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(54) Title: NOVEL USES

(57) Abstract: The disclosure provides the administration of inhibitors of phosphodiesterase 1 (PDE1) for the treatment and prophylaxis of diseases or disorders characterized by inflammation, e.g., neuroinflammation, including methods of treatment and pharmaceutical compositions for use therein.

NOVEL USES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit and priority to United States Provisional Application 62/393,386, filed September 12, 2016, as well as United States Provisional Application 62/412,739, filed October 25, 2016, as well as United States Provisional Application 62/467,218, filed March 5, 2017, the contents of each of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0001] The field relates to the administration of inhibitors of phosphodiesterase 1 (PDE1) inhibitors for the treatment and prophylaxis of inflammation and/or diseases or disorders related to inflammation, e.g., neuroinflammation.

BACKGROUND OF THE INVENTION

[0002] Eleven families of phosphodiesterases (PDEs) have been identified but only PDEs in Family I, the Ca^{2+}-calmodulin-dependent phosphodiesterases (CaM-PDEs), are activated by the Ca^{2+}-calmodulin and have been shown to mediate the calcium and cyclic nucleotide (e.g. cAMP and cGMP) signaling pathways. These PDEs are therefore active in stimulated conditions when intra-cellular calcium levels rise, leading to increased hydrolysis of cyclic nucleotides. The three known CaM-PDE genes, PDE1A, PDE1B, and PDE1C, are all expressed in central nervous system tissue. In the brain, the predominant expression of PDE1A is in the cortex and neostriatum, PDE1B is expressed in the neostriatum, prefrontal cortex, hippocampus, and olfactory tubercle, and PDE1C is more ubiquitously expressed.

[0003] PDE4 is the major cAMP-metabolizing enzyme found in inflammatory and immune cells, and PDE4 inhibitors are of interest as anti-inflammatory drugs. PDE1, however, has not been thought to play a major role in the inflammatory response, although PDE-1 is induced in monocyte-to-macrophage differentiation mediated by the cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF). The PDE1 inhibitor vinpocetine has been shown to be anti-inflammatory, but the anti-inflammatory

action of vinpocetine is believed to be caused by a direct inhibition of the IκB kinase complex (IKK) rather than PDE blockade.

[0004] Microglia have a central role in maintaining homeostasis and mediating inflammation in the brain. Microglia communicate with complex signaling to neurons and astrocytes, determining how many brains cells are needed and when to eliminate a synapse, e.g., destroying the defective or unused synapses. Microglia may exist in different states: a resting state, which is relatively inactive but may perform surveillance functions, or in one of two functionally distinct activation states, M1 and M2. The M1 state is induced by a signal such as IFN-γ or lipopolysaccharide (LPS), and responds by releasing inflammatory cytokines such as TNF-, IL-1β, and reactive oxygen species/reactive nitrogen species (ROS/NOS). The M2 state has an anti-inflammatory effect, blocking the release of pro-inflammatory cytokines, ingesting debris, promoting tissue repair and releasing neurotrophic factors. Activated microglia have been associated with a variety of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), and may contribute to the pathology of these diseases, although it is not conclusively determined whether inflammation is an underlying cause or an effect of these conditions.

[0005] It has not been previously shown that PDE1 has a significant role in mediating inflammatory cytokines, in the brain or elsewhere, or that it would have a significant effect on inflammatory diseases. Inflammatory processes in general, and diseases and disorders related to inflammation, are numerous, and the mechanisms and actions are still not well understood. Currently, there is a largely unmet need for an effective way of treating inflammation and inflammatory related diseases and disorders, especially with regard to inflammation occurring in the brain.

BRIEF DESCRIPTION OF DRAWINGS

[0006] Figure 1 depicts phosphodiesterase expression in BV2 cells treated with LPS – RNAseq analysis. FPKM refers to Fragment Reads per kilobase of exon per million reads mapped.

[0007] Figure 2 depicts that a PDE1 inhibitor (Compound 214) suppresses LPS-induced IL1β in BV2 cells

[0008] Figure 3 depicts that a PDE1 inhibitor (Compound 214) suppresses LPS-induced IL1β in mouse hippocampus, in vivo.

[0009] Figure 4a depicts that a PDE1 inhibitor (Compound 214) significantly reduces the LPS-induced increase in expression of the inflammatory cytokines IL1β, TNF- α, and Ccl2 in BV2 cells, as measured by quantitative PCR, while a PDE4 inhibitor, rolipram, displays a different profile. In a separate experiment, Figure 4b demonstrates that administration of a PDE1 inhibitor of the present invention (Compound 214) greatly reduces or blunts LPS-induced changes in proinflammatory markers in BV2 cells (Figure 4b).

[0010] Figure 5 depicts inhibition of LPS-induced TNFα release from BV2 cells.

[0011] Figure 6 depicts dose dependent reduction by a PDE1 inhibitor of LPS-stimulated TNFα mRNA expression.

[0012] Figure 7 depicts inhibition by a PDE1 inhibitor of LPS-induction of pro-inflammatory cytokine expression and an enhancement of expression of an anti-inflammatory cytokine (IL-10).

[0013] Figure 8 depicts a PDE1 inhibitor (Compound 214) prevents LPS-induced inflammatory gene expression changes in mice. Adult mice are treated with vehicle (white bars), 500 µg/kg LPS s.c. (gray bars), or 10 mg/kg ITI-214 i.p. and 500 µg /kg LPS s.c. (black bars) for 2 hours (n = 4). Striatal tissue is analyzed for mRNA levels of TNF, IL1β, Ccl2, and IL6. Expression levels are shown as change in Q-PCR signal from vehicle (ΔΔCt) and compared using an ANOVA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

[0014] Figure 9 demonstrates expression levels (FPKM) of selected genes with shared patterns of response to LPS and ITI-214. BV2 cells are treated with LPS, ITI-214, or LPS+ITI-214.

[0015] Figure 10a shows the change in expression levels of each gene in BV2 cells with 50 ng/ml LPS and the indicated dose of ITI-214 (abbreviated 214).

[0016] Figure 10b demonstrates the dose dependent effects of ITI-214 in the absence of LPS stimulation. From left to right, for Fig. 10a, the bars indicate samples treated with: LPS, 0.1 µM 214 + LPS, 0.4 µM 214 + LPS, 1.1 µM 214 + LPS, 3.3 µM 214 + LPS, 10 µM 214 + LPS, for each particular gene shown on the X-axis. From left to right, for Fig.

10b, the bars indicate samples treated with: 0.04 μM 214, 0.1 μM 214, 0.4 μM 214, 1.1 μM 214, 3.3 μM 214, 10 μM 214, for each particular gene shown on the X-axis.

[0017] Fig. 11a cGMP-dependent activity of the PDE1 inhibitor of the present invention (Compound 214), particulate guanylyl cyclase activity with atrial natriuretic peptide (ANP) or soluble guanylyl cyclase activity with nitric oxide donor DEANO can be stimulated. For each gene, from left to right, the bars indicate treatment with: LPS, 3.3 μM 214, 10 μM 214, forskolin, and DEANO.

[0018] Fig. 11b demonstrates the influence of each cyclic nucleotide (cAMP or cGMP) on the ITI-214 response by combining LPS stimulation, ITI-214 inhibition of PDE1, and either PKA inhibitor (cAMPS-Rp) (100 μM) or PKG inhibitor (-8-Br-PET-cGMPS) (100 μM). From left to right, for Fig. 11b, the bars indicate samples treated with: LPS, 3.3 μM 214, 10 μM 214, cAMPS-Rp, and RP-8-Br-PET-cGMPS.

[0019] Fig. 12 demonstrates that the tested PDE1 inhibitor is highly effective in increasing the survival of retinal ganglion cells when compared with the PBS control in the optic injury model described in Example 10.

SUMMARY OF THE INVENTION

[0020] Surprisingly, we have discovered that PDE1 mediates the expression of certain pro-inflammatory cytokines and chemokines and that PDE1 inhibitors have specific anti-inflammatory effects, which are different from the anti-inflammatory effects of PDE4 inhibitors. In one aspect, inhibition of PDE1 regulates inflammatory activity in microglia, reducing expression of pro-inflammatory genes, with a profile different from PDE4 inhibition, thereby providing novel treatments for toxic neuroinflammation.

[0021] Negative regulation of inflammatory responses in microglia by elevated intracellular cyclic nucleotide levels provides a promising area for therapeutic intervention. Cyclic guanosine monophosphate (cGMP) in microglia is produced by activation of atrial natriuretic receptors or soluble guanylyl cyclase and is hydrolyzed by phosphodiesterases (PDEs). Increasing intracellular cGMP by either stimulating production or inhibiting hydrolysis has been shown to attenuate LPS-induced responses in microglia. Additionally, cGMP has been shown to play a role in LPS-induced motility of microglia. Cyclic adenosine monophosphate (cAMP) is also a key regulator of

inflammatory responses. LPS and cytokine stimulation have been shown to increase expression of PDE4B and decrease cAMP. PDEs are proven drug-able targets. Enzymes of the PDE1 family, of which PDE1B is expressed in microglia, hydrolyze both cAMP and cGMP and are activated by calcium.

[0022] Among the roles played by the PDE1 enzyme targets of the compounds of the invention, the PDE1B isoform is found in high abundance in microglia, where it may play a role in controlling inflammatory responses, in particular under conditions of elevated intracellular calcium. This suggests that ITI-214 might prove beneficial in diseases associated with, for example, chronic neuroinflammation.

[0023] In one embodiment, therefore, the invention provides using various PDE1 inhibitory compounds to treat inflammation, and/or diseases or disorders related to inflammation. Inflammation can be neuroinflammation, and in one embodiment the PDE1 inhibitors can specifically modulate microglial activation in the brain. We have surprisingly discovered that the LPS-induced expression of certain inflammatory biomarkers (e.g., IL1β, TNF- α, and Ccl2) can be blunted or decreased with the administration of a PDE1 inhibitor as described herein. This discovery has wide-ranging applications for treating inflammatory diseases and disorders related or correlated to the expression of various inflammatory biomarkers.

[0024] Without being bound by theory, one possible mechanism for this activity is that inhibition of PDE1B may affect macrophage activation in the blood and/or microglial activation in the CNS, so as to reduce M1 activation and the release of pro-inflammatory cytokines, and to enhance the action of M2 microglia, through the up-regulation of anti-inflammatory cytokines such as IL-10. The role of neuroinflammation and microglial function in CNS pathologies is not fully understood, but we hypothesize that it is relevant to a variety of conditions, including:

a. neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases;
b. repair of damage due to stroke, cardiac arrest, hypoxia, intracerebral hemorrhage or traumatic brain injury;

c. conditions characterized by abnormal neurotransmitter production and/or response, including depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation;
d. chronic CNS infections, e.g., Lyme disease, syphilis, or CNS infection consequent to an immunosuppressive condition, e.g., HIV dementia;
e. neuroinflammation consequent to chemotherapy; .

[0025] Targeted inhibition of PDE1 in the brain with a compound of the present invention is believed to affect microglial activation and reduce damaging pro-inflammatory cytokine signaling, and at the same time, increasing production of anti-inflammatory cytokines and factors involved in microglia motility and recruitment.

[0026] Accordingly, in one embodiment, the invention provides a new method of treatment or prophylaxis of inflammation or disease associated with inflammation that may be ameliorated by administration of a specific inhibitor of phosphodiesterase type I (e.g., PDE1 inhibitor, e.g., a PDE1B inhibitor) (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described).

[0027] In one embodiment the invention provides a method of treating neuroinflammation and/or diseases or disorders associated with neuroinflammation and/or microglial function, e.g., selected from:

a. neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases;
b. stroke, cardiac arrest, hypoxia, intracerebral hemorrhage or traumatic brain injury;
c. conditions characterized by abnormal neurotransmitter production and/or response, including depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation; and

d. chronic CNS infections, e.g., Lyme disease or CNS infection consequent to an immunosuppressive condition, e.g., HIV-dementia;
e. neuroinflammation consequent to chemotherapy;

comprising administering an effective amount of a PDE1 inhibitor of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described), e.g., an amount effective to (i) reduce or inhibit activation of M1 microglia, and/or (ii) and amount effective to reduce levels of one or more pro-inflammatory cytokines (e.g., IL1β, TNF- α, and Ccl2, or combination thereof); to a patient in need thereof.

[0028] In one embodiment PDE1 inhibitors of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described) are administered to a patient with increased levels of one or more pro-inflammatory cytokines (e.g., IL1β, TNF- α, and Ccl2, or combination thereof), e.g., to a patient suffering from neuroinflammation and/or diseases or disorders associated with neuroinflammation and/or microglial function, e.g., selected from:

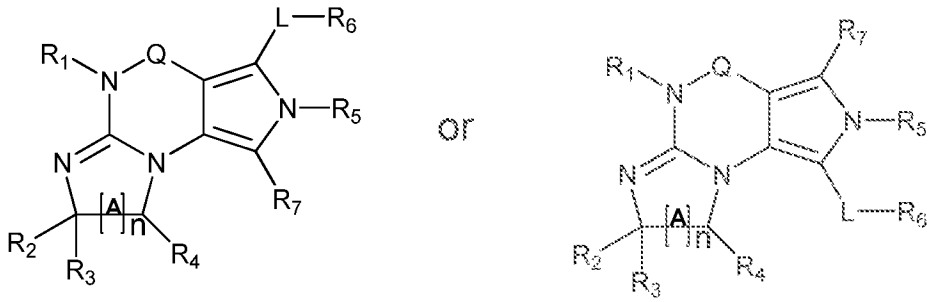
a. neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases;
b. stroke, cardiac arrest, hypoxia, intracerebral hemorrhage or traumatic brain injury;
c. conditions characterized by abnormal neurotransmitter production and/or response, including depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation; and
d. chronic CNS infections, e.g., Lyme disease or CNS infection consequent to an immunosuppressive condition, e.g., HIV-dementia;
e. neuroinflammation consequent to chemotherapy.

[0029] Further embodiments of the invention are set forth or evident from the detailed description below and the examples herein.

DETAILED DESCRIPTION OF THE INVENTION

Compounds for use in the methods of the invention

[0030] In one embodiment, the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are optionally substituted 4,5,7,8-tetrahydro-2H-imidazo[1,2-a]pyrrolo[3,4-e]pyrimidine or 4,5,7,8,9-pentahydro-2H-pyrimido[1,2-a]pyrrolo[3,4-e]pyrimidine, e.g., a Compound of Formula II, e.g., II-A or II-B: In one embodiment, the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are optionally substituted 4,5,7,8-tetrahydro-2H-imidazo[1,2-a]pyrrolo[3,4-e]pyrimidine or 4,5,7,8,9-pentahydro-2H-pyrimido[1,2-a]pyrrolo[3,4-e]pyrimidine, e.g., a Compound of Formula II, e.g., II-A or II-B:



Formula II-A Formula II-B

wherein

(i) Q is C(=O), C(=S), C(=N(R_{20})) or CH_2;

(ii) L is a single bond, -N(H)-, -CH_2-, -S-, -S(O)- or –S(O_2)-;

(iii) R_1 is H or C_{1-4} alkyl (e.g., methyl);

(iv) R_4 is H or C_{1-6} alkyl (e.g., methyl or isopropyl) and R_2 and R_3 are, independently,

H

C_{1-6}alkyl (e.g., methyl, isopropyl) optionally substituted with halo or hydroxy (e.g., R_2 and R_3 are both methyl, or R_2 is H and R_3 is methyl, ethyl, isopropyl or hydroxyethyl),

aryl,

heteroaryl,

(optionally hetero)arylalkoxy,

(optionally hetero)arylC_{1-6}alkyl; or

R_2 and R_3 together form a 3- to 6-membered ring;

or

R_2 is H and R_3 and R_4 together form a di-, tri- or tetramethylene bridge (pref. wherein the R_3 and R_4 together have the *cis* configuration, e.g., where the carbons carrying R_3 and R_4 have the R and S configurations, respectively);

or

(v) R_5 is

a) –D-E-F, wherein:

D is C_{1-4}alkylene (e.g., methylene, ethylene or prop-2-yn-1-ylene);

E is a single bond, C_{2-4}alkynylene (e.g., –C≡C–), arylene (e.g., phenylene) or heteroarylene (e.g., pyridylene);

F is

H,

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl, diazolyl, triazolyl, for example, pyrid-2-yl, imidazol-1-yl, 1,2,4-triazol-1-yl),

halo (e.g., F, Br, Cl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

-C(O)-R_{15},

-N(R_{16})(R_{17}), or

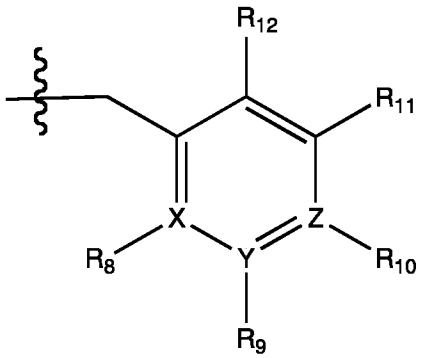
C_{3-7}cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, cyclohexyl, pyrrolidinyl (e.g., pyrrolidin-3-yl), tetrahydro-*2H*-pyran-4-yl, or morpholinyl);

wherein D, E and F are independently and optionally substituted with one or more halo (e.g., F, Cl or Br), C_{1-4}alkyl (e.g., methyl), haloC_{1-4}alkyl (e.g., trifluoromethyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, C_{1-4}carboxy, or an additional aryl or heteroaryl (e.g., biphenyl or pyridylphenyl),

for example, F is heteroaryl, e.g., pyridyl substituted with one or more halo (e.g., 6-fluoropyrid-2-yl, 5-fluoropyrid-2-yl, 6-fluoropyrid-2-yl, 3-fluoropyrid-2-yl, 4-fluoropyrid-2-yl, 4,6-dichloropyrid-2-yl), haloC_{1-4}alkyl (e.g., 5-trifluoromethylpyrid-2-yl) or C_{1-4}alkyl (e.g., 5-methylpyrid-2-yl), or F is aryl, e.g., phenyl, substituted with one or more halo (e.g., 4-fluorophenyl) or F is a C_{3-7}heterocycloalkyl (e.g., pyrrolidinyl) optionally substituted with a C_{1-6}alkyl (e.g., 1-methylpyrrolidin-3-yl); or

b) a substituted heteroarylalkyl, e.g., substituted with haloC_{1-4}alkyl;

c) attached to the nitrogen on the pyrrolo portion of Formula II-A or II-B and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F), and R_{10} is

halogen,

C_{1-4}alkyl,

haloC_{1-4}alkyl (e.g., triflouromethyl)

C_{1-4}alkoxy (e.g. methoxy),

C_{3-7}cycloalkyl,

heteroC_{3-7}cycloalkyl (e.g., pyrrolidinyl or piperidinyl),

C_{1-4}haloalkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl (for example pyrid-2-yl or pyrid-4-yl), or thiadiazolyl (e.g., 1,2,3-thiadiazol-4-yl)), diazolyl (e.g., imidazol-1-yl), triazolyl (e.g., 1,2,4-triazol-1-yl), tetrazolyl,
arylcarbonyl (e.g., benzoyl),
alkylsulfonyl (e.g., methylsulfonyl),
heteroarylcarbonyl, or
alkoxycarbonyl;

wherein the aryl, heteroaryl, cycloalkyl or heterocycloalkyl is independently, optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), halogen (e.g., chloro or fluoro), haloC_{1-4}alkyl (e.g., trifluoromethyl), hydroxy, C_{1-4}carboxy, -SH or an additional aryl, heteroaryl (e.g., biphenyl or pyridylphenyl) or C_{3-8}cycloalkyl,

preferably R_{10} is phenyl, pyridyl, piperidinyl or pyrrolidinyl optionally substituted with the substituents previously defined, e.g. optionally substituted with halo or alkyl

provided that when X, Y, or Z is nitrogen, R_8, R_9, or R_{10}, respectively, is not present;

(vi) R_6 is

H,
C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl, isobutyl),
C_{3-7}cycloalkyl (e.g., cyclopentyl or cyclohexyl),
heteroC_{3-7}cycloalkyl (e.g., pyrrolidinyl, piperidinyl, morpholinyl),
aryl (e.g., phenyl),
heteroaryl (e.g., pyrid-4-yl),
arylC_{1-4}alkyl (e.g., benzyl),
arylamino (e.g., phenylamino),
heteroarylamino,
N,N-diC_{1-4}alkylamino,
N,N-diarylamino,

N-aryl-N-(arylC_{1-4}alkyl)amino (e.g., N-phenyl-N-(1,1'-biphen-4-ylmethyl)amino), or

-N(R_{18})(R_{19}),

wherein the aryl and heteroaryl are optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), halogen (e.g., chloro or fluoro), haloC_{1-4}alkyl (e.g., trifluoromethyl), hydroxy, C_{1-4}carboxy, or an additional aryl, heteroaryl (e.g., biphenyl or pyridylphenyl) or C_{3-8}cycloalkyl;

(vii) R_7 is H, C_{1-6}alkyl (e.g., methyl or ethyl), halogen (e.g., Cl), -N(R_{18})(R_{19}), hydroxy or C_{1-6}alkoxy;

(viii) n = 0 or 1;

(ix) when n=1, **A** is -C($R_{13}R_{14}$)-, wherein R_{13} and R_{14}, are, independently, H or C_{1-4}alkyl, aryl, heteroaryl, (optionally hetero)arylC_{1-4}alkoxy, (optionally hetero)arylC_{1-4}alkyl or R_{14} can form a bridge with R_2 or R_4;

(x) R_{15} is C_{1-4}alkyl, haloC_{1-4}alkyl, -OH or –OC_{1-4}alkyl (e.g., -OCH_3)

(xi) R_{16} and R_{17} are independently H or C_{1-4}alkyl;

(xii) R_{18} and R_{19} are independently

H,

C_{1-4}alky (e.g., methyl, ethyl, n-propyl, isobutyl),

C_{3-8}cycloalky (e.g., cyclohexyl or cyclopenyl),

heteroC_{3-8}cycloalky (e.g., pyrrolidinyl, piperidinyl, morpholinyl),

aryl (e.g., phenyl) or

heteroaryl (e.g., pyridyl),

wherein said aryl and heteroaryl are optionally substituted with one or more

halo (e.g., fluorophenyl, e.g., 4-fluorophenyl),

hydroxy (e.g., hydroxyphenyl, e.g., 4-hydroxyphenyl or 2-hydroxyphenyl),

C_{1-4}alkyl (e.g., methyl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

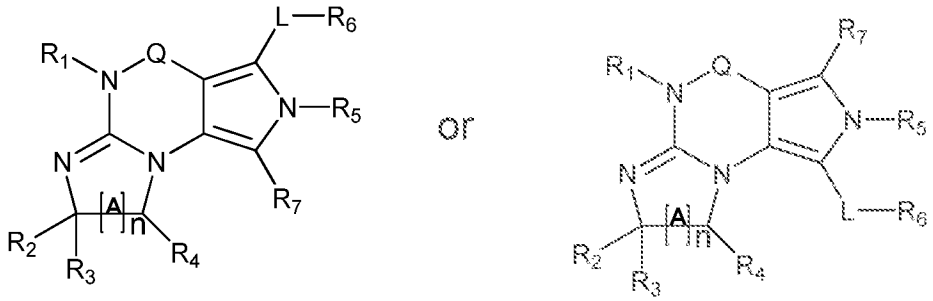
C_{1-4}carboxy, or

an additional aryl, heteroaryl (e.g., biphenyl or pyridylphenyl) or C_{3-8}cycloalkyl,

(xiii) R_{20} is H, C_{1-4}alkyl or C_{3-7}cycloalkyl;

in free or salt form.

