR⁵R⁶;

R¹ is lower alkyl, bridged alkyl (e.g., norbornyl), aralkyl (e.g., indanyl), cycloalkyl, a
 20 5- or 6-membered saturated heterocycle (e.g., 3-tetrahydrofuryl), C₁₋₃alkylenecycloalkyl (e.g., cyclopentylmethyl), aryl- or heteroaryl-substituted propargyl (e.g., -CH₂C=C-C₆H₅), aryl- or heteroaryl-substituted allyl (e.g., -CH₂CH=CH-C₆H₅), or halo-
 25 cycloalkyl (e.g., fluorocyclopentyl);

R² is hydrogen, methyl, or halo-substituted methyl, e.g., CHF₂;

R³ is C(=O)OR⁷, C(=O)R⁷, C(=NH)NR⁸R⁹, C(=O)-NR⁸R⁹, aryl, or heteroaryl;

30 R⁴ is hydrogen, lower alkyl, haloalkyl, cycloalkyl, or aryl;

R⁵ and R⁶, independently, are hydrogen, lower alkyl, haloalkyl, cycloalkyl, aryl, hetero-

- 11 -

aryl, or alkaryl, or R⁵ and R⁶ are taken together form a 5-membered or 6-membered ring;

R⁷ is branched or unbranched lower alkyl or aryl, or R⁷ can be optionally substituted with one or
5 more of OR⁸, NR⁸R⁹, or SR⁸;

R⁸ and R⁹, same or different, are selected from the group consisting of hydrogen, lower alkyl, cycloalkyl, aryl, heteroaryl, and aralkyl, or R⁸ and R⁹ are taken together form a 4-membered to 7-membered
10 ring;

R¹⁰ is hydrogen, alkyl, haloalkyl, cycloalkyl, aryl, C(=O)alkyl, C(=O)cycloalkyl, C(=O)aryl, C(=O)Oalkyl, C(=O)Ocycloalkyl, C(=O)aryl, CH₂OH, CH₂Oalkyl, CHO, CN, NO₂, or SO₂R¹¹; and

15 R¹¹ is alkyl, cycloalkyl, trifluoromethyl, aryl, aralkyl, or NR⁸R⁹.

The present invention also is directed to pharmaceutical compositions containing one or more of the compounds of structural formula (II), to use
20 of the compounds and compositions containing the compounds in the treatment of a disease or disorder, and to methods of preparing compounds and intermediates involved in the synthesis of the compounds of structural formula (II).

25 The present invention also is directed to methods of treating a mammal having a condition where inhibition of PDE4 provides a benefit, modulating cAMP levels in a mammal, reducing TNF α levels in a mammal, suppressing inflammatory cell
30 activation in a mammal, and inhibiting PDE4 function in a mammal by administering therapeutically effective amounts of a compound of structural formula

- 12 -

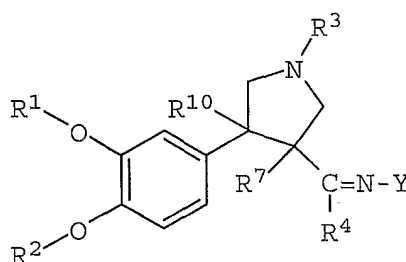
(II), or a composition containing a composition of structural formula (II) to the mammal.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5

The present invention is directed to compounds having the structural formula (II):

10



15

(II)

wherein Y is OR⁵ or NR⁵R⁶;

R¹ is lower alkyl, bridged alkyl (e.g., norbornyl), aralkyl (e.g., indanyl), cycloalkyl, a
20 5- or 6-membered saturated heterocycle (e.g., 3-tetrahydrofuryl), C₁₋₃alkylenecycloalkyl (e.g., cyclopentylmethyl), aryl- or heteroaryl-substituted propargyl (i.e., -CH₂C≡C-C₆H₅), aryl- or heteroaryl-
25 substituted allyl (e.g., -CH₂CH=CH-C₆H₅), or halocycloalkyl (e.g., fluorocyclopentyl);

R² is hydrogen, methyl, or halo-substituted methyl, e.g., CHF₂;

R³ is C(=O)OR⁷, C(=O)R⁷, C(=NH)NR⁸R⁹, C(=O)-
30 NR⁸R⁹, aryl, or heteroaryl;

R⁴ is hydrogen, lower alkyl, haloalkyl, cycloalkyl, or aryl;

- 13 -

R⁵ and R⁶, independently, are hydrogen, lower alkyl, haloalkyl, cycloalkyl, aryl, heteroaryl, or alkaryl, or R⁵ and R⁶ are taken together form a 5-membered or 6-membered ring;

5 R⁷ is branched or unbranched lower alkyl or aryl, or R⁷ can be optionally substituted with one or more of OR⁸, NR⁸R⁹, or SR⁸; and

R⁸ and R⁹, same or different, are selected from the group consisting of hydrogen, lower alkyl, 10 cycloalkyl, aryl, heteroaryl, and aralkyl, or R⁸ and R⁹ are taken together form a 4-membered to 7-membered ring;

R¹⁰ is hydrogen, alkyl, haloalkyl, cycloalkyl, aryl, C(=O)alkyl, C(=O)cycloalkyl, C(=O)aryl, 15 C(=O)Oalkyl, C(=O)Ocycloalkyl, C(=O)aryl, CH₂OH, CH₂Oalkyl, CHO, CN, NO₂, or SO₂R¹¹; and

R¹¹ is alkyl, cycloalkyl, trifluoromethyl, aryl, aralkyl, or NR⁸R⁹.

As used herein, the term "alkyl," alone or 20 in combination, is defined to include straight chain and branched chain saturated hydrocarbon groups containing one to 16 carbon atoms. The term "lower alkyl" is defined herein as an alkyl group having one through six carbon atoms (C₁-C₆). Examples of 25 lower alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, isobutyl, n-butyl, neopentyl, n-hexyl, and the like. The term "alkynyl" refers to an unsaturated alkyl group that contains a carbon-carbon triple bond.

30 The term "bridged alkyl" is defined herein as a C₆-C₁₆ bicyclic or polycyclic hydrocarbon group, for example, norboryl, adamantyl, bicyclo[2.2.2]-

- 14 -

octyl, bicyclo[2.2.1]heptyl, bicyclo[3.2.1]octyl, or decahydronaphthyl.

The term "cycloalkyl" is defined herein to include cyclic C₃-C₇ hydrocarbon groups. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, and cyclopentyl.

The term "alkylene" refers to an alkyl group having a substituent. For example, the term "C₁₋₃alkylenecycloalkyl" refers to an alkyl group containing one to three carbon atoms, and substituted with a cycloalkyl group.

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, substituents selected from halo, alkyl, 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.

- 15 -

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
5 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, alkyl-
10 sulfinyl, and alkylsulfonyl. Examples of heteroaryl groups include thienyl, furyl, pyridyl, oxazolyl, quinolyl, isoquinolyl, indolyl, triazolyl, isothiazolyl, isoxazolyl, imidazolyl, benzothiazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

15 The term "aralkyl" is defined herein as a previously defined alkyl group, wherein one of the hydrogen atoms is replaced by an aryl group as defined herein, for example, a phenyl group optionally having one or more substituents, for example,
20 halo, alkyl, alkoxy, and the like. An example of an aralkyl group is a benzyl group.

The term "alkaryl" is defined herein as a previously defined aryl group, wherein one of the hydrogen atoms is replaced by an alkyl, cycloalkyl,
25 haloalkyl, or halocycloalkyl group.

The terms "heteroaralkyl" and "hetero-alkaryl" are defined similarly as the term "aralkyl" and "alkaryl," however, the aryl group is replaced by a heteroaryl group as previously defined.

30 The term "heterocycle" is defined as a 5- or 6-membered nonaromatic ring having one or more heteroatoms selected from oxygen, nitrogen, and sulfur present in the ring. Nonlimiting examples

- 16 -

include tetrahydrofuran, piperidine, piperazine, sulfolane, morpholine, tetrahydropyran, dioxane, and the like.

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

The term "alkoxy" is defined as -OR, wherein R is alkyl.

The term "alkoxyalkyl" is defined as an
10 alkoxy group appended to an alkyl group. The terms "aryloxyalkyl" and "aralkoxyalkyl" are similarly defined as an aryloxy or aralkoxy group appended to an alkyl group.

The term "hydroxy" is defined as -OH.

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

The term "amino" is defined as -NH₂.

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

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

The term "nitro" is defined as -NO₂.

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

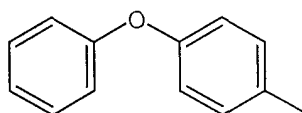
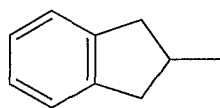
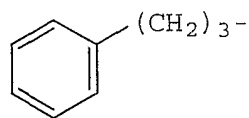
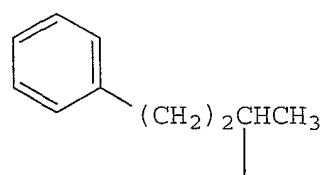
The term "alkylsulfinyl" is defined as R-SO₂, where R is alkyl.

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

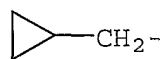
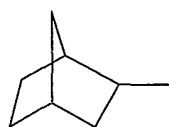
30 In preferred embodiments, R⁷ is methyl, R² is methyl or difluoromethyl, R⁴ is selected from the group consisting of hydrogen, methyl, trifluoromethyl, cyclopropyl, acetyl, ethynyl, benzyl, and

- 17 -

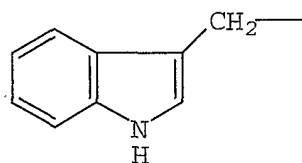
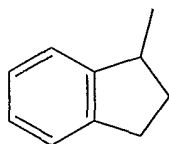
phenyl, and R^5 and R^6 are selected from the group consisting of hydrogen and methyl. R^1 is selected from the group consisting of



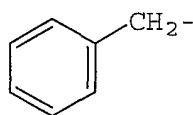
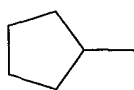
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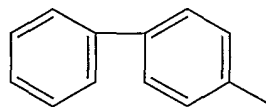
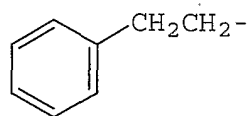
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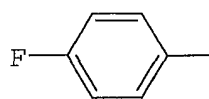
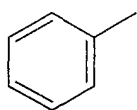


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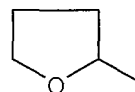
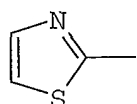


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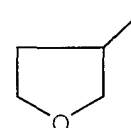
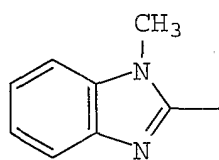
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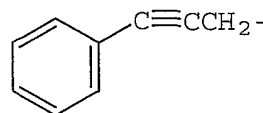
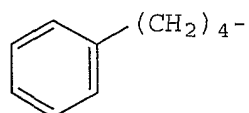