[0031] In another embodiment, the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are Compound of Formula I, e.g. Formula I-A and I-B:



Formula I-A Formula I-B

wherein

(i) Q is C(=O), C(=S), C(=N(R_{20})) or CH_2;

(ii) L is a single bond, -N(H)-, -CH_2-, -S-, -S(O)- or –S(O_2)-;

(iii) R_1 is H or C_{1-4} alkyl (e.g., methyl);

(iv) R_4 is H or C_{1-6} alkyl (e.g., methyl or isopropyl) and R_2 and R_3 are, independently,

H or C_{1-6}alkyl (e.g., methyl, isopropyl) optionally substituted with halo or hydroxy (e.g., R_2 and R_3 are both methyl, or R_2 is H and R_3 is methyl, ethyl, isopropyl or hydroxyethyl),

aryl,

heteroaryl,

(optionally hetero)arylalkoxy, or

(optionally hetero)arylC_{1-6}alkyl;

or

R_2 is H and R_3 and R_4 together form a di-, tri- or tetramethylene bridge

(pref. wherein the R_3 and R_4 together have the *cis* configuration, e.g., where the carbons carrying R_3 and R_4 have the R and S configurations, respectively);

(v) R_5 is

a) –D-E-F, wherein:

D is C_{1-4}alkylene (e.g., methylene, ethylene or prop-2-yn-1-ylene);

E is a single bond, C_{2-4}alkynylene (e.g., –C≡C–), arylene (e.g., phenylene) or heteroarylene (e.g., pyridylene);

F is

H,

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl, diazolyl, triazolyl, for example, pyrid-2-yl, imidazol-1-yl, 1,2,4-triazol-1-yl),

halo (e.g., F, Br, Cl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

-C(O)-R_{15},

-N(R_{16})(R_{17}), or

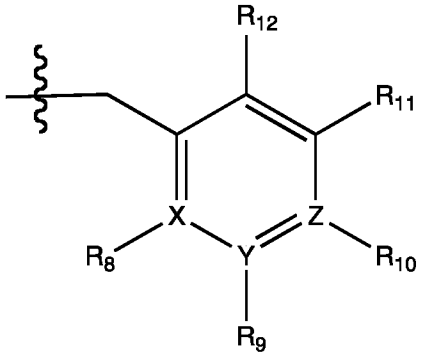
C_{3-7}cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, cyclohexyl, pyrrolidinyl (e.g., pyrrolidin-3-yl), tetrahydro-*2H*-pyran-4-yl, or morpholinyl);

wherein D, E and F are independently and optionally substituted with one or more halo (e.g., F, Cl or Br), C_{1-4}alkyl (e.g., methyl), haloC_{1-4}alkyl (e.g., trifluoromethyl), for example, F is heteroaryl, e.g., pyridyl substituted with one or more halo (e.g., 6-fluoropyrid-2-yl, 5-fluoropyrid-2-yl, 6-fluoropyrid-2-yl, 3-fluoropyrid-2-yl, 4-fluoropyrid-2-yl, 4,6-dichloropyrid-2-yl), haloC_{1-4}alkyl (e.g., 5-trifluoromethylpyrid-2-yl) or C_{1-4}alkyl (e.g., 5-methylpyrid-2-yl), or F is aryl, e.g., phenyl, substituted with one or more halo (e.g., 4-fluorophenyl) or F is a C_{3-}

$_{7}$heterocycloalkyl (e.g., pyrrolidinyl) optionally substituted with a C_{1-6}alkyl (e.g., 1-methylpyrrolidin-3-yl); or

b) a substituted heteroarylalkyl, e.g., substituted with haloalkyl;

c) attached to the nitrogen on the pyrrolo portion of Formula I-A or I-B and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F), and R_{10} is

halogen,

C_{1-4}alkyl,

C_{3-7}cycloalkyl,

C_{1-4}haloalkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl (for example pyrid-2-yl), or thiadiazolyl (e.g., 1,2,3-thiadiazol-4-yl)), diazolyl, triazolyl, tetrazolyl,

arylcarbonyl (e.g., benzoyl),

alkylsulfonyl (e.g., methylsulfonyl),

heteroarylcarbonyl, or

alkoxycarbonyl;

provided that when X, Y, or Z is nitrogen, R_8, R_9, or R_{10}, respectively, is not present;

(vi) R_6 is

H,

C_{1-4}alkyl,

C_{3-7}cycloalkyl (e.g., cyclopentyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyrid-4-yl),

arylC_{1-4}alkyl (e.g., benzyl),

arylamino (e.g., phenylamino),

heteroarylamino,

N,N-diC_{1-4}alkylamino,

N,N-diarylamino,

N-aryl-N-(arylC_{1-4}alkyl)amino (e.g., N-phenyl-N-(1,1'-biphen-4-ylmethyl)amino), or

$-N(R_{18})(R_{19})$;

wherein the aryl or heteroaryl is optionally substituted with one or more halo (e.g., F, Cl), hydroxy or C_{1-6}alkoxy;

(vii) R_7 is H, C_{1-6}alkyl, halogen (e.g., Cl), $-N(R_{18})(R_{19})$;

(viii) n = 0 or 1;

(ix) when n=1, **A** is $-C(R_{13}R_{14})-$, wherein R_{13} and R_{14}, are, independently, H or C_{1-4}alkyl, aryl, heteroaryl, (optionally hetero)arylC_{1-4}alkoxy or (optionally hetero)arylC_{1-4}alkyl;

(x) R_{15} is C_{1-4}alkyl, haloC_{1-4}alkyl, -OH or $-OC_{1-4}$alkyl (e.g., $-OCH_3$)

(xi) R_{16} and R_{17} are independently H or C_{1-4}alkyl;

(xii) R_{18} and R_{19} are independently H, C_{1-4}alky or aryl (e.g., phenyl) wherein said aryl is optionally substituted with one or more halo (e.g., fluorophenyl, e.g., 4-fluorophenyl) or hydroxy (e.g., hydroxyphenyl, e.g., 4-hydroxyphenyl or 2-hydroxyphenyl)

(xiii) R_{20} is H, C_{1-4}alkyl or C_{3-7}cycloalkyl;

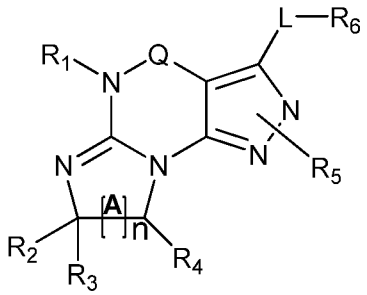
in free or salt form.

1.1 any of the preceding formulae wherein the compounds inhibit phosphodiesterase-mediated (e.g., PDE1-mediated, especially PDE1B-mediated) hydrolysis of cGMP, e.g., with an IC_{50} of less than 1μM,

preferably less than 750 nM, more preferably less than 500 nM, more preferably less than 50 nM in an immobilized-metal affinity particle reagent PDE assay,

in free or salt form.

[0032] The invention further provides optionally substituted 4,5,7,8-tetrahydro-(optionally 4-thioxo or 4-imino)-(1*H* or 2*H*)-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine or 4,5,7,8,9-pentahydro-(1*H* or 2*H*)-pyrimido[1,2-a]pyrazolo[4,3-e]pyrimidine compounds, in free or salt form, e.g., (1 or 2 and/or 3 and/or 5)-substituted 4,5,7,8-tetrahydro-1*H*-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine, 4,5,7,8-tetrahydro-2*H*-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine, 4,5,7,8-tetrahydro-(1*H* or 2*H*)-pyrimido[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-imine, 7,8-dihydro-1*H*-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-thione or 7,8-dihydro-2H-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-thione compounds, e.g., a Compound of Formula III:



Formula III

wherein

(xiv) Q is C(=S), C(=N(R_{20})) or CH_2;

(xv) L is a single bond, -N(H)-, -CH_2-;

(xvi) R_1 is H or C_{1-4} alkyl (e.g., methyl or ethyl);

(xvii) R_4 is H or C_{1-6} alkyl (e.g., methyl, isopropyl) and R_2 and R_3 are, independently:

H or C_{1-6}alkyl (e.g., methyl or isopropyl) optionally substituted with halo or hydroxy (e.g., R_2 and R_3 are both methyl, or R_2 is H and R_3 is methyl, ethyl, isopropyl or hydroxyethyl),

aryl,

heteroaryl,

(optionally hetero)arylalkoxy,

(optionally hetero)arylC_{1-6}alkyl, or

R_2 and R_3 together form a 3- to 6-membered ring;

or

R_2 is H and R_3 and R_4 together form a di-, tri- or tetramethylene bridge (pref. wherein the R_3 and R_4 together have the *cis* configuration, e.g., where the carbons carrying R_3 and R_4 have the R and S configurations, respectively);

(xviii) R_5 is

d) –D-E-F, wherein:

D is C_{1-4}alkylene (e.g., methylene, ethylene or prop-2-yn-1-ylene);

E is a single bond, C_{2-4}alkynylene (e.g., –C≡C–), arylene (e.g., phenylene) or heteroarylene (e.g., pyridylene);

F is

H,

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl, diazolyl, triazolyl, for example, pyrid-2-yl, imidazol-1-yl, 1,2,4-triazol-1-yl),

halo (e.g., F, Br, Cl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

-C(O)-R_{15},

-N(R_{16})(R_{17}),

-S(O)$_2R_{21}$ or

C_{3-7}cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, cyclohexyl, pyrrolidinyl (e.g., pyrrolidin-3-yl), tetrahydro-*2H*-pyran-4-yl, or morpholinyl);

wherein D, E and F are independently and optionally substituted with one or more :

halo (e.g., F, Cl or Br),

C_{1-4}alkyl (e.g., methyl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

C_{1-4}alkoxy) or

C_{1-4}alkyl (e.g., 5-methylpyrid-2-yl),

for example, F is heteroaryl, e.g., pyridyl substituted with one or more halo (e.g., 6-fluoropyrid-2-yl, 5-fluoropyrid-2-yl, 6-fluoropyrid-2-yl, 3-fluoropyrid-2-yl, 4-fluoropyrid-2-yl, 4,6-dichloropyrid-2-yl),

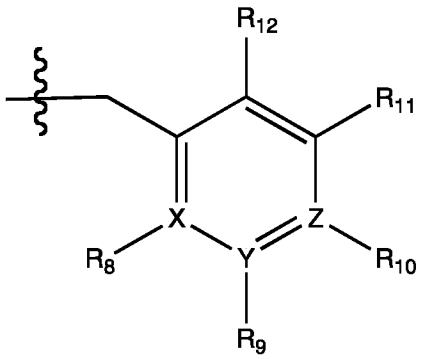
or F is aryl, e.g., phenyl, substituted with one or more halo (e.g., 4-fluorophenyl)

or F is a C_{3-7}heterocycloalkyl (e.g., pyrrolidinyl) optionally substituted with a C_{1-6}alkyl (e.g., 1-methylpyrrolidin-3-yl);

or

e) a substituted heteroarylalkyl, e.g., substituted with haloalkyl;

f) attached to one of the nitrogens on the pyrazolo portion of Formula III and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F), and R_{10} is:

halogen,

C_{1-4}alkyl,

C_{3-7}cycloalkyl,

hetC_{3-7}cycloalkyl (e.g., pyrrolidinyl or piperidinyl),

C_{1-4}haloalkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl (for example pyrid-2-yl), or thiadiazolyl (e.g., 1,2,3-thiadiazol-4-yl)), diazolyl, triazolyl, tetrazolyl,

arylcarbonyl (e.g., benzoyl),

alkylsulfonyl (e.g., methylsulfonyl),

heteroarylcarbonyl, or

alkoxycarbonyl;

wherein the aryl, heteroaryl, cycloalkyl or heterocycloalkyl is independently and optionally substituted with one or more halo (e.g., F or Cl), C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}haloalkyl (e.g., trifluoromethyl), -SH;

preferably R_{10} is phenyl, pyridyl, piperidinyl or pyrrolidinyl optionally substituted with the substituents previously defined, e.g. optionally substituted with halo or alkyl

provided that when X, Y, or Z is nitrogen, R_8, R_9, or R_{10}, respectively, is not present;

(xix) R_6 is

H,

C_{1-4}alkyl,

C_{3-7}cycloalkyl (e.g., cyclopentyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl, for example, pyrid-4-yl),

arylC_{1-4}alkyl (e.g., benzyl),

arylamino (e.g., phenylamino),

heterarylamino,

N,N-diC_{1-4}alkylamino,

N,N-diarylamino,

N-aryl-N-(arylC_{1-4}alkyl)amino (e.g., N-phenyl-N-(1,1'-biphen-4-ylmethyl)amino), or

-N(R_{18})(R_{19});

wherein the aryl or heteroaryl is optionally substituted with one or more halo (e.g., F, Cl), hydroxy, C_{1-6}alkyl, C_{1-6}alkoxy, C_{3-8}cycloalkyl, for example, R_6 is 4-hydroxyphenyl or 4-fluorophenyl,

(xx) n = 0 or 1;

(xxi) when n=1, A is -C($R_{13}R_{14}$)-, wherein R_{13} and R_{14}, are, independently, H or C_{1-4}alkyl, aryl, heteroaryl, (optionally hetero)arylC_{1-4}alkoxy,(optionally hetero)arylC_{1-4}alkyl or R_{13} or R_{14} can form a bridge with R_2 or R_4;

(xxii) R_{15} is C_{1-4}alkyl, haloC_{1-4}alkyl, -OH or –OC_{1-4}alkyl (e.g., -OCH_3)

(xxiii) R_{16} and R_{17} are independently H or C_{1-4}alkyl;

(xxiv) R_{18} and R_{19} are independently

H,

C_{1-4}alky,

C_{3-8}cycloalkyl,

heteroC_{3-8}cycloalkyl,

aryl (e.g., phenyl), or

heteroaryl,

wherein said aryl or heteroaryl is optionally substituted with one or more

halo (e.g., fluorophenyl, e.g., 4-fluorophenyl),

hydroxy (e.g., hydroxyphenyl, e.g., 4-hydroxyphenyl or 2-hydroxyphenyl),

C_{1-6}alkyl,

haloC_{1-6}alkyl,

C_{1-6}alkoxy,

aryl,

heteroaryl, or

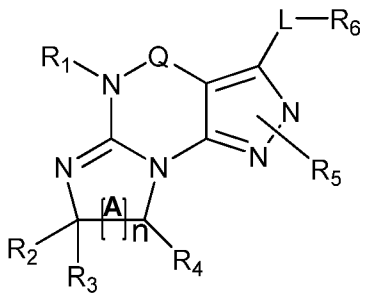
C_{3-8}cycloalkyl;

(xxv) R_{20} is H, C_{1-4}alkyl (e.g., methyl) or C_{3-7}cycloalkyl,

(xxvi) R_{21} is C_{1-6}alkyl;

in free or salt form.

[0033] In yet another embodiment, the invention also provides a Compound of Formula IV:



Formula IV

wherein

Q is C(=S), C(=N(R_{20})) or CH_2;

L is a single bond, -N(H)-, -CH_2-;

R_1 is H or C_{1-4} alkyl (e.g., methyl or ethyl);

R_4 is H or C_{1-6} alkyl (e.g., methyl, isopropyl) and R_2 and R_3 are, independently, H or C_{1-6}alkyl (e.g., methyl or isopropyl) optionally substituted with halo or hydroxy (e.g., R_2 and R_3 are both methyl, or R_2 is H and R_3 is methyl, ethyl, isopropyl or hydroxyethyl), aryl, heteroaryl, (optionally hetero)arylalkoxy, or (optionally hetero)arylC_{1-6}alkyl; or R_2 is H and R_3 and R_4 together form a di-, tri- or tetramethylene bridge (pref. wherein the R_3 and R_4 together have the *cis* configuration, e.g., where the carbons carrying R_3 and R_4 have the R and S configurations, respectively);

R_5 is

a) –D-E-F, wherein:

D is C_{1-4}alkylene (e.g., methylene, ethylene or prop-2-yn-1-ylene);

E is a single bond, C_{2-4}alkynylene (e.g., –C≡C–), arylene (e.g., phenylene) or heteroarylene (e.g., pyridylene);

F is H, aryl (e.g., phenyl), heteroaryl (e.g., pyridyl, diazolyl, triazolyl, for example, pyrid-2-yl, imidazol-1-yl, 1,2,4-triazol-1-yl), halo (e.g., F, Br, Cl), haloC_{1-4}alkyl (e.g., trifluoromethyl), -C(O)-R_{15}, -N(R_{16})(R_{17}), -S(O)$_2$$R_{21}$ or C_{3-}

$_{7}$cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, cyclohexyl, pyrrolidinyl (e.g., pyrrolidin-3-yl), tetrahydro-2*H*-pyran-4-yl, or morpholinyl);

wherein D, E and F are independently and optionally substituted with one or more :

halo (e.g., F, Cl or Br),

C_{1-4}alkyl (e.g., methyl),

haloC_{1-4}alkyl (e.g., trifluoromethyl),

for example, F is heteroaryl, e.g., pyridyl substituted with one or more halo (e.g., 6-fluoropyrid-2-yl, 5-fluoropyrid-2-yl, 6-fluoropyrid-2-yl, 3-fluoropyrid-2-yl, 4-fluoropyrid-2-yl, 4,6-dichloropyrid-2-yl), haloC_{1-4}alkyl (e.g., 5-trifluoromethylpyrid-2-yl) or C_{1-4}alkyl (e.g., 5-methylpyrid-2-yl),

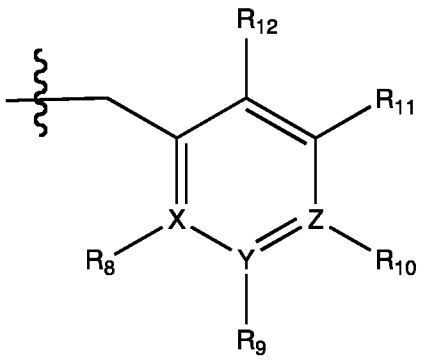
or F is aryl, e.g., phenyl, substituted with one or more halo (e.g., 4-fluorophenyl)

or F is a C_{3-7}heterocycloalkyl (e.g., pyrrolidinyl) optionally substituted with a C_{1-6}alkyl (e.g., 1-methylpyrrolidin-3-yl);

or

b) a substituted heteroarylalkyl, e.g., substituted with haloalkyl;

c) attached to one of the nitrogens on the pyrazolo portion of Formula IV and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F), and R_{10} is:

halogen,

C_{1-4}alkyl,

C_{3-7}cycloalkyl,

C_{1-4}haloalkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl (for example pyrid-2-yl), or thiadiazolyl (e.g., 1,2,3-thiadiazol-4-yl)), diazolyl, triazolyl, tetrazolyl,

arylcarbonyl (e.g., benzoyl),

alkylsulfonyl (e.g., methylsulfonyl),

heteroarylcarbonyl, or

alkoxycarbonyl;

provided that when X, Y, or Z is nitrogen, R_8, R_9, or R_{10}, respectively, is not present;

R_6 is

H,

C_{1-4}alkyl,

C_{3-7}cycloalkyl (e.g., cyclopentyl),

aryl (e.g., phenyl),

heteroaryl (e.g., pyridyl, for example, pyrid-4-yl),

arylC_{1-4}alkyl (e.g., benzyl),

arylamino (e.g., phenylamino),

heterarylamino,

N,N-diC_{1-4}alkylamino,

N,N-diarylamino,

N-aryl-N-(arylC_{1-4}alkyl)amino (e.g., N-phenyl-N-(1,1'-biphen-4-ylmethyl)amino), or

$-N(R_{18})(R_{19})$;

wherein the aryl or heteroaryl is optionally substituted with one or more halo (e.g., F, Cl), hydroxy or C_{1-6}alkoxy, for example, R_6 is 4-hydroxyphenyl or 4-fluorophenyl,

n = 0 or 1;

when n=1, **A** is -C($R_{13}R_{14}$)-, wherein R_{13} and R_{14}, are, independently, H or C_{1-4}alkyl, aryl, heteroaryl, (optionally hetero)arylC_{1-4}alkoxy or (optionally hetero)arylC_{1-4}alkyl;

R_{15} is C_{1-4}alkyl, haloC_{1-4}alkyl, -OH or –OC_{1-4}alkyl (e.g., -OCH_3)

R_{16} and R_{17} are independently H or C_{1-4}alkyl;

R_{18} and R_{19} are independently H, C_{1-4}alky or aryl (e.g., phenyl) wherein said aryl is optionally substituted with one or more halo (e.g., fluorophenyl, e.g., 4-fluorophenyl) or hydroxy (e.g., hydroxyphenyl, e.g., 4-hydroxyphenyl or 2-hydroxyphenyl)

R_{20} is H, C_{1-4}alkyl (e.g., methyl) or C_{3-7}cycloalkyl,

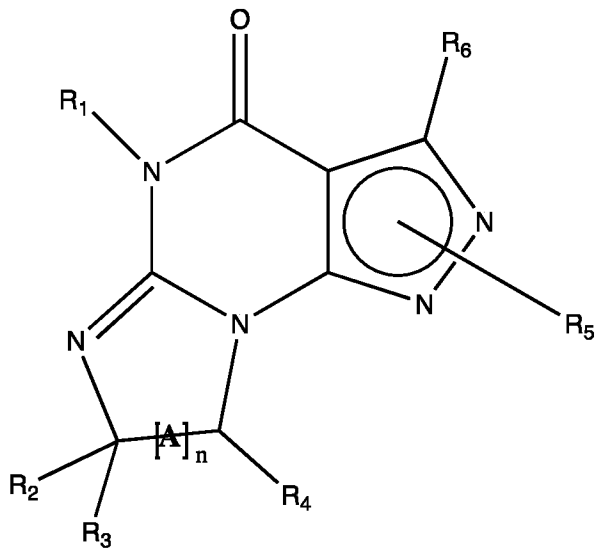
R_{21} is C_{1-6}alkyl;

in free or salt form.

[0034] In still yet another embodiment, the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis which are described herein are selected from any of the Applicant's own publications: US 2008-0188492 A1, US 2010-0173878 A1, US 2010-0273754 A1, US 2010-0273753 A1, WO 2010/065153, WO 2010/065151, WO 2010/065151, WO 2010/065149, WO 2010/065147, WO 2010/065152, WO 2011/153129, WO 2011/133224, WO 2011/153135, WO

2011/153136, and WO 2011/153138, the entire contents of each of which are incorporated herein by reference in their entireties.

[0035] In yet another embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are compounds of Formula V:



[0032]

Formula V

wherein

(i) R_1 is H or C_{1-4} alkyl (e.g., methyl);

(ii) R_4 is H or C_{1-4} alkyl and R_2 and R_3 are, independently, H or C_{1-4} alkyl (e.g., R_2 and R_3 are both methyl, or R_2 is H and R_3 is isopropyl), aryl, heteroaryl, (optionally hetero)arylalkoxy, or (optionally hetero)arylalkyl;

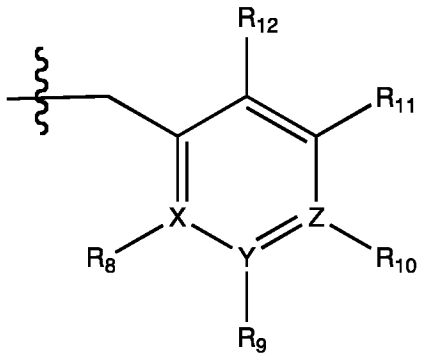
or

R_2 is H and R_3 and R_4 together form a di-, tri- or tetramethylene bridge (pref. wherein the R_3 and R_4 together have the *cis* configuration, e.g., where the carbons carrying R_3 and R_4 have the R and S configurations, respectively);

(iii) R_5 is a substituted heteroarylalkyl, e.g., substituted with haloalkyl

or

R_5 is attached to one of the nitrogens on the pyrazolo portion of Formula V and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F), and R_{10} is halogen, alkyl, cycloalkyl, haloalkyl (e.g., trifluoromethyl), aryl (e.g., phenyl), heteroaryl (e.g., pyridyl (for example pyrid-2-yl), or thiadiazolyl (e.g., 1,2,3-thiadiazol-4-yl)), diazolyl, triazolyl, tetrazolyl, arylcarbonyl (e.g., benzoyl), alkylsulfonyl (e.g., methylsulfonyl), heteroarylcarbonyl, or alkoxycarbonyl; provided that when X, Y, or Z is nitrogen, R_8, R_9, or R_{10}, respectively, is not present; and

(iv) R_6 is H, alkyl, aryl, heteroaryl, arylalkyl (e.g., benzyl), arylamino (e.g., phenylamino), heterarylamino, N,N-dialkylamino, N,N-diarylamino, or N-aryl-N-(arylakyl)amino (e.g., N-phenyl-N-(1,1'-biphen-4-ylmethyl)amino); and

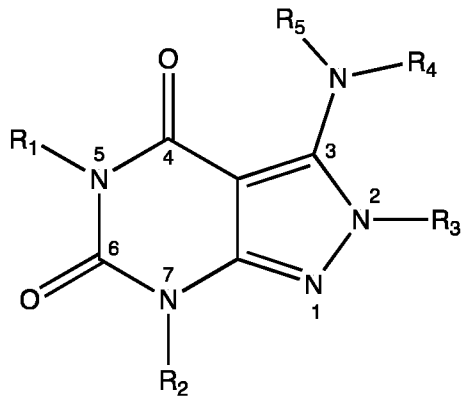
(v) n=0 or 1;

(vi) when n=1, **A** is $-C(R_{13}R_{14})-$

wherein R_{13} and R_{14}, are, independently, H or C_{1-4} alkyl, aryl, heteroaryl, (optionally hetero)arylalkoxy or (optionally hetero)arylalkyl;

in free, salt or prodrug form, including its enantiomers, diastereoisomers and racemates.

[0036] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are compounds of Formula VI:



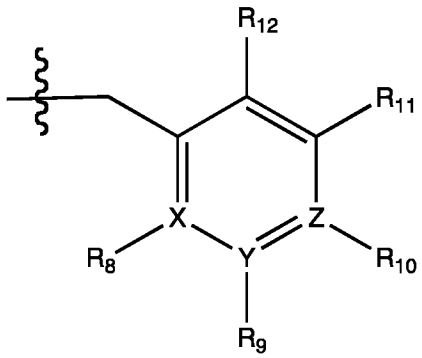
Formula VI

wherein:

(i) R_1 is H or alkyl;

(ii) R_2 is H, alkyl, cycloalkyl, haloalkyl, alkylaminoalkyl, hydroxyalkyl, arylalkyl, heteroarylalkyl, or alkoxyarylalkyl;

(iii) R_3 is heteroarylmethyl or formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen; and R_{10} is halogen, alkyl, cycloalkyl, haloalkyl, aryl,

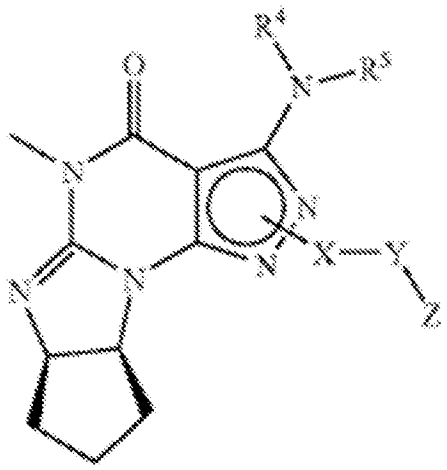
heteroaryl, alkyl sulfonyl, arylcarbonyl, heteroarylcarbonyl, alkoxycarbonyl, or aminocarbonyl;

(iv) R_4 is aryl or heteroaryl; and

(v) R_5 is H, alkyl, cycloalkyl, heteroaryl, aryl, p-benzylaryl;

provided that when X, Y or X is nitrogen, R_8, R_9 or R_{10}, respectively, is not present; wherein "alk" or "alkyl" refers to C_{1-6} alkyl and "cycloalkyl" refers to C_{3-6} cycloalkyl, in free, salt or physiologically hydrolysable and acceptable ester prodrug form.

[0037] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are compounds of Formula VII:



Formula VII

(i) X is C_{1-6}alkylene (e.g., methylene, ethylene or prop-2-yn-1-ylene);

(ii) Y is a single bond, alkynylene (e.g., —C≡C—), arylene (e.g., phenylene) or heteroarylene (e.g., pyridylene);

(iii) Z is H, aryl (e.g., phenyl), heteroaryl (e.g., pyridyl, e.g., pyrid-2-yl), halo (e.g., F, Br, Cl), haloC_{1-6}alkyl (e.g., trifluoromethyl), —C(O)—R^1, —N(R^2)(R^3), or C_{3-7}cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, cyclohexyl, tetrahydro-2H-pyran-4-yl, or morpholinyl);

(iv) R^1 is C_{1-6}alkyl, haloC_{1-6}alkyl, —OH or —OC_{1-6}alkyl (e.g., —OCH_3);

(v) R^2 and R^3 are independently H or C_{1-6}alkyl;

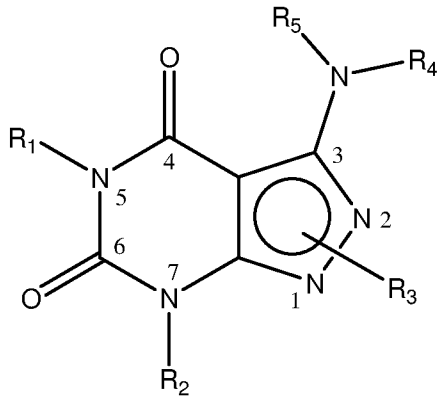
(vi) R^4 and R^5 are independently H, C_{1-6}alky or aryl (e.g., phenyl) optionally substituted with one or more halo (e.g., fluorophenyl, e.g., 4-fluorophenyl), hydroxy (e.g., hydroxyphenyl, e.g., 4-hydroxyphenyl or 2-hydroxyphenyl) or C_{1-6}alkoxy;

(vii) wherein X, Y and Z are independently and optionally substituted with one or more halo (e.g., F, Cl or Br), C_{1-6}alkyl (e.g., methyl), haloC_{1-6}alkyl (e.g., trifluoromethyl), for example, Z is heteroaryl, e.g., pyridyl substituted with one or more halo (e.g., 6-fluoropyrid-2-yl, 5-fluoropyrid-2-yl, 6-fluoropyrid-2-yl, 3-fluoropyrid-2-yl, 4-fluoropyrid-2-yl, 4,6-dichloropyrid-2-yl), haloC_{1-6}alkyl (e.g., 5-trifluoromethylpyrid-2-yl) or C_{1-6}-alkyl (e.g., 5-methylpyrid-2-yl), or Z is aryl, e.g., phenyl, substituted with one or more halo (e.g., 4-fluorophenyl),

in free, salt or prodrug form.

[0038] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are compounds of Formula VIII:

[0033]



Formula VIII

wherein

(i) R_1 is H or C_{1-6}alkyl;

(ii) R_2 is

H,

C_{1-6}alkyl,

C_{3-8}cycloalkyl optionally substituted with one or more amino,

C_{3-8}heterocycloalkyl optionally substituted with C_{1-6}alkyl,

C_{3-8}cycloalkyl-C_{1-6}alkyl,

C_{1-6}haloalkyl,

C_{0-6}alkylaminoC_{0-6}alkyl,

hydroxyC_{1-6}alkyl,

arylC_{0-6}alkyl,

heteroarylalkyl,

C_{1-6}alkoxyarylC_{1-6}alkyl, or

-G-J wherein:

G is a single bond or, alkylene;

J is cycloalkyl or heterocycloalkyl optionally substituted with alkyl;

(iii) R_3 is

a) -D-E-F wherein

1. D is single bond, C_{1-6}alkylene or arylC_{1-6}alkylene;
2. E is a C_{1-6}alkylene, arylene, C_{1-6}alkylarylene, aminoC_{1-6}alkylene- or amino; and
3. F is heteroC_{3-8}cycloalkyl optionally substituted with C_{1-6}alkyl;

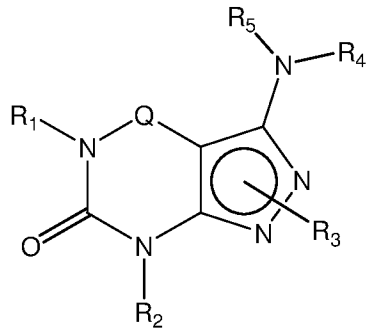
(iv) R_4 is aryl optionally substituted with one or more halo, hydroxy[[l]] or C_{1-6}alkoxy[[,]]; heteroaryl; or heteroC_{3-6}cycloalkyl; and

(v) R_5 is H, C_{1-6}alkyl, C_{3-8}cycloalkyl, heteroaryl, aryl or p-benzylaryl;

wherein "alk", "alkyl", "haloalkyl" or "alkoxy" refers to C_{1-6} alkyl and "cycloalkyl" refers to C_{3-8}cycloalkyl;

in free or salt form.

[0039] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are compounds of Formula IX:



Formula IX

wherein

(i) Q is -C(=S)-, -C(=N(R_6))- or -C(R_{14})(R_{15})-;

(ii) R_1 is H or C_{1-6}alkyl (e.g., methyl or ethyl);

(iii) R_2 is

H,

C_{1-6}alkyl (e.g., isopropyl, isobutyl, 2-methylbutyl or 2,2-dimethylpropyl) wherein said alkyl group is optionally substituted with one or more halo (e.g., fluoro) or hydroxy (e.g., hydroxyC_{1-6}alkyl, for example 1-hydroxyprop-2-yl or 3-hydroxy-2-methylpropyl),

haloC_{1-6}alkyl (e.g., trifluoromethyl or 2,2,2-trifluoroethyl),

N(R_{14})(R_{15})-C_{1-6}alkyl (e.g., 2-(dimethylamino)ethyl or 2-aminopropyl),

arylC_{0-6}alkyl (e.g., phenyl or benzyl), wherein said aryl is optionally substituted with one or more C_{1-6}alkoxy, for example, C_{1-6}alkoxyarylC_{0-6}alkyl (e.g., 4-methoxybenzyl),

heteroarylC_{0-6}alkyl (e.g., pyridinylmethyl), wherein said heteroaryl is optionally substituted with one or more C_{1-6}alkoxy (e.g., C_{1-6}alkoxyheteroarylC_{1-6}alkyl);

-G-J wherein G is a single bond or C_{1-6}alkylene (e.g., methylene) and J is C_{3-8}cycloalkyl or heteroC_{3-8}cycloalkyl (e.g., oxetan-2-yl, pyrrolidin-3-yl, pyrrolidin-2-yl) wherein the cycloalkyl and heterocycloalkyl group are optionally substituted with one or more C_{1-6}alkyl or amino, for example,

–C_{0-4}alkyl-C_{3-8}cycloalkyl (e.g., –C_{0-4}alkyl-cyclopentyl, –C_{0-4}alkyl-cyclohexyl or –C_{0-4}alkyl-cyclopropyl), wherein said cycloalkyl is optionally substituted with one or more C_{1-6}alkyl or amino (for example, 2-aminocyclopentyl or 2-aminocyclohexyl),

–C_{0-4}alkyl-C_{3-8}heterocycloalkyl (e.g., –C_{0-4}alkyl-pyrrolidinyl, for example, –C_{0-4}alkylpyrrolidin-3-yl) wherein said heterocycloalkyl is optionally substituted with C_{1-6}alkyl (e.g., methyl), for example, 1-methylpyrrolidin-3-yl, 1-methyl-pyrrolindin-2-yl, 1-methyl-pyrrolindin-2-yl-methyl or 1-methyl-pyrrolindin-3-yl-methyl);

(iv) R_3 is

1) –D-E-F wherein:

D is a single bond, C_{1-6}alkylene (e.g., methylene), or arylC_{1-6}alkylene (e.g., benzylene or –$CH_2C_6H_4$–);

E is

a single bond,

C_{1-4}alkylene (e.g., methylene, ethynylene, prop-2-yn-1-ylene),

C_{0-4}alkylarylene (e.g., phenylene or –C_6H_4–, -benzylene- or –$CH_2C_6H_4$-), wherein the arylene group is optionally substituted with halo (e.g., Cl or F),

heteroarylene (e.g., pyridinylene or pyrimidinylene),

aminoC$_{1-6}$alkylene (e.g., –CH$_2$N(H)–),

amino (e.g., –N(H)–);

C$_{3-8}$cycloalkylene optionally containing one or more heteroatom selected from N or O (e.g., piperidinylene),

F is

H,

halo (e.g., F, Br, Cl),

C$_{1-6}$alkyl (e.g., isopropyl or isobutyl),

haloC$_{1-6}$alkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

C$_{3-8}$cycloalkyl optionally containing one or more atom selected from a group consisting of N, S or O (e.g., cyclopentyl, cyclohexyl, piperidinyl, pyrrolidinyl, tetrahydro-2*H*-pyran-4-yl, or morpholinyl), and optionally substituted with one or more C$_{1-6}$alkyl (e.g., methyl or isopropyl), for example, 1-methylpyrrolidin-2-yl, pyrrolidin-1-yl, pyrrolidin-2-yl, piperidin-2-yl, 1-methylpiperidin-2-yl, 1-ethylpiperidin-2-yl,

heteroaryl (e.g., pyridyl (for example, pyrid-2-yl), pyrimidinyl (for example, pyrimidin-2-yl), thiadiazolyl (for example, 1,2,3-thiadiazol-4-yl), diazolyl (e.g., pyrazolyl (for example, pyrazol-1-yl) or imidazolyl (for example, imidazol-1-yl, 4-methylimidazolyl, 1-methylimidazol-2-yl)), triazolyl (e.g., 1,2,4-triazol-1-yl), tetrazolyl (e.g., tetrazol-5-yl), alkyloxadiazolyl (e.g., 5-methyl-1,2,4-oxadiazol), wherein said heteroaryl is optionally substituted with one or more C$_{1-6}$alkyl, halo (e.g., fluoro) or haloC$_{1-6}$alkyl;