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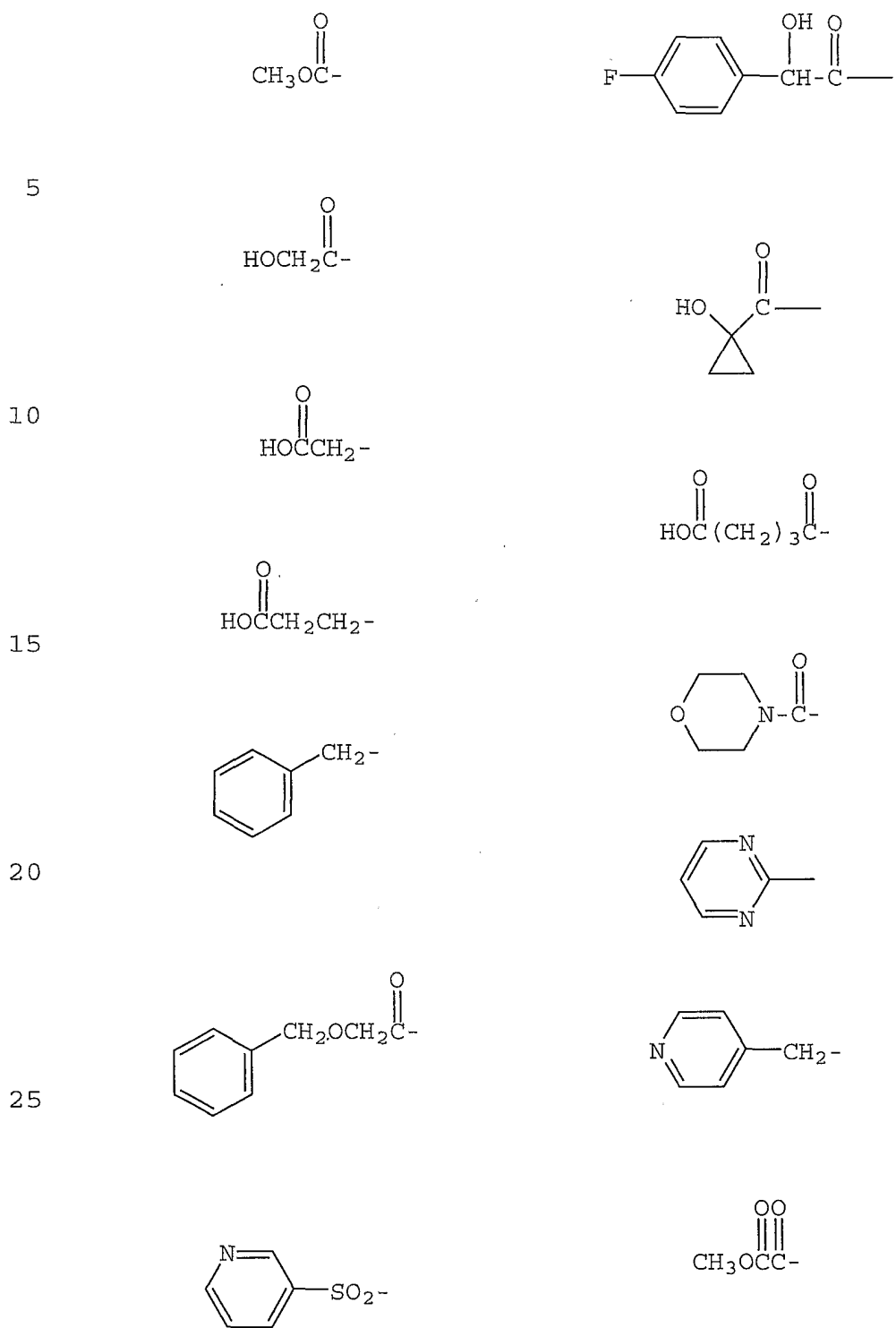


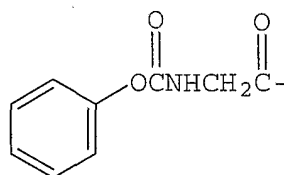
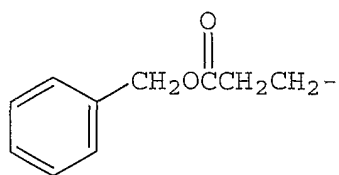
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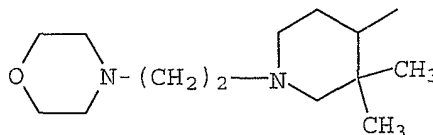
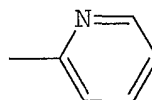
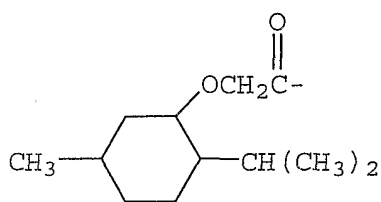
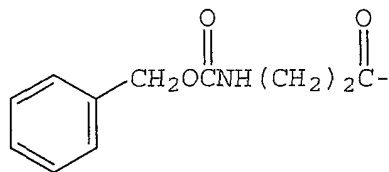
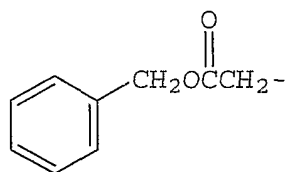


25 R³ is selected from the group consisting of

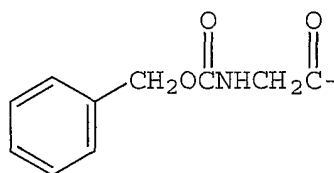
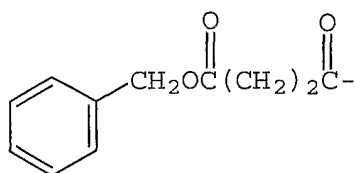
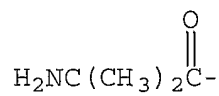
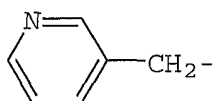




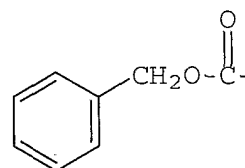
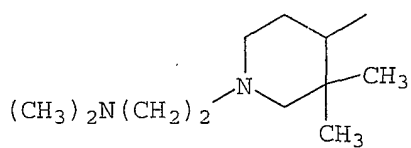
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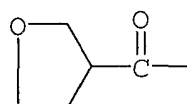
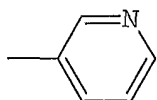
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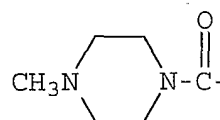
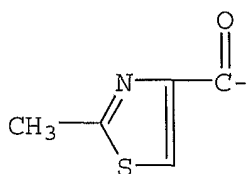
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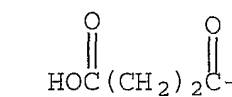
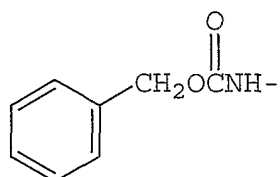
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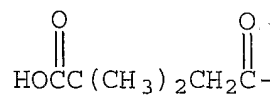
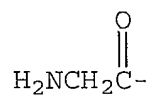
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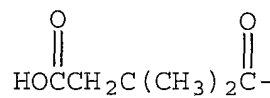


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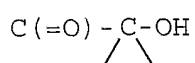
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- 23 -

In most preferred embodiments, R¹ is selected from the group consisting of cyclopentyl, tetrahydrofuryl, indanyl, norbornyl, phenethyl, and phenylbutyl; R² is selected from the group consisting of methyl and difluoromethyl; R³ is selected from the group consisting of CO₂CH₃, C(=O)CH₂OH, C(=O)CH(CH₃)-OH, C(=O)C(CH₃)₂OH, and

10



R⁴ is hydrogen or methyl; R⁵ and R⁶, independently, are hydrogen or methyl, or R⁵ and R⁶ together form a 5-membered or 6-membered ring; and R¹⁰ is hydrogen.

The present invention includes all possible stereoisomers and geometric isomers of compounds of structural formula (II), and includes not only racemic compounds but also the optically active isomers as well. When a compound of structural formula (II) is desired as a single enantiomer, it can be obtained either by resolution of the final product or by stereospecific synthesis from either isomerically pure starting material or use of a chiral auxiliary reagent, for example, see Z. Ma et al., *Tetrahedron: Asymmetry*, 8(6), pages 883-888 (1997). Resolution of the final product, an intermediate, or a starting material can be achieved by any suitable method known in the art. Additionally, in situations where tautomers of the compounds of structural formula (II) are possible, the present invention is intended to include all tautomeric

- 24 -

forms of the compounds. As demonstrated hereafter, specific stereoisomers exhibit an exceptional ability to inhibit PDE4 without manifesting the adverse CNS side effects typically associated with PDE4 inhibitors.

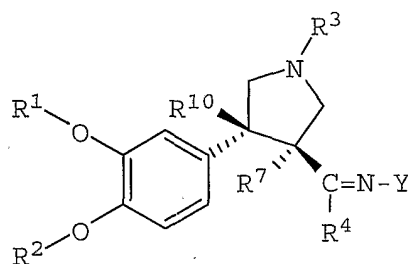
In particular, it is generally accepted that biological systems can exhibit very sensitive activities with respect to the absolute stereochemical nature of compounds. (See, E.J. Ariens, *Medicinal Research Reviews*, 6:451-466 (1986); E.J. Ariens, *Medicinal Research Reviews*, 7:367-387 (1987); K.W. Fowler, *Handbook of Stereoisomers: Therapeutic Drugs*, CRC Press, edited by Donald P. Smith, pp. 35-63 (1989); and S.C. Stinson, *Chemical and Engineering News*, 75:38-70 (1997).)

For example, rolipram is a stereospecific PDE4 inhibitor that contains one chiral center. The (-)-enantiomer of rolipram has a higher pharmacological potency than the (+)-enantiomer, which could be related to its potential antidepressant action. Schultz et al., *Naunyn-Schmiedeberg's Arch Pharmacol*, 333:23-30 (1986). Furthermore, the metabolism of rolipram appears stereospecific with the (+)-enantiomer exhibiting a faster clearance rate than the (-)-enantiomer. Krause et al., *Xenobiotica*, 18:561-571 (1988). Finally, a recent observation indicated that the (-)-enantiomer of rolipram (R-rolipram) is about ten-fold more emetic than the (+)-enantiomer (S-rolipram). A. Robichaud et al., *Neuropharmacology*, 38:289-297 (1999). This observation is not easily reconciled with differences in test animal disposition to rolipram isomers and the ability of rolipram to inhibit the PDE4 enzyme. The

- 25 -

compounds of the present invention can have three chiral centers. As shown below, compounds of a specific stereochemical orientation exhibit similar PDE4 inhibitory activity and pharmacological activity, but altered CNS toxicity and emetic potential.

Accordingly, preferred compounds of the present invention have the structural formula (III):



(III)

The compounds of structural formula (III) are potent and selective PDE4 inhibitors, and do not manifest the adverse CNS effects and emetic potential demonstrated by stereoisomers of a compound of structural formula (III).