C$_{1-6}$alkoxy,

–O-haloC$_{1-6}$alkyl (e.g., –O-CF$_3$),

C$_{1-6}$alkylsulfonyl (for example, methylsulfonyl or –S(O)$_2$CH$_3$),

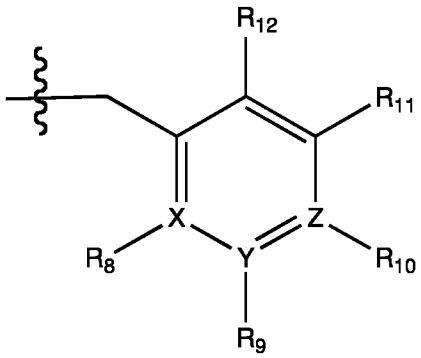
-C(O)-R_{13}, wherein R_{13} is $-N(R_{14})(R_{15})$, C_{1-6}alkyl (e.g., methyl), -OC_{1-6}alkyl (e.g., -OCH_3), haloC_{1-6}alkyl (trifluoromethyl), aryl (e.g., phenyl), or heteroaryl; -$N(R_{14})(R_{15})$;

or

2) a substituted heteroarylC_{1-6}aklyl, e.g., substituted with haloC_{1-6}alkyl;

or

3) attached to one of the nitrogens on the pyrazolo portion of Formula I and is a moiety of Formula A



Formula A

wherein:

X, Y and Z are, independently, N or C,

R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F); and

R_{10} is

halogen (e.g., fluoro or chloro),

C_{1-6}alkyl,

C_{3-8}cycloalkyl,

heteroC_{3-8}cycloalkyl (e.g., pyrrolidinyl or piperidinyl),

haloC_{1-6}alkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl) or heteroaryl (e.g., pyridyl, (for example, pyrid-2-yl) or e.g., thiadiazolyl (for

example, 1,2,3-thiadiazol-4-yl), diazolyl, triazolyl (e.g., 1,2,4-triazol-1-yl), tetrazolyl (e.g., tetrazol-5-yl), alkyloxadiazolyl (e.g., 5-methyl-1,2,4-oxadiazol), pyrazolyl (e.g., pyrazol-1-yl),

wherein said aryl, heteroaryl, cycloalkyl or heterocycloalkyl is optionally substituted with one or more C_{1-6}alkyl (e.g., methyl), halogen (e.g., chloro or fluoro), haloC_{1-6}alkyl (e.g., trifluoromethyl), hydroxy, carboxy, -SH, or an additional aryl or heteroaryl (e.g., biphenyl or pyridylphenyl),

C_{1-6}alkyl sulfonyl (e.g., methyl sulfonyl),

arylcarbonyl (e.g., benzoyl),

heteroarylcarbonyl,

C_{1-6}alkoxycarbonyl, (e.g., methoxycarbonyl),

Aminocarbonyl,

$-N(R_{14})(R_{15})$;

preferably R_{10} is phenyl, pyridyl, piperidinyl or pyrrolidinyl optionally substituted with the substituents previously defined, e.g. optionally substituted with halo or alkyl;

provided that when X, Y or X is nitrogen, R_8, R_9 or R_{10}, respectively, is not present;

(v) R_4 and R_5 are independently:

H,

C_{1-6}alkyl (e.g., methyl, isopropyl, isobutyl, n-propyl),

C_{3-8}cycloalkyl (e.g., cyclopentyl or cyclohexyl),

C_{3-8}heterocycloalkyl (e.g., pyrrolidinyl (for example pyrrolidin-3-yl or pyrrolidin-1-yl), piperidinyl (for example, piperidin-1-yl), morpholinyl),

$-C_{0-6}$alkylaryl (e.g., phenyl or benzyl) or

– C_{0-6}alkylheteroaryl (e.g., pyrid-4-yl, pyrid-2-yl or pyrazol-3-yl)

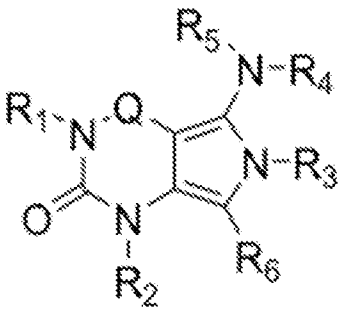
wherein said aryl or heteroaryl is optionally substituted with one or more halo (e.g., 4-fluorophenyl), hydroxy (e.g., 4-hydroxyphenyl), C_{1-6}alkyl, C_{1-6}alkoxy or another aryl group (e.g., biphenyl-4-ylmethyl);

(vi) R_6 is H, C_{1-6}alkyl (e.g., methyl or ethyl) or C_{3-8}cycloalkyl;

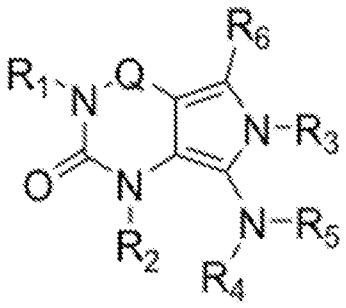
(vii) R_{14} and R_{15} are independently H or C_{1-6}alkyl,

in free or salt form.

[0040] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are Formula X, selected from Formula X-A or X-B:



or



Formula X-A Formula X-B

[0041] wherein

(i) Q is -C(=S)-, -C(=O)-, -C(=N(R_7))- or -C(R_{14})(R_{15})-;

(ii) R_1 is H or C_{1-6}alkyl (e.g., methyl or ethyl);

(iii) R_2 is H, C_{1-6}alkyl (e.g., isopropyl, isobutyl, 2-methylbutyl, 2,2-dimethylpropyl) wherein said alkyl group is optionally substituted with halo (e.g., fluoro) or hydroxy (e.g., 1- hydroxypropan-2-yl, 3-hydroxy-2-methylpropyl), for example, R_2 may be a trifluoromethyl or 2,2,2-trifluoroethyl, N(R_{14})(R_{15})- C_{1-6}alkyl (e.g., 2-(dimethylamino)ethyl or 2-aminopropyl), arylC_{1-6}alkyl (e.g., phenyl or benzyl), heteroaryl C_{1-6}alkyl

(e.g., pyridinylmethyl), C_{1-6}alkoxyaryl-C_{1-6}alkyl (e.g., 4-methoxybenzyl); -G-J wherein:

G is a single bond or, alkylene (e.g., methylene); J is cycloalkyl or heterocycloalkyl (e.g., oxetan-2-yl, pyrolyin-3-yl, pyrolyin-2-yl) optionally substituted with one or more C_{1-6}alkyl (e.g., (l-methylpyrolidin-2-yl)), amino (e.g., -NH_2), for example, -G-J may be -C_{0-4}alkyl-C_{3-8}cycloalkyl (e.g., cyclopentyl, cyclohexyl or cyclopropylmethyl) optionally substituted with one or more C_{1-6}alkyl, amino (e.g., -NH_2), for example, 2-aminocyclopentyl or 2-aminocyclohexyl,wherein said cycloalkyl optionally contains one or more heteroatom selected from N and O (e.g., pyrrolidinyl, for example, pyrrolidin-3-yl or pyrrolidin-2-yl, l-methyl-pyrrolindin-2-yl, 1-methyl- pyrrolindin-3-yl, l-methyl-pyrrolindin-2-yl-methyl or l-methyl-pyrrolindin-3-yl-methyl);

(iv) R_3 is

1) -D-E-F wherein:

D is a single bond, C_{i-6}alkylene (e.g., methylene), or arylalkylene

(e.g., p-benzylene or -$CH_2C_6H_4$-);

E is a single bond,

C_{1-6}alkylene (e.g., methylene) C_{2-6}alkynylene (e.g., ethynylene, prop-2-yn-l- ylene),ethynylene, prop-2-yn- 1 -ylene), -C_{o-4}alkylarylene (e.g., phenylene or -C_6H_4-, -benzyleή ε- or - $CH_2C_6H_4$-), wherein the arylene group is optionally substituted with halo (e.g., Cl or F), heteroarylene (e.g., pyridinylene or pyrimidinylene), aminoC_{i-6}alkylene (e.g., -$CH_2N(H)$-), amino (e.g., -N(H)-);

C_{3-8}cycloalkylene optionally containing one or more heteroatom selected from N or O (e.g., piperidinylene),

F is

H,

halo (e.g., F, Br, Cl), C_{1-6}alkyl (e.g., isopropyl or isobutyl), haloC_{1-6}alkyl (e.g., trifluoromethyl),

aryl (e.g., phenyl),

C_{3-8}cycloalkyl optionally containing at least one atom selected from a group consisting of N or O (e.g., cyclopentyl, N cyclohexyl, piperidinyl, pyrrolidinyl, tetrahydro-2H-pyran- 4-yl, or morpholinyl), said cycloalkyl is optionally substituted with C_{1-6}alkyl (e.g., methyl or isopropyl), for example, l-methylpyrrolidin-2-yl, pyrrolidin-1-yl, pyrrolidin-2-yl, piperidin-2-yl, l-methyrpiperidin-2-yl, 1- ethylpiperidin-2-yl, heteroaryl optionally substituted with C_{1-6}alkyl, (e.g., pyridyl, (for example, pyrid-2-yl), pyrimidinyl (for example, pyrimidin-2-yl), thiadiazolyl (for example, 1,2,3-thiadiazol- 4-yl), diazolyl (e.g., pyrazolyl (for example, pyrazol-1-yl) or imidazolyl (for example, imidazol-1-yl, 4- methylimidazolyl, l-methylimidazol-2-yl,), triazolyl (e.g., 1,2,4-triazol-l-yl), tetrazolyl (e.g., tetrazol-5-yl), alkoxadiazolyl (e.g., 5-methyl-l,2,4-oxadiazol), pyrazolyl (e.g., pyrazol-1-yl), wherein said

heteroaryl is optionally substituted with halo (e.g., fluoro) or haloCi_{-6}alkyl, for example, 6-fluoropyrid-2-yl; amino (e.g., $-NH_2$), C_{1-6}alkoxy, -O-haloC_{1-6}alkyl (e.g., $-0-CF_3$), C_{1-6}alkylsulfonyl (for example, methylsulfonyl or $-S(O)_2CH_3$),

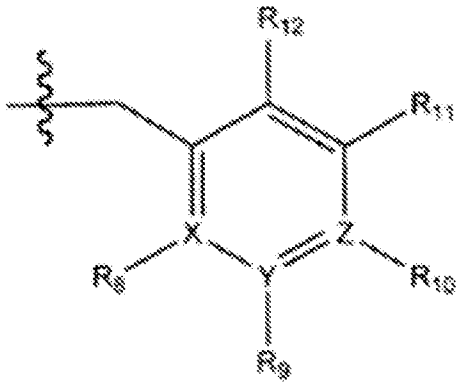
$-C(O)-R_{13}$,

$-N(R_{14})(R_{15})$; or

2) a substituted heteroarylaklyl, e.g., substituted with haloalkyl; or

3) attached to the nitrogen on the pyrrolo portion of Formula I and is a moiety of

Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9, R_{11} and R_{12} are independently H or halogen (e.g., Cl or F); and R_{10} is halogen, C_{1-6}alkyl,

C_{1-6}alkoxy (e.g., methoxy), C_{3-8}cycloalkyl, heteroC_{3-8}cycloalkyl (e.g., pyrrolidinyl) haloC_{1-6}alkyl (e.g., trifluoromethyl), aryl (e.g., phenyl), heteroaryl (e.g., pyridyl, (for example, pyrid-2-yl) or e.g., thiadiazolyl (for example, l,2,3-thiadiazol-4-yl), diazolyl (e.g., imidazolyl or pyrazolyl), triazolyl (e.g., 1,2,4-triazol-l-yl), tetrazolyl (e.g., tetrazol-5-yl), alkoxadiazolyl (e.g., 5-methyl-l,2,4-oxadiazol), pyrazolyl (e.g., pyrazol-1-yl), C_{1-6}alkyl sulfonyl (e.g., methyl sulfonyl), arylcarbonyl (e.g., benzoyl), heteroarylcarbonyl,

alkoxycarbonyl, (e.g., methoxycarbonyl), aminocarbonyl; wherein the aryl, heteroaryl, cycloalkyl or heterocycloalkyl is optionally substituted with one or more C_{1-6}alkyl (e.g., methyl), halogen (e.g., chloro or fluoro), haloC_{1-6}alkyl (e.g., trifluoromethyl), hydroxy, carboxy, -SH, or an additional aryl or heteroaryl (e.g., biphenyl or pyridylphenyl) preferably R_{10} is phenyl or pyridyl, e.g., 2-pyridyl optionally substituted with the substituents previously defined;

provided that when X, Y or X is nitrogen, R_8, R_9 or R_{10}, respectively, is not present; (v) R_4 and R_5 are independently H, C_{i-6}alkyl (e.g., methyl, isopropyl),

C_{3-8}cycloalkyl (e.g., cyclopentyl), C_{3-8}heterocycloalkyl (e.g., pyrrolidin-3-yl), aryl

(e.g., phenyl) or heteroaryl (e.g., pyrid-4-yl, pyrid-2-yl or pyrazol-3-yl) wherein said aryl or heteroaryl is optionally substituted with halo (e.g., 4-fluorophenyl), hydroxy (e.g., 4-hydroxyphenyl), C_{1-6}alkyl, C_{1-6}alkoxy or another aryl group (e.g., biphenyl-4- ylmethyl);

(vi) R_6 is H, C_{1-6}alkyl (e.g., methyl), hydroxy, Ci_{-6}alkoxy, aryloxy, -$N(R_{16})(R_{17})$, oxo (e.g., =0), or C_{3-8}Cycloalkyl;

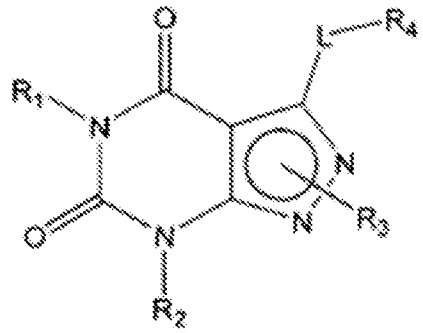
(vii) R_7 is H, C_{1-6}alkyl (e.g., methyl) or C_{3-8}cycloalkyl wherein said cycloalkyl is optionally substituted with one or more oxo (e.g., 2,5-dioxopyrrolidin-l- yl);

(viii) R_{13} is -$N(R_{14})(R_{15})$, C_{1-6}alkyl (e.g., methyl), -OC_{1-6}alkyl (e.g., -OCH_3), haloC_{1-6}alkyl (trifluoromethyl), aryl (e.g., phenyl), or heteroaryl; and

(ix) R_{14} and R_{15} are independently H or C_{1-6}alkyl;

(x) Ri_6 and R_{17} are independently H, C_{1-6}alkyl, aryl (e.g., phenyl), heteroaryl, wherein said aryl or heteroaryl is optionally substituted with halo (e.g., fluoro), C_{1-6}alkoxy (e.g.,methoxy); in free or salt form.

[0042] In one embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are Formula XI:



Formula XI

wherein

(i) L is S, SO or SO_2;

(ii) R_2 is H or C_{1-6}alkyl (e.g., methyl or ethyl);

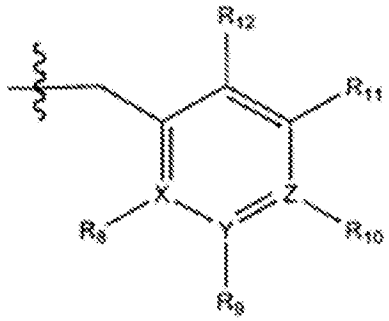
(iii) R_2 is

H,

C_{1-6}alkyl (e.g., isopropyl, isobutyl, neopentyl, 2-methylbutyl, 2,2-dimethylpropyl) wherein said alkyl group is optionally substituted with halo (e.g., fluoro) or hydroxy (e.g., 1- hydroxypropan-2-yl, 3-hydroxy-2-methylpropyl), -C_{0-4}alkyl-C_{3-8}cycloalkyl (e.g., cyclopentyl, cyclohexyl) optionally substituted with one or more amino (e.g., -NH_2), for example, 2-aminocyclopentyl or 2-aminocyclohexyl),wherein said cycloalkyl optionally contains one or more heteroatom selected from N and O and is optionally substituted with C_{1-6}alkyl (e.g., 1-methyl- pyrrolindin-2-yl, l-methyl-pyrrolindin-3-yl, 1-methyl-pyrrolindin-2-yl -methyl or 1 -methyl -pyrrolindin-3-yl- methyl), C_{3-8}heterocycloalkyl (e.g., pyrrolidinyl, for example, pyrrolidin-3-yl) optionally substituted with C_{1-6}alkyl (e.g., methyl), for example, l-methylpyrrolidin-3-yl, C_{3-8}cycloalkyl-C_{1-6}alkyl (e.g.,cyclopropylmethyl), haloC_{1-6}alkyl (e.g., trifluoromethyl, 2,2,2-trifluoroethyl), -N(R_{14})(R_{15})-C_{1-6}alkyl (e.g., 2-(dimethylamino)ethyl,2- aminopropyl), hydroxyC_{1-6}alkyl (e.g., (e.g., 3-hydroxy-2-methylpropyl, 1- hydroxyprop-2-yl), arylC_{0-6}alkyl (e.g., benzyl), heteroarylC_{1-6}alkyl (e.g., pyridinylmethyl), C_{1-6}alkoxyarylC_{1-6}alkyl (e.g., 4-methoxybenzyl); -G-J wherein: G is a single bond or, alkylene (e.g., methylene);

J is cycloalkyl or heterocycloalkyl (e.g., oxetan-2-yl, pyrolyin-3-yl, pyrolyin-2-yl) optionally substituted with C_{1-6}alkyl (e.g., (l-methylpyrolidin-2-yl));

(iv) R_3 is attached to one of the nitrogens on the pyrazolo portion of Formula I and is a moiety of Formula A



Formula A

wherein X, Y and Z are, independently, N or C, and R_8, R_9,

R_{11} and R_{12} are independently H or halogen (e.g., Cl or F); and R_{10} is halogen, C_{1-6}alkyl, C_{3-8}cycloalkyl, heteroC_{3-8}cycloalkyl (e.g., pyrrolidinyl or piperidinyl) haloC_{1-6}alkyl (e.g., trifluoromethyl), aryl (e.g., phenyl), heteroaryl (e.g., pyridyl, (for example, pyrid-2-yl) or e.g., thiadiazolyl (for example, l,2,3-thiadiazol-4- 15 yl), diazolyl, triazolyl (e.g., 1,2,4-triazol-l-yl), tetrazolyl (e.g., tetrazol-5-yl), alkoxadiazolyl (e.g., 5-methyl-l,2,4-oxadiazol), pyrazolyl (e.g., pyrazol- i-yi), alkyl sulfonyl (e.g., methyl sulfonyl),

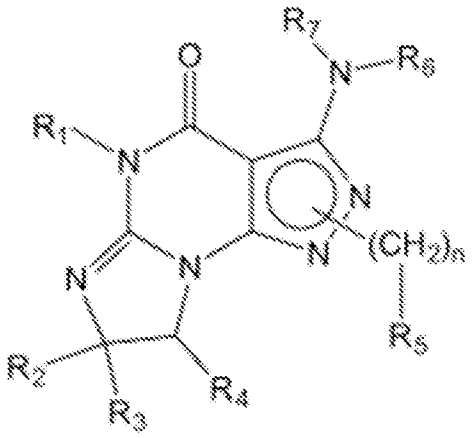
arylcarbonyl (e.g., benzoyl), or heteroarylcarbonyl, alkoxycarbonyl, (e.g., methoxycarbonyl), aminocarbonyl; preferably phenyl, pyridyl, e.g., 2-pyridyl, piperidinyl, or pyrrolidinyl; wherein the aryl, heteroaryl cycloalkyl or heterocycloalkyl is optionally substituted with one or more halo (e.g., F or Cl), C_{1-6}alkly, C_{1-6}alkoxy, C_{1-4}haloalkyl(e.g., trifluoromethyl), and/or -SH, provided that when X, Y or X is nitrogen, R_8, R_9 or R_{10}, respectively, is not present; (v) R_4 is

H, C_{1-6}alkyl (e.g., methyl, isopropyl),

C_{3-8}cycloalkyl (e.g., cyclopentyl), C_{3-8}heterocycloalkyl (e.g., pyrrolidin-3-yl), aryl (e.g., phenyl) or heteroaryl (e.g., pyrid-4-yl, pyrid-2-yl or pyrazol-3-yl) wherein said aryl or heteroaryl is optionally substituted with halo (e.g., 4-fluorophenyl), hydroxy (e.g.,

4-hydroxyphenyl), C_{1-6}alkyl, C_{1-6}alkoxy or another aryl group (e.g., biphenyl-4-ylmethyl); (vi) R_{14} and R_{15} are independently H or C_{1-6}alkyl, in free or salt form.