Compounds of structural formula (II) which contain acidic moieties can form pharmaceutically acceptable salts with suitable cations. Suitable pharmaceutically acceptable cations include alkali metal (e.g., sodium or potassium) and alkaline earth metal (e.g., calcium or magnesium) cations. The pharmaceutically acceptable salts of the compounds of structural formula (II), which contain a basic center, are acid addition salts formed with pharmaceutically acceptable acids. Examples include the

- 26 -

hydrochloride, hydrobromide, sulfate or bisulfate, phosphate or hydrogen phosphate, acetate, benzoate, succinate, fumarate, maleate, lactate, citrate, tartrate, gluconate, methanesulfonate, benzene-
5 sulphonate, and p-toluenesulphonate salts. In light of the foregoing, any reference to compounds of the present invention appearing herein is intended to include compounds of structural formula (II), as well as pharmaceutically acceptable salts and sol-
10 vates thereof.

The compounds of the present invention can be therapeutically administered as the neat chemical, but it is preferable to administer compounds of structural formula (II) as a pharmaceutical composition or formulation. Accordingly, the present
15 invention further provides for pharmaceutical formulations comprising a compound of structural formula (II), together with one or more pharmaceutically acceptable carriers and, optionally, other therapeutic and/or prophylactic ingredients. The
20 carriers are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

25 In particular, a selective PDE4 inhibitor of the present invention is useful alone or in combination with a second antiinflammatory therapeutic agent, for example, a therapeutic agent targeting TNF α , such as ENBREL[®] or REMICADE[®], which have
30 utility in treating rheumatoid arthritis. Likewise, therapeutic utility of IL-1 antagonism has also been shown in animal models for rheumatoid arthritis. Thus, it is envisioned that IL-1 antagonism, in

- 27 -

combination with PDE4 inhibition, which attenuates TNF α , would be efficacious.

The present PDE4 inhibitors are useful in the treatment of a variety of allergic, autoimmune, and inflammatory diseases.

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

In particular, inflammation is a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (i.e., sequester) both the injurious agent and the injured tissue. The term "inflammatory disease," as used herein, means any disease in which an excessive or unregulated inflammatory response leads to excessive inflammatory symptoms, host tissue damage, or loss of tissue function. Additionally, the term "autoimmune disease," as used herein, means any group of disorders in which tissue injury is associated with humoral or cell-mediated responses to the body's own constituents. The term "allergic disease," as used herein, means any symptoms, tissue damage, or loss of tissue function resulting from allergy. The term "arthritic disease," as used herein, means any of a large family of diseases that are characterized by inflammatory lesions of the joints attributable to a variety of etiologies. The term "dermatitis," as used herein, means any of a large family of diseases of the skin that are characterized by inflammation

- 28 -

of the skin attributable to a variety of etiologies. The term "transplant rejection," as used herein, means any immune reaction directed against grafted tissue (including organ and cell (e.g., bone marrow)), characterized by a loss of function of the grafted and surrounding tissues, pain, swelling, leukocytosis and thrombocytopenia.

The present invention also provides a method of modulating cAMP levels in a mammal, as well as a method of treating diseases characterized by elevated cytokine levels.

The term "cytokine," as used herein, means any secreted polypeptide that affects the functions of other cells, and that modulates interactions between cells in the immune or inflammatory response. Cytokines include, but are not limited to monokines, lymphokines, and chemokines regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a monocyte, however, many other cells produce monokines, such as natural killer cells, fibroblasts, basophils, neutrophils, endothelial cells, brain astrocytes, bone marrow stromal cells, epidermal keratinocytes, and B-lymphocytes. Lymphokines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include, but are not limited to, interleukin-1 (IL-1), interleukin-6 (IL-6), Tumor Necrosis Factor alpha (TNF α), and Tumor Necrosis Factor beta (TNF β).

The present invention further provides a method of reducing TNF levels in a mammal, which comprises administering an effective amount of a compound of structural formula (II) to the mammal.

- 29 -

The term "reducing TNF levels," as used herein, means either:

a) decreasing excessive *in vivo* TNF levels in a mammal to normal levels or below normal levels by inhibition of the *in vivo* release of TNF by all cells, including but not limited to monocytes or macrophages; or

b) inducing a down-regulation, at the translational or transcription level, of excessive *in vivo* TNF levels in a mammal to normal levels or below normal levels; or

c) inducing a down-regulation, by inhibition of the direct synthesis of TNF as a postranslational event.

Moreover, the compounds of the present invention are useful in suppressing inflammatory cell activation. The term "inflammatory cell activation," as used herein, means the induction by a stimulus (including, but not limited to, cytokines, antigens or auto-antibodies) of a proliferative cellular response, the production of soluble mediators (including but not limited to cytokines, oxygen radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatibility antigens or cell adhesion molecules) in inflammatory cells (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes, polymorphonuclear leukocytes, mast cells, basophils, eosinophils, dendritic cells, and endothelial cells). It will be appreciated by persons skilled in the art that the activation of one or a combina-

- 30 -

tion of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory condition.

The compounds of the present invention
5 also are useful in causing airway smooth muscle relaxation, bronchodilation, and prevention of bronchoconstriction.

The compounds of the present invention, therefore, are useful in treating such diseases as
10 arthritic diseases (such as rheumatoid arthritis), osteoarthritis, gouty arthritis, spondylitis, thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome,
15 asthma, chronic bronchitis, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, eosinophilic granuloma, adult (acute) respiratory distress syndrome (ARDS), chronic pulmonary inflammatory disease (such as chronic obstructive
20 pulmonary disease), silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium, brain or extremities, brain or spinal cord injury due to minor trauma, fibrosis including cystic fibrosis, keloid formation, scar tissue formation, atherosclerosis, autoimmune diseases, such as systemic
25 lupus erythematosus (SLE) and transplant rejection disorders (e.g., graft vs. host (GvH) reaction and allograft rejection), chronic glomerulonephritis, inflammatory bowel diseases, such as Crohn's disease
30 and ulcerative colitis, proliferative lymphocytic diseases, such as leukemias (e.g. chronic lymphocytic leukemia; CLL) (see Mentz et al., *Blood* 88,

- 31 -

pp. 2172-2182 (1996)), and inflammatory dermatoses, such as atopic dermatitis, psoriasis, or urticaria.

Other examples of such diseases or related conditions include cardiomyopathies, such as congestive heart failure, pyrexia, cachexia, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS-related complex), cerebral malaria, osteoporosis and bone resorption diseases, and fever and myalgias due to infection. In addition, the compounds of the present invention are useful in the treatment of diabetes insipidus and central nervous system disorders, such as depression and multi-infarct dementia.

Compounds of the present invention also have utility outside of that typically known as therapeutic. For example, the present compounds can function as organ transplant preservatives (see Pinsky et al., *J. Clin. Invest.*, 92, pp. 2994-3002 (1993)) as well.

Selective PDE4 inhibitors also can be useful in the treatment of erectile dysfunction, especially vasculogenic impotence (Doherty, Jr. et al. U.S. Patent No. 6,127,363), diabetes insipidus (*Kidney Int.*, 37, p. 362, (1990); *Kidney Int.*, 35, p. 494, (1989)), and central nervous system disorders, such as multiinfarct dementia (Nicholson, *Psychopharmacology*, 101, p. 147 (1990)), depression (Eckman et al., *Curr. Ther. Res.*, 43, p. 291 (1988)), anxiety and stress responses (*Neuropharmacology*, 38, p. 1831 (1991)), cerebral ischemia (*Eur. J. Pharmacol.*, 272, p. 107 (1995)), tardive dyskinesia (*J. Clin. Pharmacol.*, 16, p. 304 (1976)),

- 32 -

Parkinson's disease (see *Neurology*, 25, p. 722 (1975); *Clin. Exp. Pharmacol, Physiol.*, 26, p. 421 (1999)), and premenstrual syndrome. With respect to depression, PDE4-selective inhibitors show efficacy in a variety of animal models of depression such as the "behavioral despair" or Porsolt tests (*Eur. J. Pharmacol.*, 47, p. 379 (1978); *Eur. J. Pharmacol.*, 57, p. 431 (1979); *Antidepressants: neurochemical, behavioral and clinical perspectives*, Enna, Malick, and Richelson, eds., Raven Press, p. 121 (1981)), and the "tail suspension test" (*Psychopharmacology*, 85, p. 367 (1985)). Recent research findings show that chronic *in vivo* treatment by a variety of antidepressants increase the brain-derived expression of PDE4 (*J. Neuroscience*, 19, p. 610 (1999)). Therefore, a selective PDE4 inhibitor can be used alone or in conjunction with a second therapeutic agent in a treatment for the four major classes of antidepressants: electroconvulsive procedures, monoamine oxidase inhibitors, and selective reuptake inhibitors of serotonin or norepinephrine. Selective PDE4 inhibitors also can be useful in applications that modulate bronchodilatory activity via direct action on bronchial smooth muscle cells for the treatment of asthma.

Compounds and pharmaceutical compositions suitable for use in the present invention include those wherein the active ingredient is administered to a mammal 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 symptoms of, the subject being treated.

- 33 -

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.

5 The term "mammal" as used herein includes males and females, and encompasses humans, domestic animals (e.g., cats, dogs), livestock (e.g., cattle, horses, swine), and wildlife (e.g., primates, large cats, zoo specimens).

10 A "therapeutically effective dose" refers 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 cul-
15 tures or experimental animals, e.g., for determining 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
20 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 dosage range for use in humans. The dosage of such compounds prefer-
25 ably 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.

30 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

- 34 -

to provide plasma levels of the active moiety which are sufficient to maintain the therapeutic effects.

As appreciated by persons skilled in the art, reference herein to treatment extends to prophylaxis, as well as to treatment of established diseases or symptoms. It is further appreciated that the amount of a compound of the invention required for use in treatment varies with the nature of the condition being treated, and with the age and the condition of the patient, and is ultimately determined by the attendant physician or veterinarian. In general, however, doses employed for adult human treatment typically are in the range of 0.001 mg/kg to about 100 mg/kg per day. The desired dose can be conveniently administered in a single dose, or as multiple doses administered at appropriate intervals, for example as two, three, four or more subdoses per day. In practice, the physician determines the actual dosing regimen which is most suitable for an individual 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 there can be individual instances in which higher or lower dosages are merited, and such are within the scope of the present invention.

Formulations of the present invention can be administered in a standard manner for the treatment of the indicated diseases, such as orally, parenterally, transmucosally (e.g., sublingually or via buccal administration), topically, transdermally, rectally, via inhalation (e.g., nasal or deep lung inhalation). Parenteral administration in-

- 35 -

cludes, but is not limited to intravenous, intra-
arterial, intraperitoneal, subcutaneous, intramuscu-
lar, intrathecal, and intraarticular. Parenteral
administration also can be accomplished using a high
5 pressure technique, like POWDERJECT™.

For buccal administration, the composition
can be in the form of tablets or lozenges formulated
in conventional manner. For example, tablets and
capsules for oral administration can contain conven-
10 tional excipients such as binding agents (for
example, syrup, accacia, gelatin, sorbitol, traga-
canth, mucilage of starch or polyvinylpyrrolidone),
fillers (for example, lactose, sugar, microcrystal-
line, cellulose, maize-starch, calcium phosphate or
15 sorbitol), lubricants (for example, magnesium,
stearate, stearic acid, talc, polyethylene glycol or
silica), disintegrants (for example, potato starch
or sodium starch glycollate), or wetting agents (for
example, sodium lauryl sulfate). The tablets can be
20 coated according to methods well known in the art.

Alternatively, the compounds of the pres-
ent invention can be incorporated into oral liquid
preparations such as aqueous or oily suspensions,
solutions, emulsions, syrups, or elixirs, for
25 example. Moreover, formulations containing these
compounds can be presented as a dry product for
constitution with water or other suitable vehicle
before use. Such liquid preparations can contain
conventional additives, such as suspending agents,
30 such as sorbitol syrup, methyl cellulose, glucose/-
sugar syrup, gelatin, hydroxyethylcellulose,
hydroxypropylmethylcellulose, carboxymethyl cellu-
lose, aluminum stearate gel, and hydrogenated edible

- 36 -

fats; emulsifying agents, such as lecithin, sorbitan monooleate, or acacia; nonaqueous vehicles (which can include edible oils), such as almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol; and preservatives, such as methyl or propyl p-hydroxybenzoate and sorbic acid.

Such preparations also can be formulated as suppositories, e.g., containing conventional suppository bases, such as cocoa butter or other glycerides. Compositions for inhalation typically can be provided in the form of a solution, suspension, or emulsion that can be administered as a dry powder or in the form of an aerosol using a conventional propellant, such as dichlorodifluoromethane or trichlorofluoromethane. Typical topical and transdermal formulations comprise conventional aqueous or nonaqueous vehicles, such as eye drops, creams, ointments, lotions, and pastes, or are in the form of a medicated plaster, patch, or membrane.

Additionally, compositions of the present invention can be formulated for parenteral administration by injection or continuous infusion. Formulations for injection can be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulation agents, such as suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle (e.g., sterile, pyrogen-free water) before use.

A composition in accordance with the present invention also can be formulated as a depot preparation. Such long acting formulations can be

- 37 -

administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Accordingly, the compounds of the invention can be formulated with suitable polymeric or hydrophobic materials (e.g., an emulsion in an acceptable oil), ion exchange resins, or as sparingly soluble derivatives (e.g., a sparingly soluble salt).

For veterinary use, a compound of formula (II), or nontoxic salts thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can 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 compound of the formula (II), together with a pharmaceutically acceptable diluent or carrier therefor. There is further provided by the present invention a process of preparing a pharmaceutical composition comprising a compound of formula (II), which process comprises mixing a compound of formula (II), together with a pharmaceutically acceptable diluent or carrier therefor.

Specific, nonlimiting examples of compounds of structural formula (II) are provided below, the synthesis of which were performed in accordance with the procedures set forth below.

Generally, compounds of structural formula (II) can be prepared according to the following synthetic scheme. In the scheme described below, it is understood in the art that protecting groups can

- 38 -

be employed where necessary in accordance with general principles of synthetic chemistry. These protecting groups are removed in the final steps of the synthesis under basic, acidic, or hydrogenolytic conditions which are readily apparent to those skilled in the art. By employing appropriate manipulation and protection of any chemical functionalities, synthesis of compounds of structural formula (II) not specifically set forth herein can be accomplished by methods analogous to the schemes set forth below.

Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. All reactions and chromatography fractions were analyzed by thin-layer chromatography on 250-mm silica gel plates, visualized with UV (ultraviolet) light or I₂ (iodine) stain. Products and intermediates were purified by flash chromatography, or reverse-phase HPLC.

The compounds of general structural formula (II) can be prepared, for example, as set forth in the following synthetic scheme. Other synthetic routes also are known to persons skilled in the art. The following reaction scheme provides a compound of structural formula (II), wherein R¹ and R², i.e., C₂H₅ and cyclopentyl, are determined by the starting materials. Proper selection of other starting materials, or performing conversion reactions on intermediates and examples, provide compounds of general structural formula (II) having other recited R¹ through R¹¹ substituents.

- 39 -

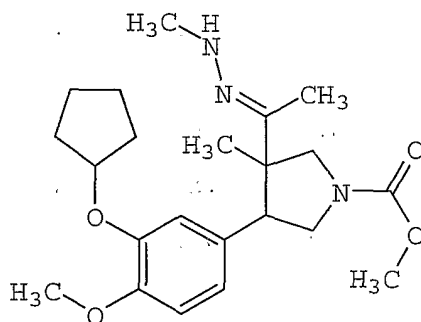
The following illustrates the synthesis of various intermediates and compounds of structural formula (II). The following examples are provided for illustration and should not be construed as
5 limiting.

Example 1

Preparation of (S)-4-(3-Cyclopentyloxy-4-methoxyphenyl)-3-methyl-3-[1-(methylhydrazono)ethyl]-pyrrolidine-1-carboxylic acid methyl ester (1a)
10 and
(R)-4-(3-Cyclopentyloxy-4-methoxyphenyl)-3-methyl-3-[1-(methylhydrazono)ethyl]-pyrrolidine-1-carboxylic acid methyl ester (1b)
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To a stirred solution of (R)-3-acetyl-4-(3-cyclopentyloxy-4-methoxyphenyl)-3-methyl-pyrrolidine-1-carboxylic acid methyl ester (see Example 12 of Feldman et al. U.S. Patent No. 5,665,654, incorporated herein by reference) (or (R)-3-acetyl-4-(3-cyclopentyloxy-4-methoxyphenyl)-3-methyl-pyrrolidine-1-carboxylic acid methyl ester) (0.133 mmol, 50 mg) in methanol (2 mL) with catalytic amount of acetic acid (20 μ L) was added methyl hydrazine (0.147 mmol, 7.8 μ L). The reaction was heated
40 gently for 36 hours, and three more portions of

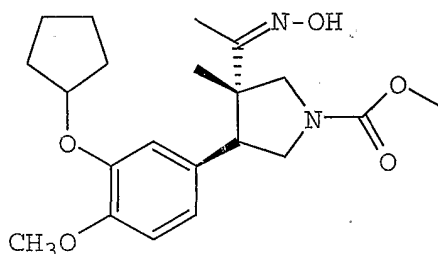
- 40 -

methyl hydrazine were added. The resulting hydrazone was purified by HPLC. The named product was confirmed by HNMR.

^1H NMR (300 MHz, CDCl_3) δ (ppm): 6.8 (d, 1H); 6.68 (m, 2H); 4.73 (bm, 1H); 3.91-4.03 (m, 2H); 3.83 (s, 3H); 3.75 (s, 3H); 3.47-3.69 (m, 2H); 2.98 (s, 3H); 1.83-1.92 (bm, 8H); 1.6 (bm, 2H); 1.0 (s, 3H).

Example 2

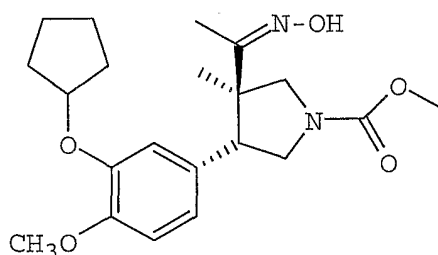
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(a)

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(b)

To a solution of (3S,4R)-3-acetyl-4-(3-cyclopentyloxy-4-methoxyphenyl)-3-methyl-pyrrolidine-1-carboxylic acid methyl ester (50 mg, 0.133 mmol) in 0.5 mL ethanol was added hydroxylamine hydrochloride (103 mg, 1.5 mmol) and 0.1 ml water.

- 41 -

The suspension was heated to 50°C and stirred overnight. The solution then was cooled to room temperature and concentrated under reduced pressure.

Purification of HPLC on a C18 column (Luna 10 μ ,
5 C128, 250x10 mm) using a gradient elution of 50-100% acetonitrile-water (0.05% TFA) gave product 2(a) as a white powder upon lyophilization (24.4 mg, 47% yield). Example 2(b) was prepared by an identical procedure.

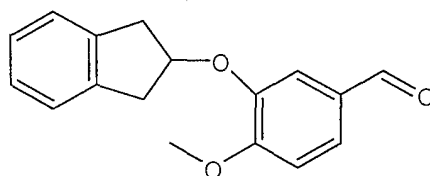
10 ¹H NMR (300 MHz, CDCl₃) δ (ppm): 6.84-6.76 (m, 1H, aromatic); 6.70-6.59 (m, 2H, aromatic); 4.87-4.57 (m, 1H); 3.96-3.60 (m, 3H); 3.83 (s, 3H, OCH₃); 3.75 (s, 3H, OCH₃); 3.59-3.45 (m, 1H); 3.40-3.21 (m, 1H);
15 1.92 (s, 3H, CH₃); 1.90-1.48 (m, 8H, cyclopentyl);
0.96 (s, 3H, CH₃).

Intermediate 1

Preparation of 3-(Indan-2-yloxy)-4-methoxybenzaldehyde

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A solution of 3-hydroxy-4-methoxy-benzaldehyde (15.2 g, 100 mmol, 1 eq), 2-indanol (12.1 g, 90 mmol, 0.9 eq), and triphenylphosphine (26.2 g, 100 mmol, 1 eq) in dry THF (300 mL) was treated dropwise with diisopropylazodicarboxylate (19.6 mL, 100 mmol, 1 eq).
35 The reaction mixture was stirred at reflux for 16 hours, then cooled and diluted with diethyl ether

- 42 -

(500 mL). The solution was washed with water (2 x 150 mL), 1 M NaOH (4 x 125 mL), and saturated NaCl (sodium chloride) (2 x 100 mL), dried with CH₂Cl₂, then concentrated to provide a syrup, which solidified upon standing. The solid was suspended in diethyl ether (350 mL) and stirred overnight to break up all chunks. The solid was collected by vacuum filtration and recrystallized from ethanol/water (21.4 g). The ethereal filtrate was concentrated and purified by flash chromatography (silica gel, 7.5 x 36 cm Biotage KP-Sil column, eluted with 25% ethyl acetate in heptane) to yield an additional 5 g of product. ¹H NMR (300 MHz, CDCl₃) δ 9.86 (s, 1H), 7.49-7.44 (m, 2H), 7.25-7.16 (m, 4H), 6.97 (d, J=8.7 Hz, 1H), 5.29-5.22 (m, 1H), 3.89 (s, 1H), 3.45 (dd, J=16.7, 6.6 Hz, 2H), 3.24 (dd, J=16.7, 3.6 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 190.9, 155.5, 147.9, 140.4, 130.0, 126.9, 126.8, 124.7, 112.1, 111.0, 78.9, 56.1, 39.7.