[0043] In yet another embodiment the invention provides that the PDE1 inhibitors for use in the methods of treatment and prophylaxis described herein are Formula XII:



Formula XII

wherein

(i) R 1 is H or C 1-4 alkyl (e.g., methyl or ethyl);

(ii) R 2 and R 3 are independently H or C 1-6 alkyl (e.g., methyl or ethyl);

(iii) R 4 is H or C 1-4 alkyl (e.g., methyl or ethyl);

(iv) R 5 is aryl (e.g., phenyl) optionally substituted with one or more groups independently selected from -C(=O)- C 1-6 alkyl (e.g., -C(=O)- CH 3) and C 1-6 -hydroxyalkyl (e.g., 1-hydroxyethyl);

(v) R 6 and R 7 are independently H or aryl (e.g., phenyl) optionally substituted with one or more groups independently selected from C 1-6 alkyl (e.g., methyl or ethyl) and halogen (e.g., F or Cl), for example unsubstituted phenyl or phenyl substituted with one or more halogen (e.g., F) or phenyl substituted with one or more C 1-6 alkyl and one or more

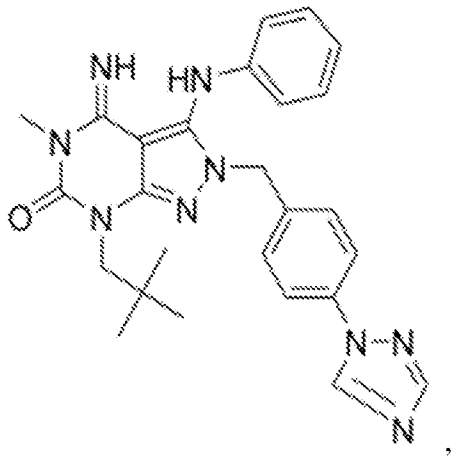
halogen or phenyl substituted with one C 1-6 alkyl and one halogen, for example 4-fluorophenyl or 3,4-difluorophenyl or 4-fluoro- 3-methylphenyl;

and

(vi) n is 1, 2, 3, or 4,

in free or salt form.

[0044] The invention further provides the use of PDE1 inhibitors of any of the preceding formulae (e.g., Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII), wherein the compound is selected from any of the following: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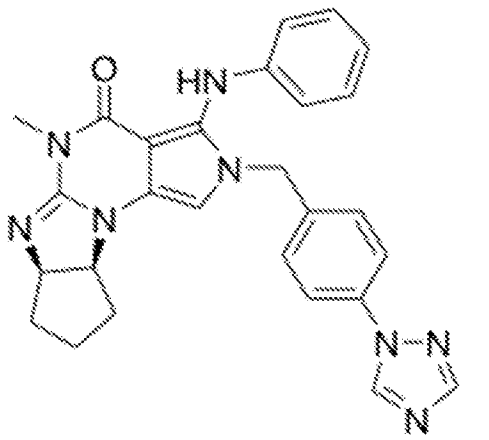
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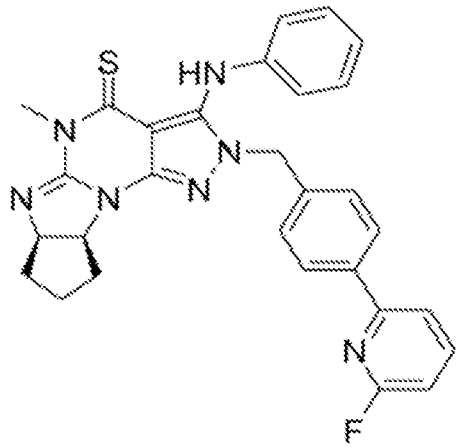
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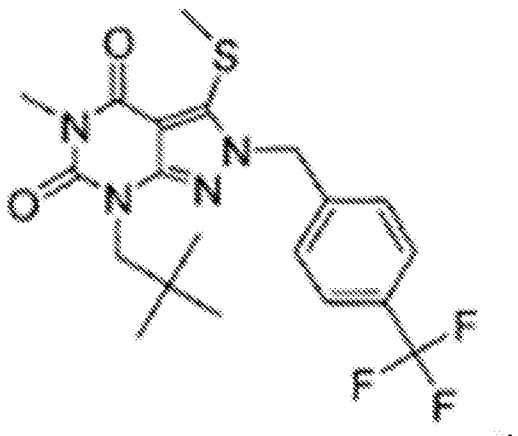
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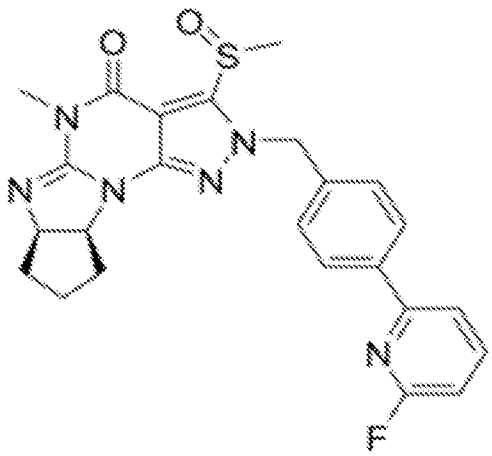

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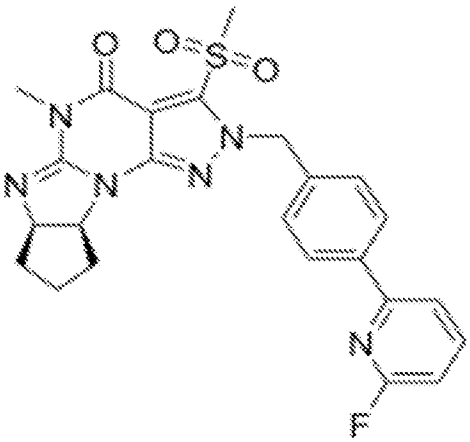

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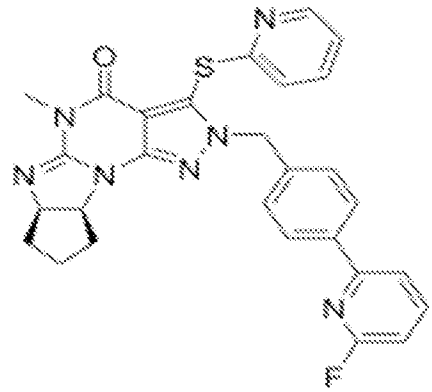

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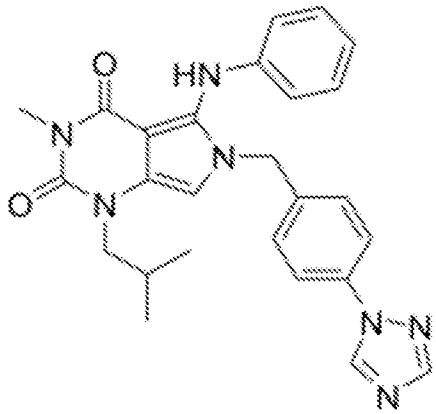

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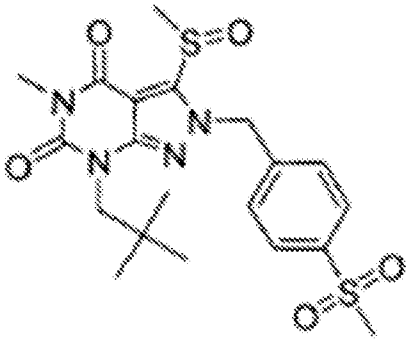
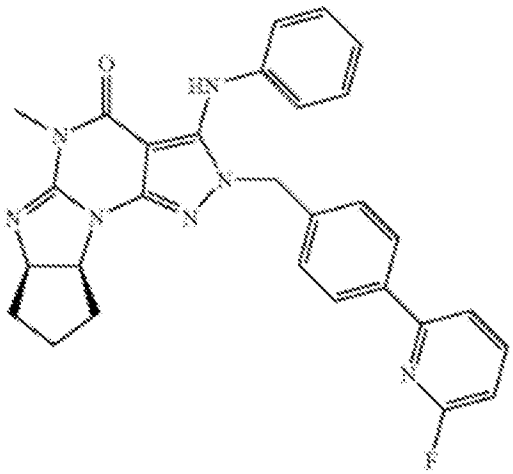
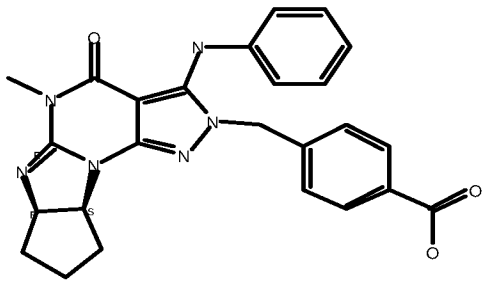
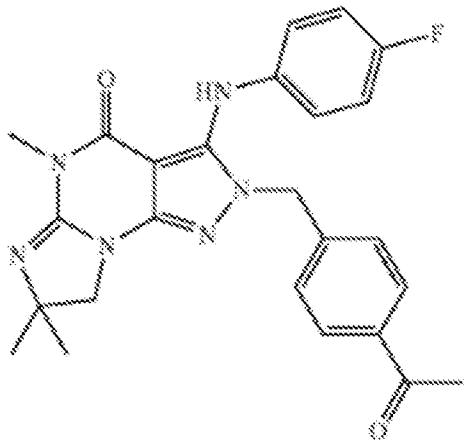
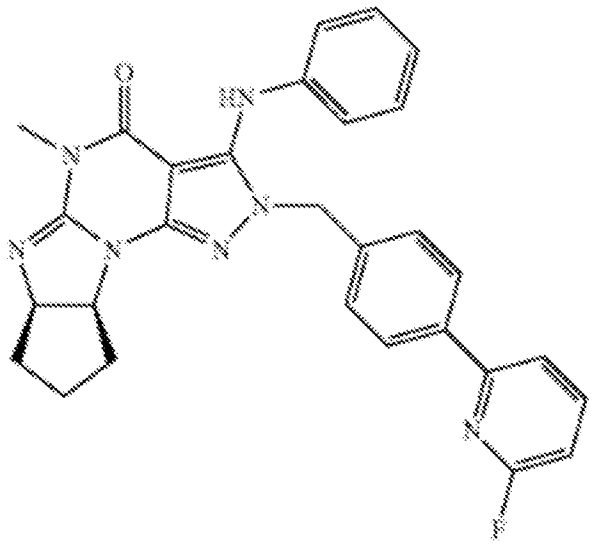




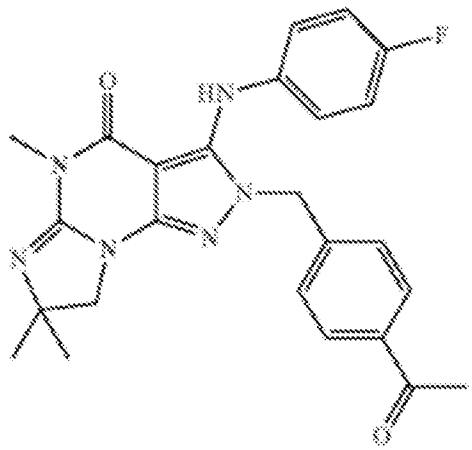




[0045] In one embodiment the invention provides administration of a PDE1 inhibitor for treatment or prophylaxis of inflammation or an inflammatory related disease or disorder, wherein the inhibitor is a compound according to the following:



[0046] In still another embodiment, the invention provides administration of a PDE1 inhibitor for treatment or prophylaxis of inflammation or an inflammatory related disease or disorder, wherein the inhibitor is a compound according to the following:



[0047] In one embodiment, selective PDE1 inhibitors of the any of the preceding formulae (e.g., Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII) are compounds that inhibit phosphodiesterase-mediated (e.g., PDE1-mediated, especially PDE1B-mediated)

hydrolysis of cGMP, e.g., the preferred compounds have an IC_{50} of less than 1μM, preferably less than 500 nM, preferably less than 50 nM, and preferably less than 5nM in an immobilized-metal affinity particle reagent PDE assay, in free or salt form.

[0048] If not otherwise specified or clear from context, the following terms herein have the following meanings:

"Alkyl" as used herein is a saturated or unsaturated hydrocarbon moiety, preferably saturated, preferably having one to six carbon atoms, which may be linear or branched, and may be optionally mono-, di- or tri- substituted, e.g., with halogen (e.g., chloro or fluoro), hydroxy, or carboxy.

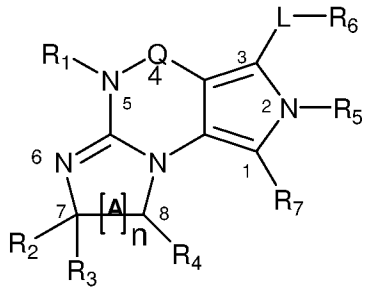
"Cycloalkyl" as used herein is a saturated or unsaturated nonaromatic hydrocarbon moiety, preferably saturated, preferably comprising three to nine carbon atoms, at least some of which form a nonaromatic mono- or bicyclic, or bridged cyclic structure, and which may be optionally substituted, e.g., with halogen (e.g., chloro or fluoro), hydroxy, or carboxy. Wherein the cycloalkyl optionally contains one or more atoms selected from N and O and/or S, said cycloalkyl may also be a heterocycloalkyl.

"Heterocycloalkyl" is, unless otherwise indicated, saturated or unsaturated nonaromatic hydrocarbon moiety, preferably saturated, preferably comprising three to nine carbon atoms, at least some of which form a nonaromatic mono- or bicyclic, or bridged cyclic structure, wherein at least one carbon atom is replaced with N, O or S, which heterocycloalkyl may be optionally substituted, e.g., with halogen (e.g., chloro or fluoro), hydroxy, or carboxy.

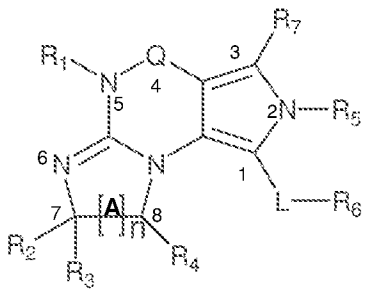
"Aryl" as used herein is a mono or bicyclic aromatic hydrocarbon, preferably phenyl, optionally substituted, e.g., with alkyl (e.g., methyl), halogen (e.g., chloro or fluoro), haloalkyl (e.g., trifluoromethyl), hydroxy, carboxy, or an additional aryl or heteroaryl (e.g., biphenyl or pyridylphenyl).

"Heteroaryl" as used herein is an aromatic moiety wherein one or more of the atoms making up the aromatic ring is sulfur or nitrogen rather than carbon, e.g., pyridyl or thiadiazolyl, which may be optionally substituted, e.g., with alkyl, halogen, haloalkyl, hydroxy or carboxy.

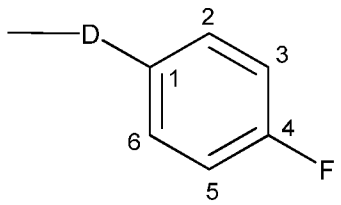
[0049] For ease of reference, the atoms on the pyrazolo-pyrimidine core of the Compounds of the Invention are numbered in accordance with the numbering depicted in below for Formula I, unless otherwise noted or evident from the context.



or



When E is phenylene, the numbering is as follows:



[0050] It is intended that wherein the substituents end in "ene", for example, alkylene, phenylene or arylalkylene, said substitutents are intended to bridge or be connected to two other substituents. Therefore, methylene is intended to be $–CH_2–$ and phenylene intended to be $–C_6H_4–$ and arylalkylene is intended to be $–C_6H_4–CH_2–$ or $–CH_2–C_6H_4–$.

[0051] Compounds of the Invention, e.g., substituted 4,5,7,8-tetrahydro-2H-imidazo[1,2-a]pyrrolo[3,4-e]pyrimidine or 4,5,7,8,9-pentahydro-2H-pyrimido[1,2-a]pyrrolo[3,4-e]pyrimidine, e.g., Compounds of Formula I (Formula I-A and I-B), or a Compound of Formula II (e.g., II-A or II-B), may exist in free or salt form, e.g., as acid addition salts. In this specification unless otherwise indicated, language such as "Compounds of the Invention" is to be understood as embracing the compounds in any form, for example free or acid addition salt form, or where the compounds contain acidic substituents, in base addition salt form. The Compounds of the Invention are intended for use as pharmaceuticals, therefore pharmaceutically acceptable salts are preferred. Salts which are unsuitable for pharmaceutical uses may be useful, for example, for the

isolation or purification of free Compounds of the Invention or their pharmaceutically acceptable salts, are therefore also included.

[0052] Compounds of the Invention, encompassing any of the compounds disclosed herein, e.g., optionally substituted 4,5,7,8-tetrahydro-(optionally 4-thioxo or 4-imino)-(1*H* or 2*H*)-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine or 4,5,7,8,9-pentahydro-(1*H* or 2*H*)-pyrimido[1,2-a]pyrazolo[4,3-e]pyrimidine compounds, e.g., (1 or 2 and/or 3 and/or 5)-substituted 4,5,7,8-tetrahydro-1*H*-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine, 4,5,7,8-tetrahydro-2H-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine, 4,5,7,8-tetrahydro-(1*H* or 2*H*)-pyrimido[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-imine, 7,8-dihydro-1*H*-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-thione or 7,8-dihydro-2H-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidine-4(5H)-thione compounds, e.g., Compounds of Formula III, or Compound of Formula IV as described herein, may exist in free or salt form, e.g., as acid addition salts.

[0053] Compounds of the Invention may in some cases also exist in prodrug form. A prodrug form is compound which converts in the body to a Compound of the Invention. For example when the Compounds of the Invention contain hydroxy or carboxy substituents, these substituents may form physiologically hydrolysable and acceptable esters. As used herein, "physiologically hydrolysable and acceptable ester" means esters of Compounds of the Invention which are hydrolysable under physiological conditions to yield acids (in the case of Compounds of the Invention which have hydroxy substituents) or alcohols (in the case of Compounds of the Invention which have carboxy substituents) which are themselves physiologically tolerable at doses to be administered. Therefore, wherein the Compound of the Invention contains a hydroxy group, for example, Compound-OH, the acyl ester prodrug of such compound, i.e., Compound-O-C(O)-C_{1-4}alkyl, can hydrolyze in the body to form physiologically hydrolysable alcohol (Compound-OH) on the one hand and acid on the other (e.g., HOC(O)-C_{1-4}alkyl). Alternatively, wherein the Compound of the Invention contains a carboxylic acid, for example, Compound-C(O)OH, the acid ester prodrug of such compound, Compound-

C(O)O-C_{1-4}alkyl can hydrolyze to form Compound-C(O)OH and HO-C_{1-4}alkyl. As will be appreciated the term thus embraces conventional pharmaceutical prodrug forms.

[0054] In another embodiment, the invention further provides a pharmaceutical composition comprising a Compound of the Invention, in free or pharmaceutically acceptable salt form, in admixture with a pharmaceutically acceptable carrier, for use as an anti-inflammatory agent.

[0055] Compounds of the Invention may in some cases also exist in prodrug form. A prodrug form is compound which converts in the body to a Compound of the Invention. For example when the Compounds of the Invention contain hydroxy or carboxy substituents, these substituents may form physiologically hydrolysable and acceptable esters. As used herein, "physiologically hydrolysable and acceptable ester" means esters of Compounds of the Invention which are hydrolysable under physiological conditions to yield acids (in the case of Compounds of the Invention which have hydroxy substituents) or alcohols (in the case of Compounds of the Invention which have carboxy substituents) which are themselves physiologically tolerable at doses to be administered. Therefore, wherein the Compound of the Invention contains a hydroxy group, for example, Compound-OH, the acyl ester prodrug of such compound, i.e., Compound-O-C(O)-C_{1-4}alkyl, can hydrolyze in the body to form physiologically hydrolysable alcohol (Compound-OH) on the one hand and acid on the other (e.g., HOC(O)-C_{1-4}alkyl). Alternatively, wherein the Compound of the Invention contains a carboxylic acid, for example, Compound-C(O)OH, the acid ester prodrug of such compound, Compound-C(O)O-C_{1-4}alkyl can hydrolyze to form Compound-C(O)OH and HO-C_{1-4}alkyl. As will be appreciated the term thus embraces conventional pharmaceutical prodrug forms.

[0056] In another embodiment, the invention further provides a pharmaceutical composition comprising a Compound of the Invention, in free, pharmaceutically

acceptable salt or prodrug form, in admixture with a pharmaceutically acceptable carrier, for use as an anti-inflammatory agent.

Methods of Making Compounds of the Invention

[0057] The compounds of the Invention and their pharmaceutically acceptable salts may be made using the methods as described and exemplified herein and by methods similar thereto and by methods known in the chemical art. Such methods include, but not limited to, those described below. If not commercially available, starting materials for these processes may be made by procedures, which are selected from the chemical art using techniques which are similar or analogous to the synthesis of known compounds.

[0058] Various starting materials and/or Compounds of the Invention may be prepared using methods described in US 2008-0188492 A1, US 2010-0173878 A1, US 2010-0273754 A1, US 2010-0273753 A1, WO 2010/065153, WO 2010/065151, WO 2010/065151, WO 2010/065149, WO 2010/065147, WO 2010/065152, WO 2011/153129, WO 2011/133224, WO 2011/153135, WO 2011/153136, WO 2011/153138, and US Pat. 9,073,936, the contents of each of which herein are hereby incorporated by reference in their entireties.

[0059] The Compounds of the Invention include their enantiomers, diastereoisomers and racemates, as well as their polymorphs, hydrates, solvates and complexes. Some individual compounds within the scope of this invention may contain double bonds. Representations of double bonds in this invention are meant to include both the E and the Z isomer of the double bond. In addition, some compounds within the scope of this invention may contain one or more asymmetric centers. This invention includes the use of any of the optically pure stereoisomers as well as any combination of stereoisomers.

[0060] It is also intended that the Compounds of the Invention encompass their stable and unstable isotopes. Stable isotopes are nonradioactive isotopes which contain one additional neutron compared to the abundant nuclides of the same species (i.e., element). It is expected that the activity of compounds comprising such isotopes would be retained, and such compound would also have utility for measuring pharmacokinetics of the non-

isotopic analogs. For example, the hydrogen atom at a certain position on the Compounds of the Invention may be replaced with deuterium (a stable isotope which is non-radioactive). Examples of known stable isotopes include, but not limited to, deuterium, ^{13}C, ^{15}N, ^{18}O. Alternatively, unstable isotopes, which are radioactive isotopes which contain additional neutrons compared to the abundant nuclides of the same species (i.e., element), e.g., ^{123}I, ^{131}I, ^{125}I, ^{11}C, ^{18}F, may replace the corresponding abundant species of I, C and F. Another example of useful isotope of the compound of the invention is the ^{11}C isotope. These radio isotopes are useful for radio-imaging and/or pharmacokinetic studies of the compounds of the invention.

[0061] Melting points are uncorrected and (dec) indicates decomposition. Temperature are given in degrees Celsius (°C); unless otherwise stated, operations are carried out at room or ambient temperature, that is, at a temperature in the range of 18-25 °C. Chromatography means flash chromatography on silica gel; thin layer chromatography (TLC) is carried out on silica gel plates. NMR data is in the delta values of major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Conventional abbreviations for signal shape are used. Coupling constants (J) are given in Hz. For mass spectra (MS), the lowest mass major ion is reported for molecules where isotope splitting results in multiple mass spectral peaks. Solvent mixture compositions are given as volume percentages or volume ratios. In cases where the NMR spectra are complex, only diagnostic signals are reported.

[0062] Terms and abbreviations:

BuLi = n-butyllithium

Bu^tOH = *tert*-butyl alcohol,

CAN = ammonium cerium (IV) nitrate,

DIPEA = diisopropylethylamine,

DMF = N,N-dimethylforamide,

DMSO = dimethyl sulfoxide,

Et_2O = diethyl ether,

EtOAc = ethyl acetate,

equiv. = equivalent(s),

h = hour(s),

HPLC =high performance liquid chromatography,

LDA = lithium diisopropylamide

MeOH = methanol,

NBS = N-bromosuccinimide

NCS = N-chlorosuccinimide

$NaHCO_3$ = sodium bicarbonate,

NH_4OH = ammonium hydroxide,

$Pd_2(dba)_3$ = tris[dibenzylideneacetone]dipalladium(0)

PMB = p-methoxybenzyl,

$POCl_3$ = phosphorous oxychloride,

$SOCl_2$ = thionyl chloride,

TFA = trifluoroacetic acid,

TFMSA = trifluoromethanesulfonic acid

THF = tetrahedrofuran.

Methods of using Compounds of the Invention

[0063] The Compounds of the Invention are useful in the treatment of inflammatory diseases or conditions, particularly neuroinflammatory diseases or conditions. Therefore, administration or use of a preferred PDE1 inhibitor as described herein, e.g., a PDE1 inhibitor as hereinbefore described, e.g., a Compound of Formula Ia, Ib, IIa, IIb, III, IV, V, VI, VII, VIII, IX, X, XI, XII provides a means to regulate inflammation (e.g., prevent, reduce, and/or reverse neuroinflammation, and diseases or disorders related to neuroinflammation), and in certain embodiments provide a treatment for various inflammatory diseases and disorders.

[0064] For example, in one embodiment the invention provides a method (Method 1) of treatment or prophylaxis of inflammation or disease associated with inflammation comprising administering an effective amount of a specific inhibitor of phosphodiesterase type I (PDE1), to a patient in need thereof, for example:

1.1. Method 1 which is a method of treating neuroinflammation and/or diseases or disorders associated with neuroinflammation and/or microglial function.

1.2. Method 1 or 1.1 wherein the disease or condition to be treated is selected from:

a. neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases;
b. stroke, cardiac arrest, hypoxia, intracerebral hemorrhage or traumatic brain injury;
c. conditions characterized by abnormal neurotransmitter production and/or response, including depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation; and
d. chronic CNS infections, e.g., Lyme disease or CNS infection consequent to an immunosuppressive condition, e.g., HIV-dementia;
e. neuroinflammation consequent to chemotherapy;

comprising administering an effective amount of a PDE1 inhibitor of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described), e.g., an amount effective to (i) reduce or inhibit activation of M1 microglia, and/or (ii) and amount effective to reduce levels of one or more pro-inflammatory cytokines (e.g., IL1β, TNF- α, and Ccl2, or combination thereof); to a patient in need thereof.

1.3. Any foregoing method wherein the disease or condition to be treated is a neurodegenerative conditions , e.g., selected from Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases.

1.4. Any foregoing method wherein the disease or condition to be treated is selected from stroke, cardiac arrest, hypoxia, intracerebral hemorrhage and traumatic brain injury.

1.5. Any foregoing method wherein the disease or condition to be treated is a condition characterized by abnormal neurotransmitter production and/or response, e.g., selected from depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation.

1.6. Any foregoing method wherein the disease or condition to be treated is selected from chronic CNS infections, e.g., Lyme disease or CNS infection consequent to an immunosuppressive condition, e.g., HIV-dementia.

1.7. Any foregoing method wherein the disease or condition to be treated is neuroinflammation consequent to chemotherapy.

1.8. Any foregoing method comprising administering an effective amount of a PDE1 inhibitor of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described), e.g., an amount effective to (i) reduce or inhibit activation of M1 microglia, and/or (ii) and amount effective to reduce levels of one or more pro-inflammatory cytokines (e.g., IL1β, TNF- α, IL6 and Ccl2, or combination thereof); to a patient in need thereof.

1.9. Any foregoing method comprising administering an effective amount of a PDE1 inhibitor of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described) to a patient in need thereof, in an amount effective to anti-inflammatory cytokines (e.g., IL-10).

1.10. Any foregoing method comprising administering an effective amount of a PDE1 inhibitor of the current invention (e.g., a PDE1 inhibitor of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as herein described) to a patient in need thereof, in an amount effective to reduce levels of microglial M1 phenotype and/or enhance levels of microglial M2 phenotype.

1.11. Any foregoing method wherein the PDE1 inhibitor is a Compound of Formula Ia, Ib, IIa, IIb, III, IV, V, VI, VII, VIII, IX, X, XI, XII.

1.12. Any foregoing method wherein the neuroinflammation is associated with increased expression and/or activation of microglial cells (e.g., M1 microglial cells) in the brain.

1.13. Any foregoing method wherein the PDE1 inhibitor blunts or inhibits the expression and/or activity of pro-inflammatory cytokines in the brain, e.g., selected from the group consisting of: IL1B, IL-6, TNF- α, Ccl2, Nitric Oxide (NO), and Reactive Oxygen Species (ROS).

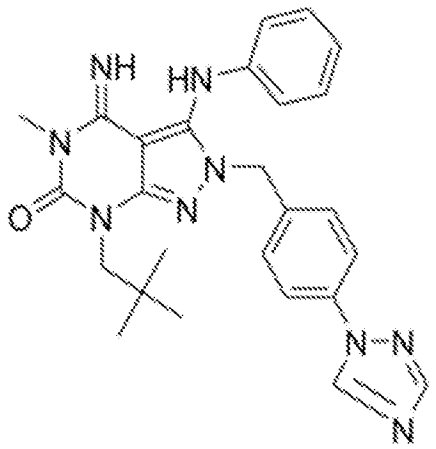
1.14. Any foregoing method wherein the PDE1 inhibitor in administered in combination with a PDE4 inhibitor (e.g., rolipram).

1.15. Any foregoing method wherein the patient exhibits increased levels of pro-inflammatory cytokines (e.g., IL1B, IL6, TNF-alpha, Ccl2).

1.16. Any foregoing method wherein "PDE1 inhibitor" describes a compound(s) which selectively inhibit phosphodiesterase-mediated (e.g., PDE1-mediated, especially PDE1B-mediated) hydrolysis of cGMP, e.g., with an IC_{50} of less than 1μM, preferably less than 750 nM, more preferably less than 500 nM, more preferably less than 50 nM in an immobilized-metal affinity particle reagent PDE assay.

1.17. Any foregoing method wherein the PDE1 inhibitor inhibits the activity of PDE1 (e.g., bovine PDE1 in the assay described in Example 1) with an IC_{50} of less than 10 nM, e.g., wherein the PDE1 inhibitor does not inhibit the activity of PDE types other than PDE1, e.g., has an IC_{50} at least 1000 times greater for PDE types other than PDE1.

1.18. Any foregoing method, wherein the PDE1 inhibitor is selected from any of the following:



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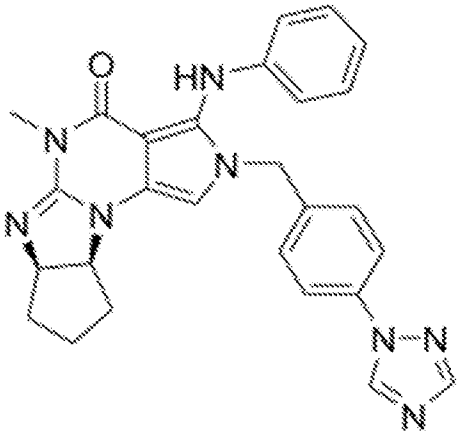

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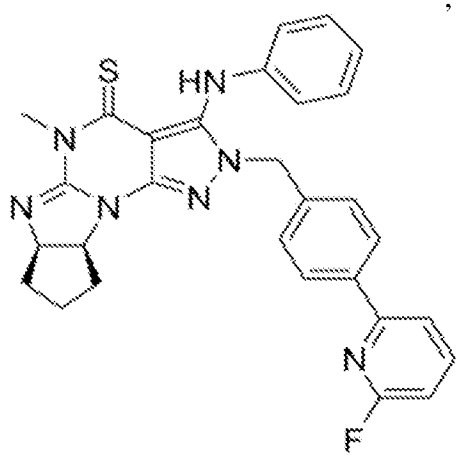

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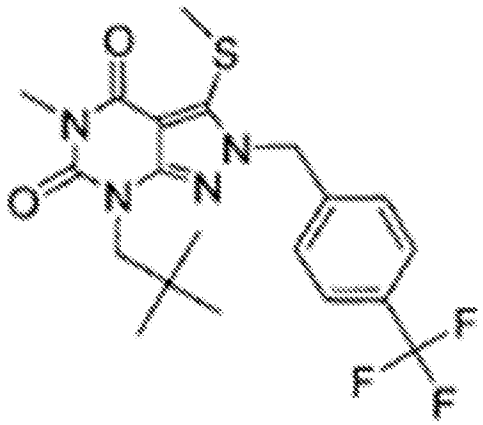

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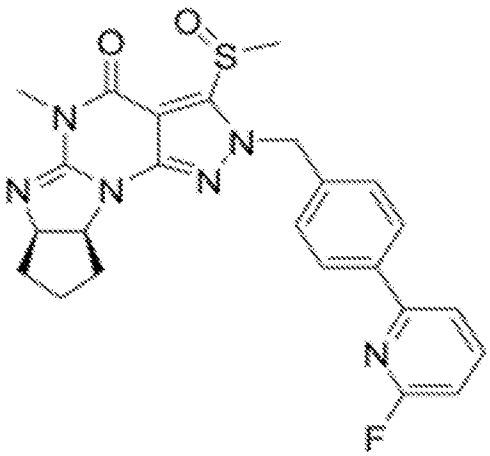

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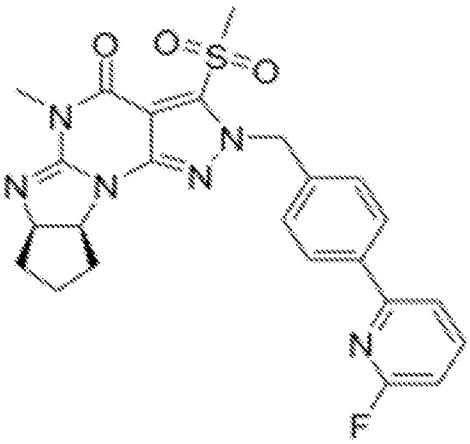

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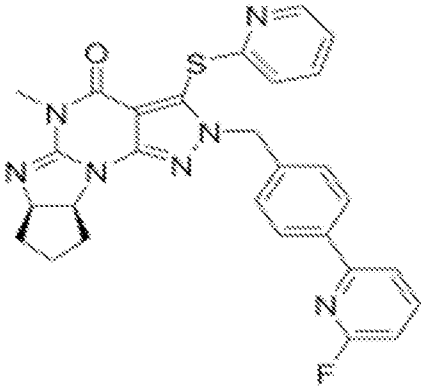

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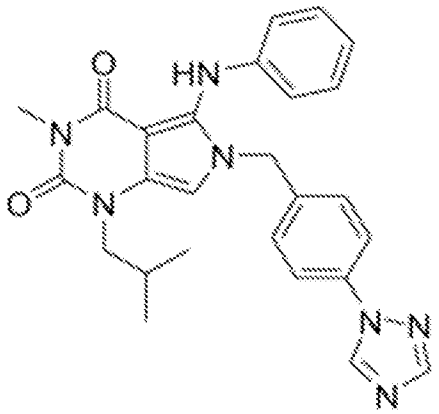

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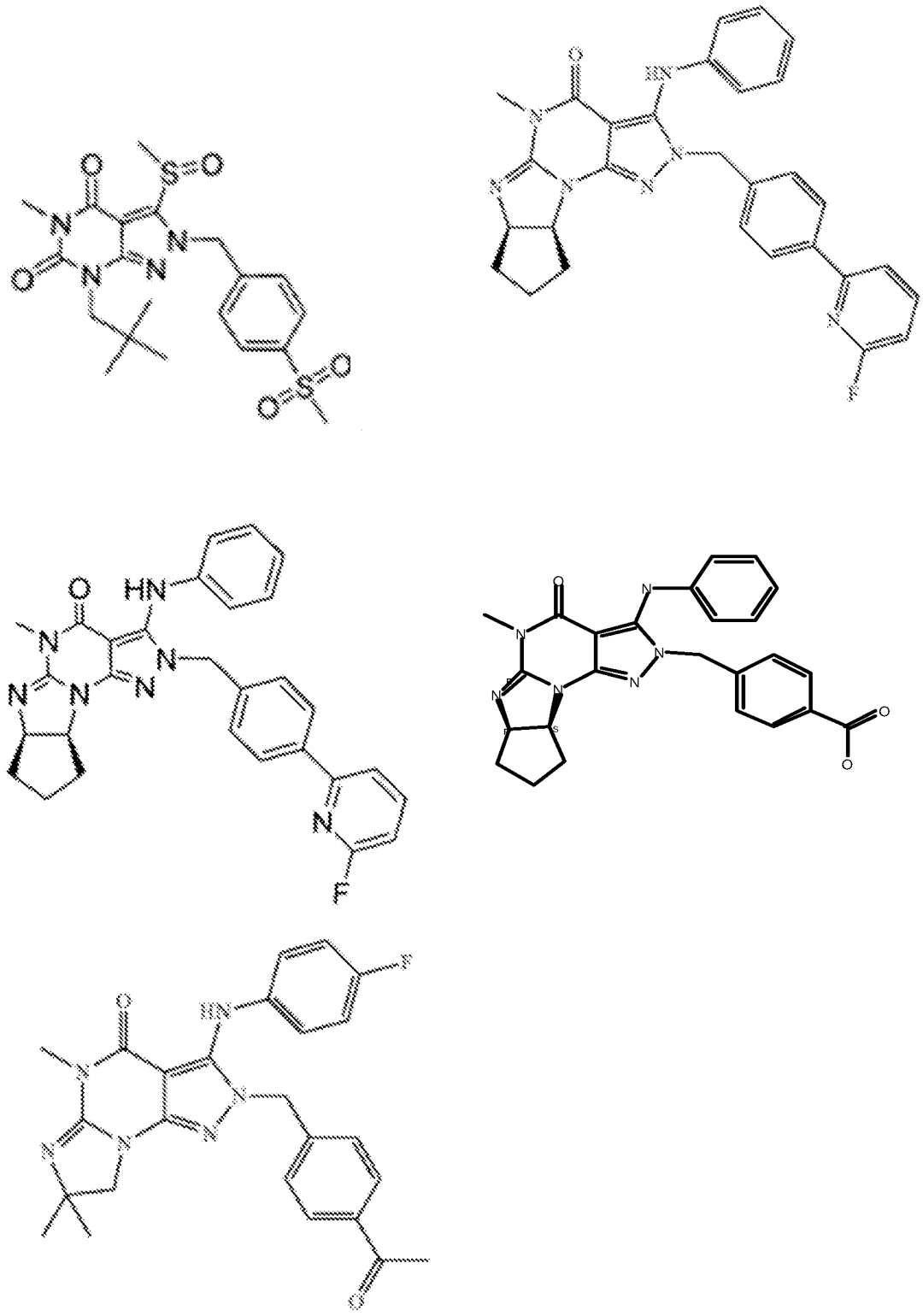
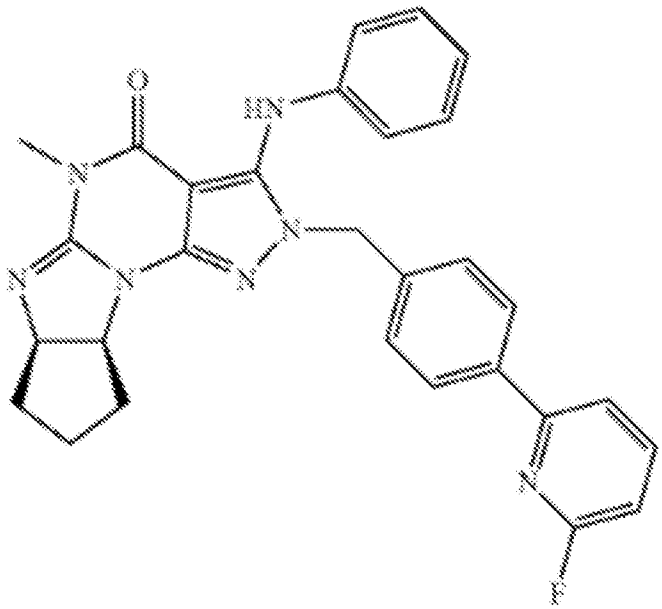
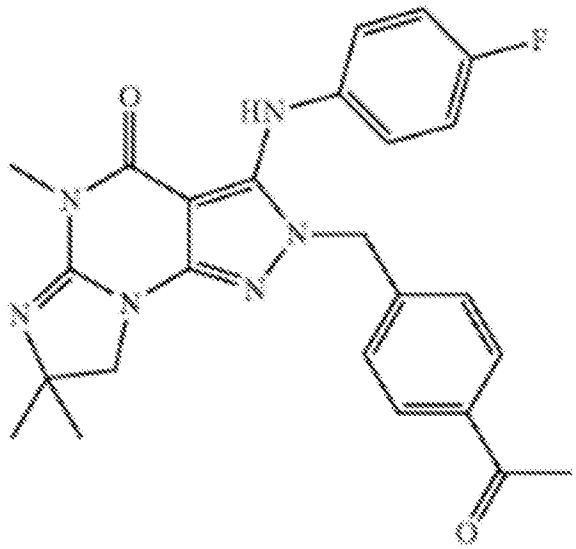


1.19. Any foregoing method,, wherein the PDE1 inhibitor is the following:



1.20. Any foregoing method, wherein the PDE1 inhibitor is the following:



1.21. Any foregoing method, wherein the PDE1 inhibitor is administered in combination (e.g. administered sequentially or simultaneously or within a 24 hour period) with an effective amount of one or more antidepressant agents, e.g., with one or more compounds in free or pharmaceutically acceptable salt form, selected from selective serotonin reuptake inhibitors (SSRIs), serotonin-

norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and atypical antipsychotics, e.g. one or more compounds in free or pharmaceutically acceptable salt form, selected from

(a) Selective serotonin reuptake inhibitors (SSRIs), e.g., Citalopram (Celexa), Escitalopram (Lexapro, Cipralex), Paroxetine (Paxil, Seroxat), Fluoxetine (Prozac), Fluvoxamine (Luvox) Sertraline (Zoloft, Lustral);

(b) Serotonin-norepinephrine reuptake inhibitors (SNRIs), e.g., Desvenlafaxine (Pristiq), Duloxetine (Cymbalta), Levomilnacipran (Fetzima), Milnacipran (Ixel, Savella), Tofenacin (Elamol, Tofacine), Venlafaxine (Effexor);

c) Tricyclic antidepressants (TCAs), e.g., Amitriptyline (Elavil, Endep), Amitriptylinoxide (Amioxid, Ambivalon, Equilibrin), Clomipramine (Anafranil), Desipramine (Norpramin, Pertofrane), Dibenzepin (Noveril, Victoril), Dimetacrine (Istonil), Dosulepin (Prothiaden), Doxepin (Adapin, Sinequan), Imipramine (Tofranil), Lofepramine (Lomont, Gamanil), Melitracen (Dixeran, Melixeran, Trausabun), Nitroxazepine (Sintamil), Nortriptyline (Pamelor, Aventyl), Noxiptiline (Agedal, Elronon, Nogedal), Pipofezine (Azafen/Azaphen), Protriptyline (Vivactil), Trimipramine (Surmontil);

d) Atypical antipsychotics, e.g., Aripiprazole (Abilify), Asenapine (Saphris), Brexpiprazole (Rexulti), Clozapine (Clozaril), Lumateperone, Lurasidone (Latuda), Olanzapine (Zyprexa), Paliperidone (Invega), Quetiapine (Seroquel), Risperidone (Risperdal), Sertindole (Serdolect, Serlect) Ziprasidone (Geodon)

1.22. Method 1.21 wherein the antidepressant agent is an atypical antipsychotic agent, e.g., Lumateperone, in free or pharmaceutically acceptable salt form.