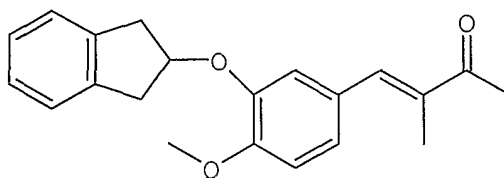
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Intermediate 2

(E)-4-[3-(Indan-2-yloxy)-4-methoxyphenyl]-3-methylbut-3-en-2-one

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30



A solution of Intermediate 1 (0.14 mol, 1 eq) and 2-butanone (50 mL, 0.56 mol, 4 eq) in dry THF (50 mL) was cooled to -4°C. Hydrogen chloride gas was passed through the well-stirred solution for several

- 43 -

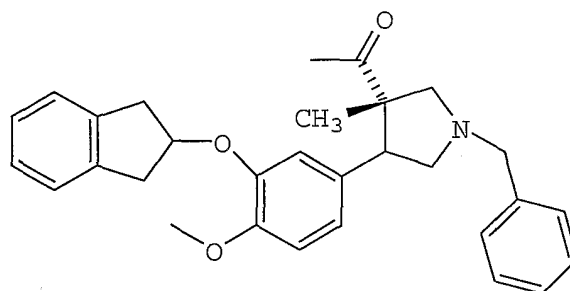
minutes, and the reaction mixture was capped and stored at -4°C for 16 hours. The mixture was poured into a well stirred solution of ice-cold saturated NaHCO_3 (about 2 L). If necessary, the pH was
5 adjusted to >7 with saturated NaHCO_3 , and the mixture was extracted with ethyl acetate (3 x 300 mL). The ethyl acetate layer was washed with NaHCO_3 (2 x 200 mL), water (2 x 200 mL), and saturated NaCl (2 x 200 mL), dried with CH_2Cl_2 , then concentrated to a syrup.
10 The crude mixture was purified by flash chromatography (silica gel, 7.5 x 36 cm Biotage KP-Sil column, eluted with 25% ethyl acetate in heptane, to yield a solid product.

^1H NMR (300 MHz, CDCl_3) δ 7.49-7.43 (m, 2H), 7.26-
15 7.15 (m, 5H), 6.90 (d, $J=8.2$ Hz, 1H), 5.25-5.16 (M, 1H), 3.85 (s, 3H), 3.38 (dd, $J=16.7, 6.5$ Hz, 2H), 3.25 (dd, $J=16.7, 3.8$ Hz, 2H), 2.45 (s, 3H), 2.10 (d, $J=1.1$ Hz).

20 Intermediate 3

1-{(3SR, 4RS)-1-Benzyl-4-[3-(indan-2-yloxy)-4-methoxyphenyl]-3-methyl-pyrrolidin-3-yl}-ethanone

25



30

(racemic)

- 44 -

A solution of Intermediate 2 (50.6 mmol, 1 eq) and N-methoxymethyl-N-benzyl-trimethylsilylmethylamine (50.6 mmol, 1 eq) in dichloromethane (85 mL) at 0°C was treated dropwise with a solution of trifluoroacetic acid (1 M in dichloromethane, 5 mL, 5.1 mmol, 0.1 eq). After stirring at the same temperature for 30 minutes, the reaction mixture was stirred at room temperature for 16 hours. The solution was treated with additional N-methoxymethyl-N-benzyltrimethylsilylmethylamine (25.3 mmol, 0.5 eq), stirred 1 hour at room temperature, and treated for a third time with N-methoxymethyl-N-benzyl-trimethylsilylmethylamine (25.3 mmol, 0.5 eq). The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate (500 mL). This solution was washed with 1 N HCl (2 x 60 mL with 10 mL saturation NaCl added), water (250 mL), 1 M NaOH (250 mL), water (250 mL), saturated NaCl (2 x 100 mL), dried with CH₂Cl₂, and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 7.5 x 36 cm Biotage KP-Sil column, eluted with 5-10% diethyl ether in dichloromethane) to yield the product.

¹H NMR (300 MHz, CDCl₃) δ 7.38-7.16 (m, 9H), 6.88 (br s, 1H), 6.78 (br s, 2H), 5.18-5.13 (m, 1H), 3.82-3.73 (m, 2H), 3.79 (s, 3H), 3.60 (d, J=13.0 Hz, 1H), 3.41-3.17 (m, 4H), 3.14 (d, J=9.7 Hz, 1H), 3.05 (t, J=8.3 Hz, 1H), 2.84 (t, J=8.3 Hz, 1H), 2.44 (d, J=9.7 Hz, 1H), 2.24 (s, 3H), 0.86 (s, 3H).

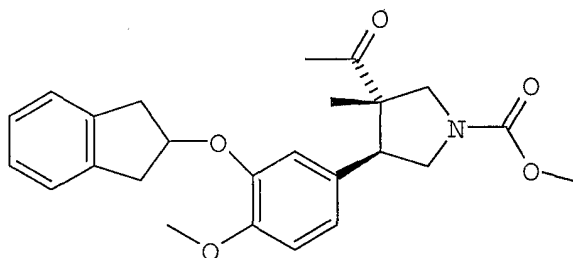
- 45 -

Intermediate 4

trans-(±)-3-Acetyl-4-[3-(indan-2-yloxy)-4-methoxy-phenyl]-3-methyl-pyrrolidine-1-carboxylic acid methyl ester

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racemic

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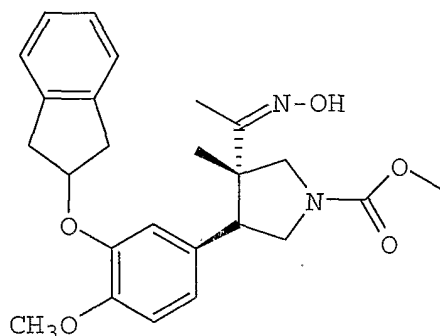
A solution of Intermediate 3 (40.5 mmol, 1 eq) in acetonitrile (150 mL) was treated with methyl chloroformate (15.6 mL, 202.5 mmol, 5 eq), and the mixture was stirred at reflux 1 hour. The reaction mixture was concentrated, and the residue was purified by flash chromatography (silica gel, 7.5 x 36 cm Biotage KP-Sil column, eluted with 50-60% ethyl acetate in heptane) to afford the product.

^1H NMR (300 MHz, CDCl_3) δ 7.24-7.16 (m, 4H), 6.82 (d, $J=8.8$ Hz, 1H), 6.75-6.72 (m, 2H), 5.18-5.10 (m, 1H), 3.91 (t, $J=11.2$ Hz, 1H), 3.80 (s, 3H), 3.77-3.65 (m, 3H), 3.74 (s, 3H), 3.42-3.16 (m, 5H), 2.17 (d, $J=6.8$ Hz, 3H), 1.04 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 210.1/209.9, 155.3, 149.4, 146.9/146.8, 140.5/140.4, 130.5/130.0, 126.7, 124.7, 121.3/121.1, 116.1/115.8, 111.9, 79.2, 58.2/57.4, 55.9, 54.7/54.2, 52.6, 50.2/50.0, 48.5/48.1, 39.7, 26.6/26.5, 17.8.

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Example 3

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To a solution of Intermediate 4 (102.4 mg, 0.24 mmol) in 0.5 mL ethanol was added hydroxylamine hydrochloride (83.8 mg, 1.2 mmol) and 0.1 mL water. The suspension was heated to 50°C and stirred overnight. The solution then was cooled to room temperature and concentrated under reduced pressure. Purification by HPLC on a C18 column (Luna 10 μ , C18, 250x10 mm) using a gradient elution of 50-100% acetonitrile-water (0.05% TFA) gave solid product (60 mg, 57% yield).

^1H NMR (300 MHz, CDCl_3) δ (ppm): 7.26-7.16 (m, 4H, aromatic), 6.83-6.77 (m, 1H, aromatic), 6.74-6.71 (m, 2H), 5.14-5.11 (m, 1H), 3.92-3.64 (m, 3H), 3.80 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.58-3.48 (m, 1H), 3.38-3.16 (m, 5H), 1.92 (s, 3H, CH_3), 0.96 (s, 3H, CH_3).

The compounds of structural formula (II) were tested for an ability to inhibit PDE4. The ability of a compound to inhibit PDE4 activity is related to the IC_{50} value for the compound, i.e., the concentration of inhibitor required for 50% inhibi-

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- 47 -

tion of enzyme activity. The IC_{50} value for compounds of structural formula (II) were determined using recombinant human PDE4.

The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE4 of less than about 50 μM , and preferably less than about 25 μM , and more preferably less than about 15 μM . The compounds of the present invention typically exhibit an IC_{50} value against recombinant human PDE4 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 PDE4 inhibitor has an IC_{50} of about 1 nM to about 15 μM .

The IC_{50} values for the compounds were determined from concentration-response curves typically using concentrations ranging from 0.1 pM to 500 μM . Tests against other PDE enzymes using standard methodology, as described in Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996), also showed that compounds of the present invention are highly selective for the cAMP-specific PDE4 enzyme.

The compounds of structural formula (II) also were tested for an ability to reduce $TNF\alpha$ secretion in human peripheral blood lymphocytes. The ability to reduce $TNF\alpha$ secretion is related to the EC_{50} values (i.e., the effective concentration of the compound capable of inhibiting 50% of the total $TNF\alpha$).

The compounds of the present invention typically exhibit an EC_{50} value of less than about 50 μM , and preferably less than about 25 μM , and more preferably less than about 15 μM . The compounds of the present invention preferably exhibit a PBL/ $TNF\alpha$

- 48 -

EC₅₀ value of less than about 1 μ M, and often less than about 0.05 μ M. To achieve the full advantages of the present invention, a present PDE4 inhibitor has an EC₅₀ value of about 1 nM to about 500 nM.

5 The production of recombinant human PDEs and the IC₅₀ and EC₅₀ determinations can be accomplished by well-known methods in the art. Exemplary methods are described as follows:

10 **EXPRESSION OF HUMAN PDEs**

Expression in Baculovirus-Infected *Spodoptera frugiperda* (Sf9) Cells

15 Baculovirus transfer plasmids were constructed using either pBlueBacIII (Invitrogen) or pFastBac (BRL-Gibco). The structure of all plasmids was verified by sequencing across the vector junctions and by fully sequencing all regions generated by PCR. Plasmid pBB-PDE1A3/6 contained the complete open reading frame of PDE1A3 (Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996)) in pBlueBacIII. Plasmid Hcam3aBB contained the complete open reading frame of PDE1C3 (Loughney et al. 20 (1996)) in pBlueBacIII. Plasmid pBB-PDE3A contained the complete open reading frame of PDE3A (Meacci et al., *Proc. Natl. Acad. Sci., USA*, 89, pp. 3721-3725 (1992)) in pBlueBacIII.

25 Recombinant virus stocks were produced using either the MaxBac system (Invitrogen) or the FastBac system (Gibco-BRL) according to the manufacturer's protocols. In both cases, expression of recombinant human PDEs in the resultant viruses was

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- 49 -

driven off the viral polyhedron promoter. When using the MaxBac[®] system, virus was plaque purified twice in order to insure that no wild type (occ+) virus contaminated the preparation. Protein expression was carried out as follows. Sf9 cells were grown at 27°C in Grace's Insect culture medium (Gibco-BRL) supplemented with 10% fetal bovine serum, 0.33% TC yeastolate, 0.33% lactalbumin hydrolysate, 4.2 mM NaHCO₃, 10 µg/mL gentamycin, 100 units/mL penicillin, and 100 µg/mL streptomycin. Exponentially growing cells were infected at a multiplicity of approximately 2 to 3 virus particles per cell and incubated for 48 hours. Cells were collected by centrifugation, washed with nonsupplemented Grace's medium, and quick-frozen for storage.

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

- 50 -

hours, YEP medium-containing glycerol was added to a final concentration of 2X YET/3% glycerol. Approximately 24 hr later, cells were harvested, washed, and stored at -70°C.