1.23. Method 1.21 wherein the antidepressant agent is an SSRI, e.g., Fluoxetine or Escitalopram, in free or pharmaceutically acceptable salt form.

1.24. Any of the foregoing method wherein the patient has elevated levels of one or more pro-inflammatory cytokines (e.g., selected from IL1β, TNFα, Ccl2, IL-6, and combinations thereof).

1.25. Any of the foregoing method wherein the patient has reduced levels of one or more anti-inflammatory cytokines (e.g., IL-10).

1.26. Any of the foregoing method wherein the patient has elevated levels of microglial M1 phenotype compared to microglial M2 phenotype.

1.27. Any of the foregoing methods, wherein the patient has abnormal levels (e.g., abnormal levels relative to a reference standard) of one or more of the cytokines described in Fig 10 or Fig. 11.

1.28. Any of the foregoing methods, wherein the PDE1 inhibitor is administered to treat or prevent chronic neuroinflammation or a disease associated with chronic neuroinflammation.

1.29. Any of the foregoing methods, wherein the PDE1 inhibitor is administered to a patient with an optic nerve injury.

1.30. The method of 1.29, wherein the PDE1 inhibitor increases expression of PDE1 in retinal ganglion.

1.31. The method of 1.29 or 1.30, wherein the administration of the PDE1 inhibitor increases the survival of retinal ganglion cells (e.g., increased as compared to a reference standard or control).

[0065] The invention further provides the use of a PDE1 inhibitor, e.g., any of a Compound of Formula I, Formula II, Formula III, Formula IV, Formula V, Formula VI, Formula VII, Formula VIII, Formula IX, Formula X, Formula XI, or Formula XII in the manufacture of a medicament for use in any of Methods 1, et seq.

[0066] The invention further provides a PDE1 inhibitor, e.g., any of a Compound of Formula I, Formula II, Formula III, Formula IV, Formula V, Formula VI, Formula VII, Formula VIII, Formula IX, Formula X, Formula XI, or Formula XII for use in any of Methods 1, et seq.

[0067] The invention further provides a pharmaceutical composition comprising a PDE1 inhibitor, e.g., any of a Compound of Formula I, Formula II, Formula III, Formula IV, Formula V, Formula VI, Formula VII, Formula VIII, Formula IX, Formula X, Formula XI, or Formula XII for use in any of Methods 1 et seq.

[0068] The phrase "Compounds of the Invention" or "PDE 1 inhibitors of the Invention", or like terms, encompasses any and all of the compounds disclosed herewith, e.g., a Compound of Formula I, Formula II, Formula III, Formula IV, Formula V, Formula VI, Formula VII, Formula VIII, Formula IX, Formula X, Formula XI, or Formula XII.

[0069] The words "treatment" and "treating" are to be understood accordingly as embracing prophylaxis and treatment or amelioration of symptoms of disease as well as treatment of the cause of the disease.

[0070] For methods of treatment, the word "effective amount" is intended to encompass a therapeutically effective amount to treat or mitigate a specific disease or disorder, and/or a symptom thereof, and/or to reduce inflammatory cytokines, e.g., as produced by microglia, and/or to reduce M1 microglia activation, and/or to increase anti-inflammatory cytokines, e.g., as produced by microglia, and/or to enhance M2 microglia activation.

[0071] The term "patient" includes a human or non-human (i.e., animal) patient. In a particular embodiment, the invention encompasses both humans and nonhuman animals. In another embodiment, the invention encompasses nonhuman animals. In other embodiments, the term encompasses humans.

[0072] The term "comprising" as used in this disclosure is intended to be open-ended and does not exclude additional, unrecited elements or method steps.

[0073] Compounds of the Invention, e.g., Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII as hereinbefore described, in free or pharmaceutically acceptable salt form, may be used as a sole therapeutic agent, but may also be used in combination or for co-administration with other active agents.

[0074] For example, in certain embodiments, the Compounds of the Invention, e.g., Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII as hereinbefore described, in free or pharmaceutically acceptable salt form, may be administered in combination (e.g.

administered sequentially or simultaneously or within a 24 hour period) with other active agents, e.g., with one or more antidepressant agents, e.g., with one or more compounds in free or pharmaceutically acceptable salt form, selected from selective serotonin reuptake inhibitors (SSRIs),) serotonin-norepinephrine reuptake inhibitors (SNRIs), c) tricyclic antidepressants (TCAs), and atypical antipsychotics.

[0075] Dosages employed in practicing the present invention will of course vary depending, e.g. on the particular disease or condition to be treated, the particular Compound of the Invention used, the mode of administration, and the therapy desired. Compounds of the Invention may be administered by any suitable route, including orally, parenterally, transdermally, or by inhalation, but are preferably administered orally. In general, satisfactory results, e.g. for the treatment of diseases as hereinbefore set forth are indicated to be obtained on oral administration at dosages of the order from about 0.01 to 2.0 mg/kg. In larger mammals, for example humans, an indicated daily dosage for oral administration will accordingly be in the range of from about 0.75 to 150 mg (depending on the drug to be administered and the condition to be treated, for example in the case of Compound 214, 0.5 to 25 mg, e.g., 1 to 10 mg, per diem, e.g., in monophosphate salt form, for treatment of neuroinflammatory conditions), conveniently administered once, or in divided doses 2 to 4 times, daily or in sustained release form. Unit dosage forms for oral administration thus for example may comprise from about 0.2 to 75 or 150 mg, e.g. from about 0.2 or 2.0 to 50, 75 or 100 mg (e.g., 1, 2.5, 5, 10, or 20 mg) of a Compound of the Invention, e.g., together with a pharmaceutically acceptable diluent or carrier therefor.

[0076] Pharmaceutical compositions comprising Compounds of the Invention may be prepared using conventional diluents or excipients and techniques known in the galenic art. Thus oral dosage forms may include tablets, capsules, solutions, suspensions and the like.

EXAMPLE 1

Measurement of PDEIB inhibition in vitro using IMAP Phosphodiesterase Assay Kit

[0077] Phosphodiesterase I B (PDEIB) is a calcium/calmodulin dependent phosphodiesterase enzyme that converts cyclic guanosine monophosphate (cGMP) to 5'-guanosine monophosphate (5'-GMP). PDEIB can also convert a modified cGMP

substrate, such as the fluorescent molecule cGMP-fluorescein, to the corresponding GMP-fluorescein. The generation of GMP-fluorescein from cGMP-fluorescein can be quantitated, using, for example, the IMAP (Molecular Devices, Sunnyvale, CA) immobilized-metal affinity particle reagent.

[0078] Briefly, the IMAP reagent binds with high affinity to the free 5'- phosphate that is found in GMP-fluorescein and not in cGMP-fluorescein. The resulting GMP-fluorescein— IMAP complex is large relative to cGMP-fluorescein. Small fluorophores that are bound up in a large, slowly tumbling, complex can be distinguished from unbound fluorophores, because the photons emitted as they fluoresce retain the same polarity as the photons used to excite the fluorescence.

[0079] In the phosphodiesterase assay, cGMP-fluorescein, which cannot be bound to IMAP, and therefore retains little fluorescence polarization, is converted to GMP-fluorescein, which, when bound to IMAP, yields a large increase in fluorescence polarization (Amp). Inhibition of phosphodiesterase, therefore, is detected as a decrease in Amp.

Enzyme assay

[0080] Materials: All chemicals are available from Sigma-Aldrich (St. Louis, MO) except for IMAP reagents (reaction buffer, binding buffer, FL-GMP and IMAP beads), which are available from Molecular Devices (Sunnyvale, CA).

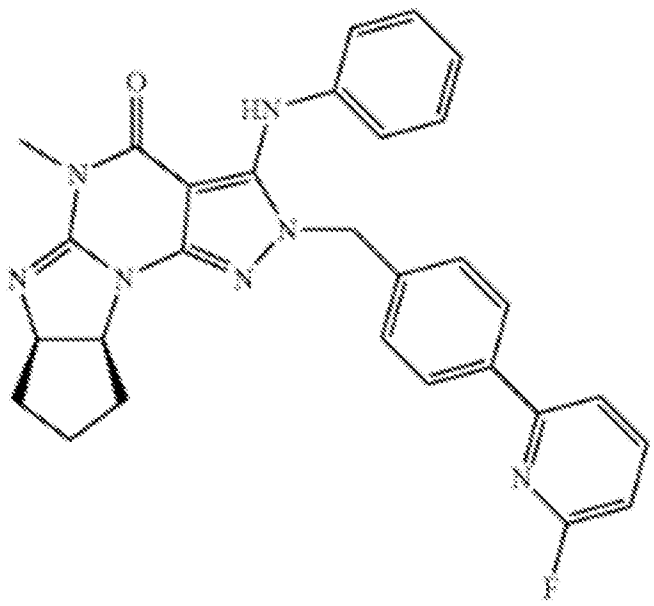
[0081] Assay: The following phosphodiesterase enzymes may be used: 3',5'-cyclic-nucleotide-specific bovine brain phosphodiesterase (Sigma, St. Louis, MO) (predominantly PDEIB) and recombinant full length human PDEl A and PDE1B (r-hPDEl A and r-hPDElB respectively) which may be produced e.g., in HEK or SF9 cells by one skilled in the art. The PDEl enzyme is reconstituted with 50% glycerol to 2.5 U/ml. One unit of enzyme will hydrolyze 1.0 μm of 3',5'-cAMP to 5'-AMP per min at pH 7.5 at 30°C. One part enzyme is added to 1999 parts reaction buffer (30 μM $CaCl_2$, 10 U/ml of calmodulin (Sigma P2277), 10mM Tris-HCl pH 7.2, 10mM $MgCl_2$, 0.1% BSA, 0.05% NaN_3) to yield a final concentration of 1.25mU/ml. 99 μl of diluted enzyme solution is added into each well in a flat bottom 96-well polystyrene plate to which 1 μĩ

of test compound dissolved in 100% DMSO is added. The compounds are mixed and pre-incubated with the enzyme for 10 min at room temperature.

[0082] The FL-GMP conversion reaction is initiated by combining 4 parts enzyme and inhibitor mix with 1 part substrate solution (0.225 μM) in a 384-well microtiter plate. The reaction is incubated in dark at room temperature for 15 min. The reaction is halted by addition of 60 μL of binding reagent (1:400 dilution of IMAP beads in binding buffer supplemented with 1:1800 dilution of antifoam) to each well of the 384-well plate. The plate is incubated at room temperature for 1 hour to allow IMAP binding to proceed to completion, and then placed in an Envision multimode microplate reader (PerkinElmer, Shelton, CT) to measure the fluorescence polarization (Amp).

[0083] A decrease in GMP concentration, measured as decreased Amp, is indicative of inhibition of PDE activity. IC50 values are determined by measuring enzyme activity in the presence of 8 to 16 concentrations of compound ranging from 0.0037 nM to 80,000 nM and then plotting drug concentration versus AmP, which allows IC50 values to be estimated using nonlinear regression software (XLFit; IDBS, Cambridge, MA).

[0084] The Compounds of the Invention are tested in an assay as described or similarly described herein for PDE1 inhibitory activity. For example, Compound 214, is identified as a specific PDE1 inhibitor of formula:



This compound has efficacy at sub-nanomolar levels vs PDE1 (IC_{50} of 0.058nM for bovine brain PDE1 in the assay described above) and high selectivity over other PDE families, as depicted on the following table:

PDE Target	IC50 (nM)	ratio PDEx/PDE1
bovine brain PDE1	0.058	1
hPDE2A	3661	63121
hPDE3B	3120	53793
hPDE4A	158	2724
r-bovine PDE5A	632	10897
bovine retina PDE6	324	5586
hPDE7B	355	6121
hPDE8A	3001	51741
hPDE9A	16569	285672
hPDE10A	1824	31448
hPDE11A	1313	22638

The compound is also highly selective versus a panel of 63 receptors, enzymes, and ion channels. These data, and data for other PDE1 inhibitors described herein, are described in Li et al., J. Med. Chem. 2016: 59, 1149−1164, the contents of which are incorporated herein by reference.

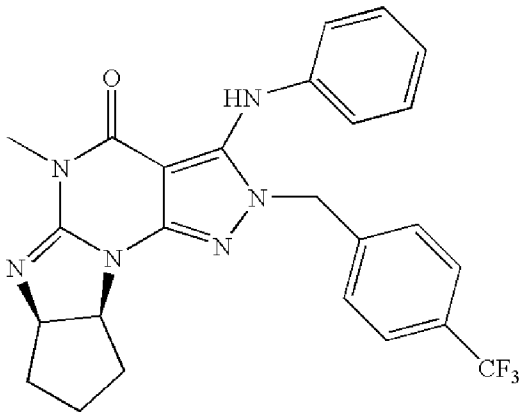
EXAMPLE 2

Inhibition of Monocyte to Activated Macrophage Transition and Interaction with ANP

[0085] PDE1 is induced in the inflammatory monocyte-to-activated-macrophage transition mediated by GM-CSF, and this transition can be inhibited by PDE1 knockdown. Bender and Beavo, 2006 PNAS **103**, 460-5. Atrial natriuretic peptide (ANP) elevates cGMP levels, by activating the ANP catalytic receptor, which stimulates intracellular guanylyl cyclase activity to convert GTP to cGMP. ANP has an anti-inflammatory effect on macrophages, reducing the secretion of inflammatory mediators in macrophages. Kiemer, et al., Ann Rheum Dis. 2001 Nov; 60(Suppl 3): iii68–iii70. Specifically, ANP inhibits the lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS) in macrophages, reduces the activation of NF-κB, inhibits the macrophage release of TNFα and interleukin 1β (IL1β), but not secretion of the anti-inflammatory cytokines IL10 and IL1 receptor antagonist (IL1ra).

[0086] We have shown that there is a synergistic effect between ANP and PDE1 inhibition. An immortalized human pro-myeloid cell line (HL60 from ATCC) is grown, differentiated and harvested as described in Bender, AT, and Beavo, JA, 2006, *PNAS* 103, 460-465. The cells are grown in HEPES buffered RPMI 1640 medium with penicillin, streptomycin, and 10% fetal bovine serum. Phorbol-12-myristate-13-acetate (PMA), at 100 nM for 3 days, is used to differentiate the HL60 cells into macrophage-like cells. Following differentiation, the cells are incubated with a PDE1 inhibitor or vehicle (DMSO) beginning at time 0. At 40 minutes, 5 μM ionomycin (a calcium ionophore) is added. At 50 minutes, 100 nM ANP was added. At 60 minutes, the cells are harvested. Total cGMP levels are measured using a competitive ELISA (Bender and Beavo, 2006).

[0087] A representative PDE1 inhibitor, (6aR,9aS)-3-(phenylamino)-5,6a,7,8,9,9a-hexahydro-5-methyl-2-(4-(trifluoromethyl)-benzyl)-cyclopent[4,5]imidazo[1,2-a]pyrazolo[4,3-e]pyrimidin-4(2H)-one, disclosed as Example 20 of US 8,273,750, having the following structure:



is tested for its effect on cGMP levels in this system. Like Compound 214, this compound is a potent and selective inhibitor of PDE1 (Ki = 0.68 nM bovine brain PDE1 assay described above). The cGMP level induced in the HL60 cells by treatment with 100 nM ANP in combination with 100 nM of the PDE1 inhibitor is greater than that induced by either the ANP alone or the PDE1 inhibitor alone. In addition, the cGMP level attained by co-treatment with ANP and the PDE1 inhibitor is much greater than that obtained by co-treatment with ANP and a mixed PDE1/PDE5 inhibitor, SCH 51866 (used at 5 μM). In this experiment, the calcium ionophore ionomycin (used at 5 μM) is used to raise the intracellular calcium level and to counteract the cGMP rise induced by ANP. The decreasing cGMP signal caused by the activation of PDE1 by ionomycin is synergistically prevented by the combination of a PDE1 inhibitor and sub-optimal levels of ANP. Addition of ionomycin has only a weak cGMP lowering effect when combined with ANP and the PDE1 inhibitor.

EXAMPLE 3

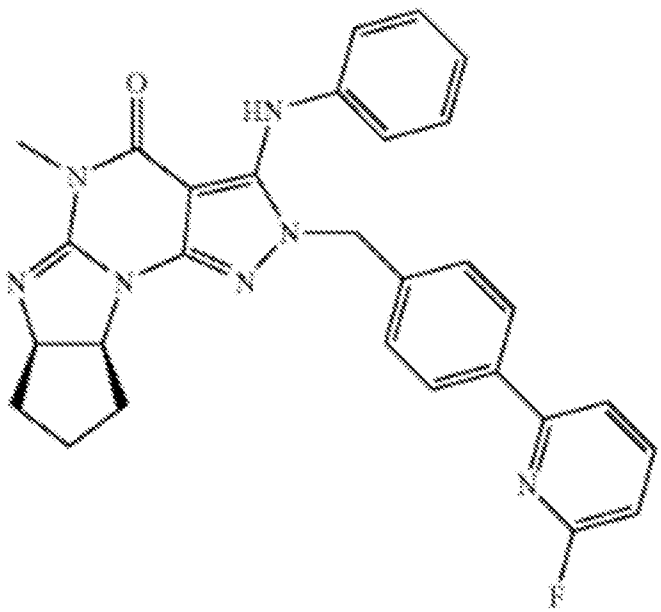
Effect of PDE1 inhibitors on microglia-derived cells

[0088] Neuroinflammatory processes are regulated largely by microglia. Microglia have activation states somewhat similar to macrophages and in response to IFN-γ or lipopolysaccharide (LPS), they will be activated to release pro-inflammatory cytokines such as TNF-, IL-1β, and reactive oxygen species/reactive nitrogen species (ROS/NOS). Under other circumstances, they can be activated to release *anti*-inflammatory cytokines, such as IL-10, and to participate in tissue repair. The immortalized murine microglial cell

line BV-2 is used as a model for microglia signaling. Stansley et al. Journal of Neuroinflammation 2012, 9:115.