5

HUMAN PHOSPHODIESTERASE PREPARATIONS

Phosphodiesterase Activity Determinations

10 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
15. [32P]cAMP or [32P]cGMP to the corresponding [32P]5'-AMP or [32P]5'-GMP in proportion to the amount of PDE activity present. The [32P]5'-AMP or [32P]5'-GMP then was quantitatively converted to free [32P]phosphate and unlabeled adenosine or guanosine
20. by the action of snake venom 5'-nucleotidase. Hence, the amount of [32P]phosphate liberated is proportional to enzyme activity. The assay was performed at 30°C in a 100 μ L reaction mixture containing (final concentrations) 40 mM Tris HCl (pH
25 8.0), 1 μ M ZnSO₄, 5 mM MgCl₂, and 0.1 mg/mL bovine serum albumin (BSA). Alternatively, in assays assessing PDE1-specific activity, incubation mixtures further incorporated the use of 0.1 mM CaCl₂ and 10 μ g/mL calmodulin. PDE enzyme was present in
30 quantities that yield <30% total hydrolysis of substrate (linear assay conditions). The assay was initiated by addition of substrate (1 mM [32P]cAMP or cGMP), and the mixture was incubated for 12

- 51 -

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 μL of
5 activated charcoal (25 mg/mL suspension in 0.1 M NaH_2PO_4 , pH 4). After centrifugation (750 X g for 3 minutes) to sediment the charcoal, a sample of the supernatant was taken for radioactivity determination in a scintillation counter and the PDE activity
10 was calculated.

Inhibitor analyses were performed similarly to the method described in Loughney et al., *J. Biol. Chem.*, 271, pp. 796-806 (1996), except both cGMP and cAMP were used, and substrate concentrations were kept below 32 nM, which is far below the
15 K_m of the tested PDEs.

Human PDE4A, 4B, 4C, 4D Preparations

20 Preparation of PDE4A from *S. cerevisiae*

Yeast cells (50 g of yeast strain YI26 harboring HDUN1.46) were thawed at room temperature by mixing with 50 mL of Lysis Buffer (50 mM MOPS pH
25 7.5, 10 μM ZnSO_4 , 2 mM MgCl_2 , 14.2 mM 2-mercaptoethanol, 5 $\mu\text{g}/\text{mL}$ each of pepstatin, leupeptin, aprotinin, 20 $\mu\text{g}/\text{mL}$ each of calpain inhibitors I and II, and 2 mM benzamidine HCl). Cells were lysed in a French® pressure cell (SLM-Aminco®, Spectronic
30 Instruments) at 10°C. The extract was centrifuged in a Beckman JA-10 rotor at 9,000 rpm for 22 minutes at 4°C. The supernatant was removed and centrifuged

- 52 -

in a Beckman TI45 rotor at 36,000 rpm for 45 minutes at 4°C.

PDE4A was precipitated from the high-speed supernatant by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. The precipitated proteins containing PDE4A were collected via centrifugation in a Beckman JA-10 rotor at 9,000 rpm for 22 minutes. The precipitate was resuspended in 50 mL of Buffer G (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 5 mM MgCl₂, 100 mM NaCl, 14.2 mM 2-mercaptoethanol, 2 mM benzamidine HCl, 5 μ g/mL each of leupeptin, pepstatin, and aprotinin, and 20 μ g/mL each of calpain inhibitors I and II) and passed through a 0.45 μ m filter.

The resuspended sample (50 to 100 mL) was loaded onto a 5 X 100 cm column of Pharmacia SEPHACRYL® S-300 equilibrated in Buffer G. Enzyme activity was eluted at a flow rate of 2 mL/min and pooled for later fractionation.

The PDE4A isolated from gel filtration chromatography was applied to a 1.6 X 20 cm column of Sigma Cibacron Blue Agarose-type 300 (10 mL) equilibrated in Buffer A (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 5 mM MgCl₂, 14.2 mM 2-mercaptoethanol, and 100 mM benzamidine HCl). The column was washed in succession with 50 to 100 mL of Buffer A, 20 to 30 mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL of Buffer A containing 1.5 M NaCl, and 10 to 20 mL of Buffer C (50 mM Tris HCl pH 8, 10 μ M ZnSO₄, 14.2 mM 2-mercaptoethanol, and 2 mM benzamidine HCl). The enzyme was eluted with 20 to 30 mL of Buffer C containing 20 mM cAMP.

- 53 -

The PDE activity peak was pooled, and precipitated with ammonium sulfate (0.33 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.5, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl), and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific activities of about 10 to 40 μ mol cAMP hydrolyzed per minute per milligram protein.

15 Preparation of PDE4B from *S. cerevisiae*

Yeast cells (150 g of yeast strain YI23 harboring HDUN2.32) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a Bead-Beater[®], and the cells lysed by rapid mixing for 6 cycles of 30 seconds each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE4B was precipitated by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stir-

- 54 -

ring in an ice bath and maintaining the pH between 7.0 and 7.5. This mixture was then centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant
5 was discarded and the pellet was dissolved in 200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl₂, 1 mM DTT, 1 mM benzamidine HCl, and 5 µg/mL each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

10 The resuspended sample was loaded onto a 1.6 X 200 cm column (25 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The sample was cycled through the column 4 to 6 times over the course of 12 hours. The column was washed
15 in succession with 125 to 250 mL of Buffer A, 125 to 250 mL of Buffer A containing 1.5 M NaCl, and 25 to 50 mL of Buffer A. The enzyme was eluted with 50 to 75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and 20 mM
20 cAMP) and 50 to 75 mL of Buffer E containing 1 M NaCl. The PDE activity peak was pooled, and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM
25 MOPS pH 7.5, 5 µM ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl) and desalted via gel filtration on a Pharmacia PD-10® column per manufacturer's instructions. The enzyme pool was dialyzed overnight against Buffer X containing 50% glycerol.
30 This enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >90% pure by SDS-PAGE. These preparations had specific

- 55 -

activities of about 10 to 50 μmol cAMP hydrolyzed per minute per milligram protein.

Preparation of PDE4C from *S. cerevisiae*

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Yeast cells (150 g of yeast strain YI30 harboring HDUN3.48) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 $\mu\text{g}/\text{mL}$ each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a BEAD-BEATER®, and the cells lysed by rapid mixing for 6 cycles of 30 sec each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C.

The supernatant was recovered and PDE4C was precipitated by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. Thirty minutes later, this mixture was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in 200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl_2 , 1 mM DTT, 2 mM benzamidine HCl, and 5 $\mu\text{g}/\text{mL}$ each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

- 56 -

The resuspended sample was loaded onto a 1.6 X 20 cm column (25 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The sample was cycled through the column 4 to 6 times
5 over the course of 12 hours. The column was washed in succession with 125 to 250 mL of Buffer A, 125 to 250 mL of Buffer A containing 1.5 M NaCl, and then 25 to 50 mL of Buffer A. The enzyme was eluted with
10 50 to 75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and 20 mM cAMP) and 50 to 75 mL of Buffer E containing 1 M NaCl. The PDE4C activity peak was pooled, and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The pre-
15 cipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.2, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl) and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme pool was
20 dialyzed overnight against Buffer X containing 50% glycerol. This enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific
25 activities of about 10 to 20 μ mol cAMP hydrolyzed per minute per milligram protein.

- 57 -

Preparation of PDE4D from *S. cerevisiae*

Yeast cells (100 g of yeast strain YI29 harboring HDUN4.11) were thawed by mixing with 150 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 10 μ M ZnSO₄, 2 mM MgCl₂, 14.2 mM 2-mercaptoethanol, 2 mM benzamidine HCl, 5 μ g/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a Bead-Beater®, and the cells lysed by rapid mixing for 6 cycles of 30 sec each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE4D was precipitated by the addition of solid ammonium sulfate (0.33 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. Thirty minutes later, this mixture was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in 100 mL of Buffer A (50 mM MOPS pH 7.5, 10 μ M ZnSO₄, 5 mM MgCl₂, 14.2 mM 2-mercaptoethanol, 100 mM benzamidine HCl, and 5 μ g/mL each of leupeptin, pepstatin, aprotinin, calpain inhibitor I and II). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

At a flow rate of 0.67 mL/min, the resuspended sample was loaded onto a 1.6 X 20 cm column

- 58 -

(10 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The column was washed in succession with 50 to 100 mL of Buffer A, 20 to 30 mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL of Buffer A containing 1.5 M NaCl, and then 10 to 20 mL of Buffer C (50 mM Tris HCl pH 8, 10 μ M ZnSO₄, 14.2 mM 2-mercaptoethanol, 2 mM benzamidine HCl). The enzyme was eluted with 20 to 30 mL of Buffer C containing 20 mM cAMP.

10 The PDE4D activity peak was pooled and precipitated with ammonium sulfate (0.4 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.2, 5 μ M ZnSO₄, 50 mM NaCl, 1 mM DTT, 15 and 1 mM benzamidine HCl) and desalted via gel filtration on a Pharmacia PD-10[®] column per manufacturer's instructions. The enzyme pool was dialyzed overnight against Buffer X containing 50% glycerol. This enzyme preparation was quick-frozen 20 in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific activities of about 20 to 50 μ mol cAMP hydrolyzed per minute per milligram protein.

25

Lipopolysaccharide-Stimulated TNF α Release from Human Peripheral Blood Lymphocytes

30

To assess the ability of a compound to reduce TNF α secretion in human peripheral blood lymphocytes (PBL), the following tests were performed. Previous studies have demonstrated that incubation of human PBL with cAMP-elevating agents,

- 59 -

such as prostaglandin E2, forskolin, 8-bromo-cAMP, or dibutryl-cAMP, inhibits the secretion of TNF α by the cells when stimulated by lipopolysaccharide (LPS; endotoxin). Accordingly, preliminary experiments have been performed to demonstrate that selective PDE4 inhibitors, such as rolipram, inhibit LPS-induced TNF α secretion from human lymphocytes in a dose-dependent fashion. Hence, TNF α secretion from human PBL was used as a standard for the ability of a compound to elevate intracellular cAMP concentrations and/or to inhibit PDE4 activity within the cell.

Heparinized blood (approximately 30 mL) drawn from human volunteers was mixed 1:1 with Dulbecco's modified phosphate-buffered saline. This mixture was mixed 1:1 with HISTOPAQUE[®] and centrifuged at 1,500 rpm at room temperature without braking in the swinging bucket of a Beckman model TJ6 centrifuge. Erythrocytes were centrifuged to the bottom of the tubes, and serum remained at the surface of the tubes. A layer containing lymphocytes sedimented between the serum and HISTOPAQUE[®] layers, and was removed by aspiration to a fresh tube. The cells were quantified and adjusted to 3×10^6 cells/mL and a 100 μ L aliquot is placed into the wells of a 96 well plate. Test compounds and RPMI media (Gibco/BRL Life Sciences) are added to each of the wells 15 minutes prior to addition of bacterial LPS (25 mg/mL). The mixture was allowed to incubate for 20 hours at 37°C in a humidified chamber. The cells then were separated by centrifuging at 800 rpm for 5 minutes at room temperature. An aliquot of 180 μ L of supernatant was transferred to a new plate

- 60 -

for determination of TNF α concentration. TNF α protein in the cell supernatant fluids was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (CYTOSCREEN[®] Immunoassay Kit
5 from Biosource International).

The cell-based assay provided the following results for various pyrrolidine compounds of the present invention. The EC₅₀ values (i.e., effective concentration of the compound capable of inhibiting
10 50% of the total TNF α) illustrate the ability of the present compounds to inhibit LPS-stimulated TNF α release from human PBL.

The table below illustrates the ability of compounds of formula (II) to inhibit PDE4 activity
15 and TNF α release *in vitro*. In the following table, the IC₅₀ values were determined against human recombinant PDE4.

Example Number	PDE4 IC ₅₀ (Mx10 ⁻⁹)	PBL/TNF α EC ₅₀ (Mx10 ⁻⁹)
20 1a	115.9	76.0
1b	2.2	2.2
2a	49.0	281.0
2b	2.2	23.0
25 3	189.3	551.0

The data presented above shows that the present compounds are potent inhibitors of PDE4,
30 e.g., the compounds have an IC₅₀ vs. human recombinant PDE4 of about 1 nM to about 15 μ M. Preferred compounds have an IC₅₀ of about 50 nM or less, and especially preferred compounds have an IC₅₀ of about 25 nM or less.

- 61 -

Similarly, preferred compounds have a PBL/TNF α EC₅₀ about 1000 nM or less, and preferably about 500 nM or less. More preferred compounds have a PBL/TNF α EC₅₀ of about 100 nM or less.

5 To achieve the full advantages of the present invention, the compound has an IC₅₀ vs. human recombinant PDE4 of about 50 nM or less and a PBL/TNF α EC₅₀ of about 1000 nM or less. More preferably, the compound has an IC₅₀ of about 25 nM or
10 less and a PBL/TNF α EC₅₀ of about 500 nM or less.

Animal Models

15 Combined Mouse endotoxin-stimulated TNF α Release and Locomotor Activity Assay

The purpose of this study was to determine the efficacy of PDE4 inhibitors *in vivo* in an LPS
20 mouse model together with a determination with respect to central nervous system (CNS) side-effects manifested by a decrease in spontaneous mobility.

The test animals were female Balb/c mice, having an average weight of about 20 g. The PDE4
25 inhibitors, formulated in 30% Cremophor[®] EL, were administered via intraperitoneal (i.p.) injections at doses of 0.1, 1.0, 10.0, and 100 mg/kg. Individual dose volumes (about 150 μ L) were adjusted based on the body weights measured. One hour later,
30 5 mg/kg LPS in a final volume of 200 μ L was injected via the tail vein to each animal. Ninety minutes following the LPS treatment, the animals were bled and serum samples were collected before being stored at -70°C until assayed.

- 62 -

For efficacy determination, the serum samples were diluted two-fold and TNF α levels were determined using the CYTOSCREEN[®] Immunoassay Kit (Biosource International). The data were averaged
5 between triplicate sample subjects for each of the tested compounds.

Movement of the X-Y plane, or rearing up on the hind legs, was quantified by counting the number of "light-beam" crosses per unit of time. A
10 decrease in the number of activity events is directly proportional to the mobility or immobilization of the animal. The quantitative scoring correlated well with the subjective measurements described above.

15 The following table summarizes the locomotor activity assay (mobility, % activity) results obtained by the above-described method:

Example Number	Mobility (% activity at 50 mg/kg dose) or ED ₅₀ (mg/kg)
1a	64% activity
1b	6% activity
2a	ED ₅₀ ¹⁾ (mg/kg) > 50 mg/kg
2b	ED ₅₀ (mg/kg) = 7 mg/kg

1) effective dose, in mg/kg, that decreases spontaneous mobility 50% of control.

30

It also was determined that compounds of formula (II) have fewer central nervous system side
35 effects compared to rolipram and to compounds disclosed in Feldman et al. U.S. Patent No. 5,665,754. It also was found that central nervous system

- 63 -

activity is related to the absolute stereochemistry of the present compounds.

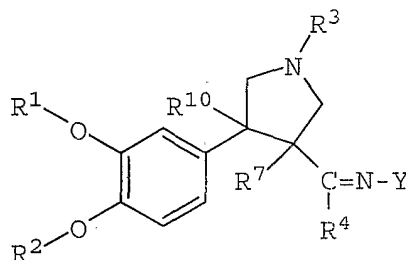
The results summarized above show that the compounds of the present invention are useful for
5 selectively inhibiting PDE4 activity in a mammal, without exhibiting the adverse CNS and emetic effects associated with prior PDE4 inhibitors.

Obviously, many modifications and variations of the invention as hereinbefore set
10 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.

- 64 -

WHAT IS CLAIMED IS:

1. A compound having the formula:



wherein Y is OR^5 or NR^5R^6 ;

R^1 is lower alkyl, bridged alkyl, aralkyl, cycloalkyl, a 5- or 6-membered saturated heterocycle, C_{1-3} alkylenecycloalkyl, aryl- or heteroaryl-substituted propargyl, aryl- or heteroaryl-substituted allyl, or halocycloalkyl;

R^2 is hydrogen, methyl, or halo-substituted methyl;

R^3 is $C(=O)OR^7$, $C(=O)R^7$, $C(=NH)NR^8R^9$, $C(=O)NR^8R^9$, aryl, or heteroaryl;

R^4 is hydrogen, lower alkyl, haloalkyl, cycloalkyl, or aryl;

R^5 and R^6 , independently, are hydrogen, lower alkyl, haloalkyl, cycloalkyl, aryl, heteroaryl, or alkaryl, or R^5 and R^6 are taken together form a 5-membered or 6-membered ring;

R^7 is branched or unbranched lower alkyl or aryl, and R^7 can be optionally substituted with one or more of RO^8 , NR^8R^9 , or SR^8 ; and

R^8 and R^9 , same or different, are selected from the group consisting of hydrogen, lower alkyl,

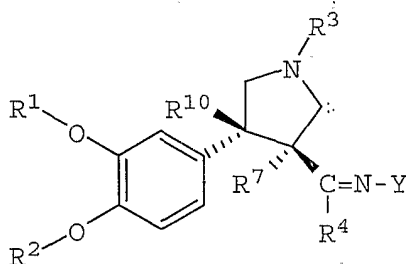
- 65 -

cycloalkyl, aryl, heteroaryl, and aralkyl, or R^8 and R^9 are taken together form a 4-membered to 7-membered ring;

R^{10} is hydrogen, alkyl, haloalkyl, cycloalkyl, aryl, $C(=O)$ alkyl, $C(=O)$ cycloalkyl, $C(=O)$ aryl, $C(=O)$ Oalkyl, $C(=O)$ Ocycloalkyl, $C(=O)$ aryl, CH_2OH , CH_2O alkyl, CHO , CN , NO_2 , or SO_2R^{11} ; and

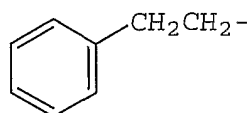
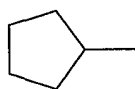
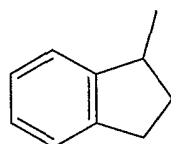
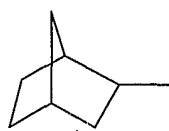
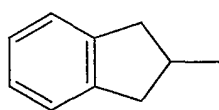
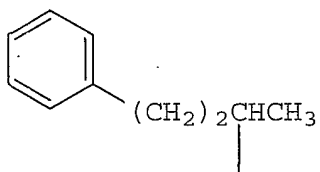
R^{11} is alkyl, cycloalkyl, trifluoromethyl, aryl, aralkyl, or NR^8R^9 .

2. The compound of claim 1 having the structure:

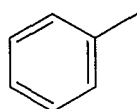
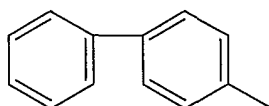
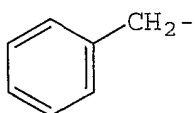
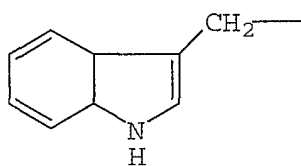
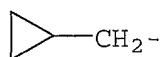
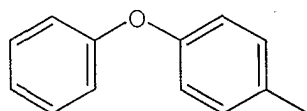
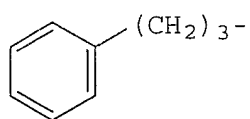


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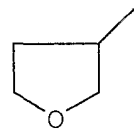
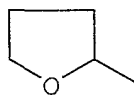
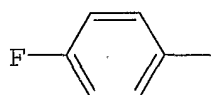
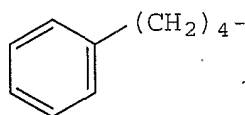
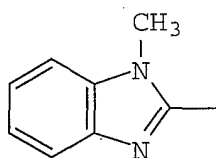
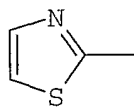
3. The compound of claim 1 wherein R¹ is selected from the group consisting of:



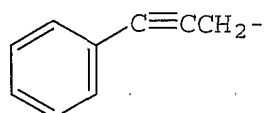
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- 68 -

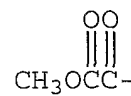
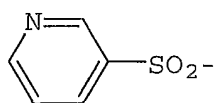
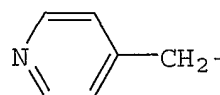
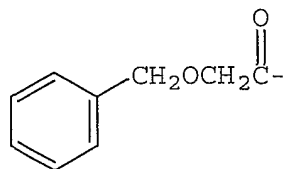
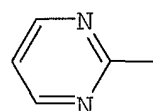
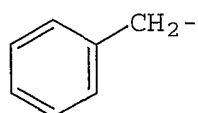
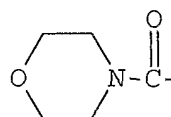
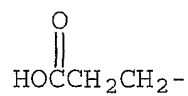
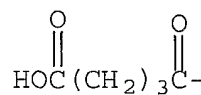
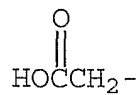
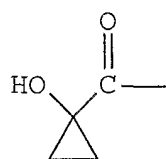
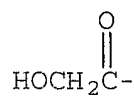
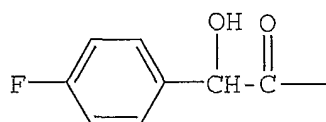
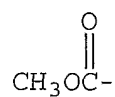


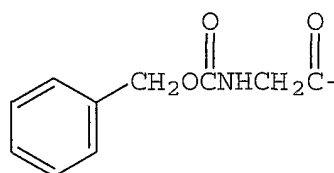
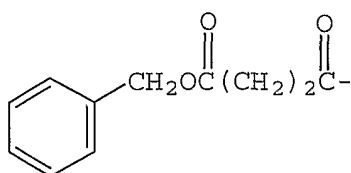
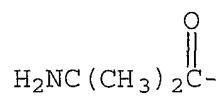
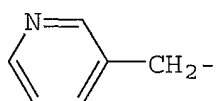
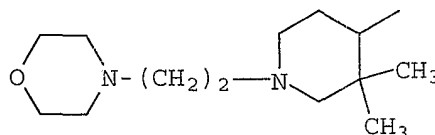
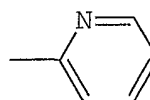
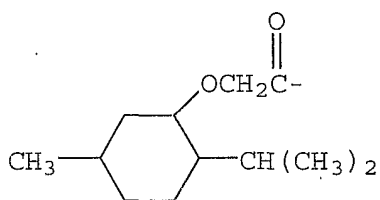
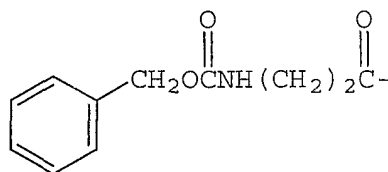
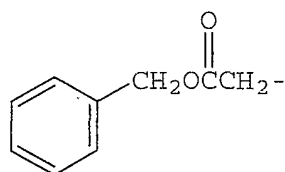
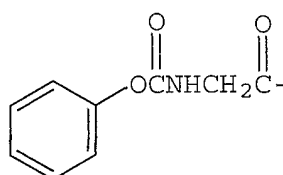
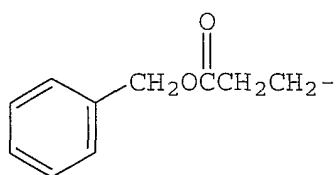
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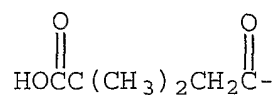
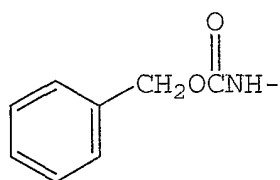
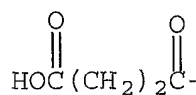
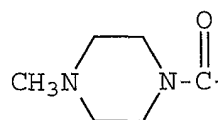
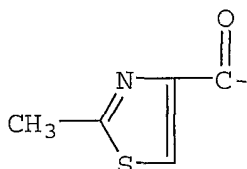
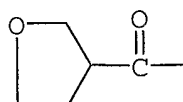
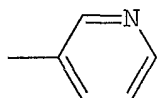
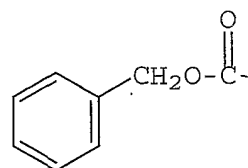
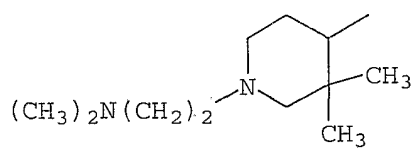


- 69 -

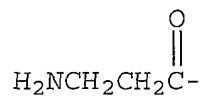
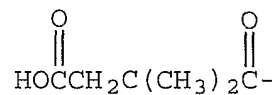
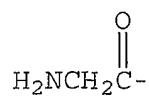
4. The composition of claim 1 wherein R³ is selected from the group consisting of:







and



- 72 -

5. The compound of claim 1 wherein R^4 is selected from the group consisting of hydrogen, methyl, trifluoromethyl, cyclopropyl, benzyl, and phenyl.

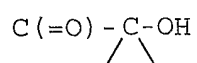
6. The compound of claim 1 wherein R^5 and R^6 , independently, are hydrogen or lower alkyl.

7. The compound of claim 1 wherein R^7 is lower alkyl.

8. The compound of claim 1 wherein R^8 and R^9 , independently, are hydrogen or lower alkyl, or are taken together form a 5-membered or 6-membered ring.

- 73 -

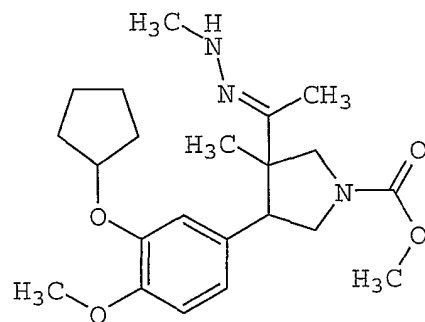
9. The compound of claim 1 wherein R¹ is selected from the group consisting of cyclopentyl, tetrahydrofuryl, indanyl, norbornyl, phenethyl, and phenylbutyl; R² is selected from the group consisting of methyl and difluoromethyl; R³ is selected from the group consisting of CO₂CH₃, C(=O)CH₂OH, C(=O)CH(CH₃)-OH, C(=O)C(CH₃)₂OH, and



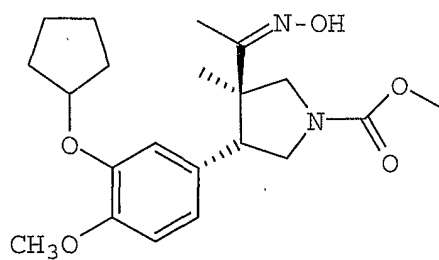
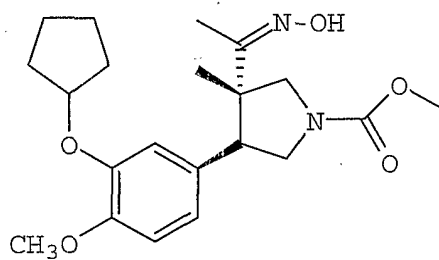
R⁴ is hydrogen or methyl; R⁵ and R⁶, independently, are hydrogen or methyl, or R⁵ and R⁶ are taken together to form a 5-membered or 6-membered ring; R⁷ is methyl; and R¹⁰ is hydrogen.

- 74 -

10. The compound of claim 1 having the structure:

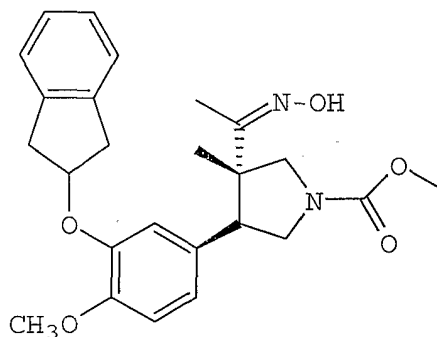


(R and S isomers)



, and

- 75 -



11. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 1 nM to about 15 μ M.

12. The compound of claim 1 having a PBL/TNF α EC_{50} of about 1 nM to about 20 μ M.

13. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 1 nM to about 15 μ M, and a PBL/TNF α EC_{50} of about 1 nM to about 25 μ M.

14. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 100×10^{-6} M or less.

15. The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 50×10^{-6} M or less.

- 76 -

16. A pharmaceutical composition comprising a compound of claim 1, a pharmaceutically acceptable carrier, and, optionally, a second anti-inflammatory therapeutic agent.

17. The composition of claim 16 wherein the second antiinflammatory therapeutic agent is capable of targeting TNF α .

18. A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of therapeutic benefit, said method comprising administering to said mammal at therapeutically effective amount of a compound of claim 1.

19. A method of modulating cAMP levels in a mammal comprising administering to said mammal an effective amount of a compound of claim 1.

20. A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of a therapeutic benefit comprising administering to said mammal an effective amount of a pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

21. The method of claim 20 wherein the condition is an allergic disease, an autoimmune disease, an inflammatory disease, an arthritic disease, or dermititis.

- 77 -

22. The method of claim 20 wherein the condition is rheumatoid arthritis, osteoarthritis, gouty arthritis, or spondylitis.

23. The method of claim 20 wherein the condition is thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome, allergic conjunctivitis, vernal conjunctivitis, or eosinophilic granuloma.

24. The method of claim 20 wherein the condition is asthma, chronic bronchitis, allergic rhinitis, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, chronic obstructive pulmonary disease, silicosis, or pulmonary sarcoidosis.

25. The method of claim 20 wherein the condition is reperfusion injury of the myocardium, brain or extremities as a brain or spinal cord injury due to trauma.

26. The method of claim 20 wherein the condition is a fibrosis, keloid formation, or scar tissue formation.

27. The method of claim 20 wherein the condition is systemic lupus erythematosus, a transplant rejection disorder, a graft vs. host reaction, or an allograft rejection.

- 78 -

28. The method of claim 20 wherein the condition is chronic glomerulonephritis, an inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

29. The method of claim 20 wherein the condition is proliferative lymphocytic disease or a leukemia.

30. The method of claim 20 wherein the condition is an inflammatory dermatosis, atopic dermatitis, psoriasis, or urticaria.

31. The method of claim 20 wherein the condition is a cardiomyopathy, congestive heart failure, atherosclerosis, pyrexia, cachexia, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome, ARC, cerebral malaria, osteoporosis, a bone resorption disease, fever and myalgias due to infection, erectile dysfunction, diabetes insipidus, a central nervous system disorder, depression, multi-infarct dementia, an anxiety or stress response, cerebral ischemia, tardive dyskinesia, Parkinson's disease, or premenstrual syndrome.

33. The method of claim 20 wherein the mammal exhibits a minimal emetic response.

34. The method of claim 20 wherein the mammal is free of an emetic response.

- 79 -

35. The method of claim 20 wherein the mammal exhibits minimal adverse central nervous system side effects.

36. The method of claim 20 wherein the mammal is free of adverse central nervous system side effects.

37. The method of reducing TNF levels in a mammal comprising administering to said mammal therapeutically effective amount of a compound of claim 1.

38. A method of suppressing inflammatory cell activation in a mammal comprising administering to said mammal a therapeutically effective amount of a compound of claim 1.

37. A method of inhibiting PDE4 function in a mammal comprising administering to said mammal a therapeutically effective amount of a compound of claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/42316

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D207/09 A61K31/40 A61P29/00 A61P19/02

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 A61K A61P

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)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 08534 A (GLAXO INC ;FELDMAN PAUL LAWRENCE (US); STAFFORD JEFFREY ALAN (US)) 30 March 1995 (1995-03-30) cited in the application page 16; claim 28	1,16,20
A	EP 0 671 389 A (MITSUBISHI CHEM CORP) 13 September 1995 (1995-09-13) example 1 page 5, line 15 - line 30	1,16,20
A	WO 92 19594 A (SMITHKLINE BEECHAM CORP) 12 November 1992 (1992-11-12) claims 1,8	1,16,20

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

25 April 2001

Date of mailing of the international search report

16/05/2001

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Seitner, I

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/42316

Patent document cited in search report	Publication date	Patent family member(s)	Publication date		
WO 9508534 A	30-03-1995	US 5665754 A	09-09-1997		
		AT 194593 T	15-07-2000		
		AU 685170 B	15-01-1998		
		AU 7839694 A	10-04-1995		
		CA 2171448 A	30-03-1995		
		DE 69425267 D	17-08-2000		
		DE 69425267 T	18-01-2001		
		EP 0720600 A	10-07-1996		
		ES 2149888 T	16-11-2000		
		JP 9502979 T	25-03-1997		
		NZ 274381 A	26-02-1998		

		EP 0671389 A	13-09-1995	CA 2143143 A	09-09-1995
				CN 1121917 A	08-05-1996
FI 951052 A	09-09-1995				
HU 71891 A	28-02-1996				
JP 7300455 A	14-11-1995				
NO 950879 A	11-09-1995				
US 5545647 A	13-08-1996				

WO 9219594 A	12-11-1992	AU 1917092 A	21-12-1992		
		CA 2102106 A	03-11-1992		
		CN 1067244 A	23-12-1992		
		EP 0584208 A	02-03-1994		
		IE 921376 A	04-11-1992		
		JP 6507405 T	25-08-1994		
		MX 9202090 A	01-11-1992		
		PT 100441 A	30-09-1993		
		ZA 9203210 A	31-03-1993		