[0089] BV2 cells are treated with lipopolysaccharide (LPS) and the level of expression of PDE expression is measured using RNAseq analysis. The data are presented in Figure 1. "FPKM" represents the "Fragment Reads per kilobase of exon per million reads mapped". After treatment with LPS, an endotoxin associated with the inflammatory response, levels of PDE1B, show relatively large increases in RNA expression when compared to other members of the PDE family of enzymes. PDE1B is among the phosphodiesterase subfamilies to show large increases in RNA expression upon LPS administration.

[0090] BV2 cells are further assessed by RNAseq analysis with LPS stimulation in the presence or absence of rolipram (a specific PDE4 inhibitor), and in the presence or absence of Compound 214, which is a specific PDE1 inhibitor of formula:



209 mRNA transcripts are decreased in the presence of LPS + rolipram vs. LPS alone; 138 transcripts are decreased in the presence of Compound 214 + LPS vs. LPS alone. The overlap between the two sets is 48 transcripts. Similarly 156 transcripts are elevated in the presence of LPS + rolipram vs. LPS alone; 149 transcripts are elevated in the

presence of Compound 214 + LPS vs. LPS alone. The overlap between the two sets is 45 transcripts.

[0091] A further RNASeq analysis of expression in LPS-stimulated BV2 cells in the presence and absence of Compound 214 and Rolipram (see Example 7) shows 293 genes significantly affected by Compound 214 but not Rolipram, 251 significantly affected by Rolipram but not Compound 214, and only 114 affected by both, an overlap of only about 17%.

[0092] In still a further experiment, an analysis of expression in in LPS-stimulated BV2 cells in the presence and absence of Compound 214 and Rolipram, shows that the differentially expressed genes for each pair ((LPS vs LPS + rolipram, LPS vs LPS + ITI-214) share about half of the most highly significant genes. For example, the assay demonstrates 1240 genes significantly affected by Compound 214 but not Rolipram, 1463 significantly affected by Rolipram but not Compound 214, and only 683 affected by both, an overlap of only about half.

[0093] The relatively small overlaps indicate that the effects of PDE1 inhibitors on these cells in response to LPS stimulation are very different from the effects of PDE4 inhibitors. While PDE4 inhibitors are often considered to be anti-inflammatory, the two types of inhibitors in this case are, for the most part, affecting expression of completely different genes.

[0094] Moreover, the expression levels of PDEs in BV2 cells to that of mouse brain microglia as determined by RNAseq quantitation of gene transcripts are compared. As detailed in Table A, PDE1B is the second most abundant PDE transcript in freshly isolated mouse microglia, and the most abundant PDE transcript in BV2 cells. PDE4B, PDE4A, PDE7A, and PDE8a expression is also substantial (≥0.7 FPKM/RPKM) in both cell types. Among the several PDEs enzymes detected by RNA-Seq in the BV2 cells, PDE1 is the only one with the ability to hydrolyze both cAMP and cGMP. The relative abundance of PDE1B and PDE4 isoenzymes in BV2 cells potentially indicate to that these are an adequate model for inhibitor studies (Table A):

Table A

X = FPKM or RPKM ≤0.1

Expression retained

	Pde1b	Pde4b	Pde4a	Pde7a	Pde8a
Microglia	9.9	8.1	2.3	1.8	0.4
BV2 cells	4.2	1.5	2.8	0.7	0.4

Abundant, expression lost

	Pde3b	Pde2a	Pde8b	Pde3a
Microglia	33.6	9.6	2	0.5
BV2 cells	X	0.2	X	X

Other, expression lost

	Pde4d	Pde1a	Pde10a	Pde7b
Microglia	0.4	0.3	0.3	0.3
BV2 cells	X	X	X	X

Not in microglia

	Pde1c	Pde4c	Pde11a
Microglia	X	X	X
BV2 cells	X	1.2	X

EXAMPLE 4

Effect on IL1β expression in microglia-derived cells

[0095] BV2 cells are incubated with (i) LPS (10 microgram/ml), (ii) Compound 214 (10 microgram/ml), or (iii) LPS and Compound 214. Levels of IL1β are measured using quantitative PCR of IL1β mRNA. IL1β is considered a marker of inflammation. Results are depicted in Figure 2 (RQ: relative quantification of changes in gene expression in treated versus control samples; ***$p<0.01$ vs control, √ $p<0.01$ vs LPS alone; ANOVA with Newman-Keuls post-hoc test). Administration of a PDE1 inhibitor of the present invention thus significantly blunts the LPS-induced increase in expression of IL1β in microglia-derived cells.

EXAMPLE 5

Effect on IL1β expression in hippocampus in vivo

[0096] Mice are injected with (i) LPS (2mg/kg, s.c.), (ii) Compound 214 (10mg/kg, i.p.), or (iii) LPS and Compound 214. At six hours post injection, levels of IL1β in the hippocampus are measured via quantitative PCR of IL1β mRNA. IL1β is considered a marker of inflammation. Data are presented in Figure 3 (***$p<0.01$ vs LPS alone, ANOVA with Newman-Keuls post-hoc test). The effect *in vivo* is similar to that seen in the BV2 cells: administration of a PDE1 inhibitor of the present invention significantly blunts the LPS-induced increase in expression of IL1β in the brain.

EXAMPLE 6 - *Effect on neuroinflammatory gene expression in BV2 cells*

[0097] Administration of a PDE1 inhibitor of the present invention (Compound 214) at 10 μM significantly reduces the LPS-induced increase in expression of the inflammatory cytokines IL1β, TNFα, and Ccl2 in BV2 cells, as measured by quantitative PCR, as described in Example 4 with respect to IL1β. The PDE4 inhibitor, rolipram, displays a different profile, increasing IL1β expression, while reducing expression of TNF- α and Ccl2. Data are presented in Figure 4a.

[0098] In a separate experiment, administration of a PDE1 inhibitor of the present invention (Compound 214) greatly reduces or blunts LPS-induced changes in proinflammatory markers in BV2 cells (Figure 4b). BV2 cells are pretreated with compound, ITI-214 or rolipram, a PDE4 inhibitor, then stimulated with 50 ng/ml LPS for 4 hours. Expression levels of TNF, IL1β, Ccl2, and IL6 are measured. Normalized mRNA levels are shown as change from vehicle (ΔΔCt) and compared using a one-way ANOVA. Lines denote the mean. * Significantly different from vehicle, # Significantly different from LPS. * $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. The mRNA transcripts for TNF, IL1β, and Ccl2 - three of the four cytokines studied *in vivo* - were elevated in BV2 cells treated with 50 ng/ml LPS. The mRNA signals for TNF and Ccl2 were significantly decreased by treatment with ITI-214 (10 μM), and IL1β mRNA signal trended downward.

EXAMPLE 7 – *Inhibition of LPS-induced TNFα release from BV2 cells*

[0099] PDE1 inhibition reduces LPS-induced TNFα gene expression and release from BV2 cells.

[00100] *TNFα release:* TNFα levels are measured in BV2 conditioned media and normalized to cell protein levels. Inhibition of LPS-induced TNFα release from BV2 cells is depicted in Figure 5. The left panel shows BV2 cells treated with 10μM Compound 214 and 50 ng/ml LPS (one-way ANOVA, *p<0.05, Vehicle n=16, LPS n=31, LPS+214 n=14). The right panel shows a dose-dependent inhibition of LPS-induced TNFα release (50ng/ml LPS stimulation) in response to Compound 214 (measured as % inhibition of total LPS response per experiment).

[00101] *TNFα gene expression*: Figure 6 shows that this dose dependent reduction of TNFα release as measured in the media corresponds to a reduction in TNFα mRNA expression.

EXAMPLE 8 -

Effect on neuroinflammatory gene expression in BV2 cells and to in vivo mouse striatum

[00102] The effects of a selective PDE1 inhibitor (Compound 214) are tested in LPS stimulated BV2 cells by *measuring* changes in cytokine release using ELISA and in gene expression using RT-qPCR and RNA-Seq. (Experiment A (n = 4): (1) Vehicle; (2) 10 μM Compound 214;(3) 50 ng/ml LPS;(4) 50 ng/ml LPS + 10 μM Compound 214; Experiment B (n = 2): (1) 50 ng/ml LPS; (2) 50 ng/ml LPS + 10 μ M Compound 214; (3) 50 ng/ml LPS + 10 μM Rolipram (a known potent PDE4 inhibitor)). The test compound is added first, the LPS is added one hour later, and the cells and/or media are harvested at five hours from commencement of the experiment.

[00103] PDE1 inhibition prevents LPS-induced increases in TNFα release in BV2 cells. Similarly, LPS-induced increases in TNFα, IL-1β, and Ccl2 mRNA expression are reduced by >50% both in BV2 cells and in mice ($p<0.01$) upon PDE1 inhibition. To better understand the actions of PDE1 inhibition on resting and LPS-activated microglia, we examine transcriptional regulation using RNA-Seq. A subset of genes whose transcript expression is significantly changed with PDE1 inhibition is identified. Using gene ontology software (AmiGO 2), it is seen that these genes are significantly ($p<0.05$) enriched in cell migration and extravasation pathways as well as inflammatory pathways.

Of the genes induced by LPS, a subset is attenuated by PDE1 inhibition, all of which are significantly associated with inflammatory pathways ($p<0.05$). PDE4 inhibition attenuates a *different* subset of LPS-induced genes, demonstrating the unique properties of our target (about 17% overlap with PDE1 inhibition).

[00104] **Cells:** BV2 mouse microglial cell line (ICLC, Italy) grown in 2% or 10% heat-inactivated FBS.

[00105] **TNFαELISA:** Thermo Fisher mouse TNFα colorimetric, sandwich ELISA kit. Interpolatee values from standard curve. Dose response is fit to a 4-parameter logistic curve.

[00106] **RTqPCR:** RNA from BV2 cells purified using RNeasyKit(Qiagen) and from mouse tissue using TRIzol (Ambion).TaqMan primer-probe assays from Thermo Fisher. mRNA levels for all conditions are normalized to GAPDH and to vehicle control ($\Delta\Delta Ct$). Data are analyzed statistically using one-way ANOVA with the Bonferroni post-test for multiple comparisons.

[00107] Our results indicate that inhibition of PDE1 regulates activity in microglia, reducing expression of inflammatory genes, providing a rationale to use PDE1 inhibitors to treat toxic neuroinflammation.

[00108] **RNASeq:** Flashfreeze BV2cells, isolate RNA, prepare a library using polyA selection, and conduct 1x50bp single read sequencing on the Illumina HiSeq 2500 in High Output mode (using V4 chemistry). Genes are mapped to reference genome (GRCm38) using CLC Genomics Server. Number of reads per sample average ~17million. Differential gene expression analysis is performed using DESeq2software (Bioconductor.org). Differentially expressed genes ($p<0.01$,Waldtest) are reported as log2(fold change).

[00109] The following table demonstrates a summary of initial results of neuroinflammatory biomarker expression in both BV2 cells and mouse striatum subject to LPS administration in the presence or absence of a PDE1 inhibitor (Compound 214) or a PDE4 inhibitor (rolipram). The results are based upon an evaluation of samples using Q-PCR.

Biomarker	BV2 + LPS	PDE1 Inhibitor	rolipram	Striatum Mouse + LPS	PDE1 Inhibitor	rolipram
Tumor necrosis factor	UP	Down	Down	UP	NC	Down
Interleukin 1 beta	Up	Down	UP UP	UP	NC	UP-UP
Interleukin 6	NC	NC	NC	NC	NC	UP-UP
Chemokine (C-C) motif Ligand 2	UP	Down	Down	UP	Down	Down
Leukemia inhibitory factor	NC	NC	UP	NC	NC	NC
Oncostatin M	NC	UP	UP	UP	Down	Down

Administering a PDE1 inhibitor of the present invention correlates with either a decrease or no change in the expression of biomarkers: IL1β, TNF- α, and Ccl_2 compared to samples treated only with LPS. Interestingly, the anti-inflammatory profile of the PDE1 inhibitor is quite different from that of the PDE4 inhibitor.

[00110] Data from a further experiment measuring the effect of PDE1 inhibition on inflammatory gene expression in mouse striatum is depicted in Figure 7. Adult mice (at least 2 month old, male C57BL/6) are treated with both Compound 214 (10 mg/kg i.p.) and LPS (500 µg/kg s.c.), and sacrificed after two hours. Striatal tissue is isolated and flash frozen, and RNA is extracted. Measurements from RT-qPCR are shown as log2 fold change (2ΔΔCt). n=4 *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

[00111] In addition to confirming that the PDE1 inhibitor inhibits LPS stimulation of the pro-inflammatory cytokines TNFα, Ccl2, IL-1β, and IL-6, the PDE1 inhibitor is seen to significantly enhance expression of the anti-inflammatory cytokine IL-10.

Further Effects on neuroinflammatory gene expression in BV2 cells and to in vivo mouse striatum

[00112] Data from yet another experiment measuring the effect of PDE1 inhibition on inflammatory gene expression in mouse striatum is depicted in Figure 8. Adult mice are treated with vehicle (white bars), 500 µg/kg LPS s.c. (gray bars), or 10 mg/kg (Compound 214) i.p. and 500 µg /kg LPS s.c. (black bars) for 2 hours (n = 4). Striatal tissue is analyzed for mRNA levels of TNF, IL1β, Ccl2, and IL6. Expression levels are shown as change in Q-PCR signal from vehicle (ΔΔCt) and compared using an ANOVA. * p<0.05, **p<0.01, ***p<0.001.

[00113] The Applicants measure mRNA levels of four common inflammatory markers (TNF, IL1β, Ccl2, and IL6) using quantitative PCR (Figure 8). In adult mice treated with

500 μg/kg LPS s.c. for 2 hours, mRNA expression levels of all four markers increases significantly as measured in isolated tissue samples from the striatum (Fig 8). A dose of 10 mg/kg (Compound 214) which is delivered i.p. attenuates the expression of TNF and Ccl2. In this experiment, levels of IL1-beta and IL6 trended to lower levels as well, but do not reach significance.

Biological Processes Associated with PDE1 inhibition in BV2 Cells

Experiment 1

[00114] The following table (Table 1) highlights biological processes associated with PDE1 inhibition in BV2 cells, by analyzing differentially expressed genes in Compound 214 comparisons from Experiment A and Rolipram comparisons from Experiment B, against a database of gene functions. Gene ontology analysis is generated using the AmiGO2 software version: 2.4.24 running PANTHER.PANTHER Overrepresentation Test (release20160715). GO Ontology database Released 2016-10-27. Reference List = Mus musculus (all genes in database). Bonferroni Correction. Biological processes shown are Headers in Hierarchy View ($p<0.05$).

Table 1

Increase with Compound 214 vs Vehicle

Biological Process	**Pathway ID**	**P-Value**
positive regulation of inflammatory response	GO:0050729	3.83E-05
leukocyte migration	GO:0050900	3.15E-04
leukocyte migration involved in inflammatory response	GO:0002523	1.09E-03
positive regulation of interleukin-8 production	GO:0032757	3.00E-03
regulation of cellular extravasation	GO:0002691	5.90E-03
leukocyte chemotaxis	GO:0030595	8.30E-03
positive regulation of cellular extravasation	GO:0002693	3.89E-02

Decrease with Compound 214 vs Vehicle

Biological Process	Pathway ID	P-Value
cellular response to lipopolysaccharide	GO:0071222	2.15E-02
regulation of lymphocyte activation	GO:0051249	3.29E-02
leukocyte differentiation	GO:0002521	3.45E-02
regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	GO:0002822	3.58E-02

Increase with Compound 214 vs Vehicle -Subset of genes highly expressed in microglia

(FPKM >10, Brain RNA-Seq database)

Biological Process	Pathway ID	P-Value
positive regulation of inflammatory response	GO:0050729	7.50E-04
leukocyte chemotaxis	GO:0030595	2.63E-02
positive regulation of cell-cell adhesion	GO:0022409	2.88E-02
inflammatory response	GO:0006954	1.36E-02
cellular metal ion homeostasis	GO:0006875	2.42E-02
positive regulation of apoptotic process	GO:0043065	1.29E-02

Increase with LPS vs Vehicle, Decrease with LPS + ITI-214 vs LPS

Biological Process	Pathway ID	P-Value
positive regulation of immune system process	GO:0002684	3.98E-08
cellular response to lipopolysaccharide	GO:0071222	4.69E-05
inflammatory response	GO:0006954	4.87E-05
response to cytokine	GO:0034097	3.68E-03
positive regulation of inflammatory response	GO:0050729	1.08E-02

positive regulation of cytokine biosynthetic process	GO:0042108	1.59E-02
positive regulation of cell death	GO:0010942	1.94E-02
positive regulation of tumor necrosis factor production	GO:0032760	2.45E-02

Increase with LPS vs Vehicle, Decrease with LPS + Rolipram vs LPS

Biological Process	**Pathway ID**	**P-Value**
cellular response to cytokine stimulus	GO:0071345	4.31E-03
macrophage chemotaxis	GO:0048246	6.39E-03
leukocyte migration	GO:0050900	2.18E-02
positive regulation of GTPase activity	GO:0043547	3.92E-02

Increase with LPS vs Vehicle, Increase with LPS + Rolipram vs LPS

Biological Process	**Pathway ID**	**P-Value**
negative regulation of secretion by cell	GO:1903531	2.73E-02

Experiment 2

In a still further experiment, using AmiGO, the following table (Table 2) analyzes regulatory pathways impacted by LPS and ITI-214 in RNAseq experiments in BV2 cells. Overall, the primary process type changes by LPS, and can be attenuated with inclusion of ITI-214, are believed to relate to inflammation, cytokine expression and cellular responses to LPS (Table 2). Generally, differentially expressed genes associated with ITI-214 treatment relative to vehicle are believed to associate with processes related to chemotaxis and migration. The genes that respond only to the combination of LPS and ITI-214 are believed to demonstrate enrichment for biological processes related to DNA replication, mitotic cycle, and DNA repair.

Table 2

Vehicle vs Compound 214

Biological Process	**Pathway ID**	**Fold Enrichment**	**p-value**
positive regulation of tumor necrosis factor biosynthetic process	GO:0042535	15.17	3.15E-02
toll-like receptor signaling pathway	GO:0002224	7.53	3.40E-03
DNA-dependent DNA replication	GO:0006261	5.94	8.05E-05
neutrophil chemotaxis	GO:0030593	5.83	3.88E-02
cellular response to lipopolysaccharide	GO:0071222	5.82	3.14E-07
regulation of bone mineralization	GO:0030500	5.48	2.71E-02
positive regulation of inflammatory response	GO:0050729	5.43	2.97E-04
positive regulation of leukocyte migration	GO:0002687	4.27	8.12E-03
double-strand break repair	GO:0006302	3.78	4.03E-02
regulation of reactive oxygen species metabolic process	GO:2000377	3.76	6.50E-03
inflammatory response	GO:0006954	3.5	1.69E-08
positive regulation of leukocyte cell-cell adhesion	GO:1903039	3.45	4.07E-02
response to metal ion	GO:0010038	3.2	2.46E-02
leukocyte differentiation	GO:0002521	3.04	4.63E-04
regulation of MAP kinase activity	GO:0043405	2.91	2.71E-02
cellular response to organonitrogen compound	GO:0071417	2.82	5.72E-03
regulation of hemopoiesis	GO:1903706	2.79	6.79E-03
lymphocyte activation	GO:0046649	2.79	2.00E-03
negative regulation of immune system process	GO:0002683	2.71	3.69E-03
negative regulation of cell cycle	GO:0045786	2.64	2.13E-02
positive regulation of cell activation	GO:0050867	2.5	2.84E-02
positive regulation of apoptotic process	GO:0043065	2.48	5.73E-04
carboxylic acid metabolic process	GO:0019752	2.41	4.72E-05
apoptotic process	GO:0006915	2.34	5.79E-05
organophosphate metabolic process	GO:0019637	2.2	4.38E-03
response to abiotic stimulus	GO:0009628	2.08	4.63E-03
positive regulation of cell proliferation	GO:0008284	2.06	4.99E-03
cellular lipid metabolic process	GO:0044255	2.04	4.09E-02
phosphate-containing compound metabolic process	GO:0006796	2.04	9.60E-08
positive regulation of catalytic activity	GO:0043085	2	3.14E-03
immune response	GO:0006955	2	1.07E-03

LPS vs. LPS + Compound 214

Biological Process	**Pathway ID**	**Fold Enrichment**	**p-value**
T cell costimulation	GO:0031295	10.39	1.28E-02
toll-like receptor signaling pathway	GO:0002224	9.52	2.99E-07
leukocyte mediated cytotoxicity	GO:0001909	7.93	7.24E-03
positive regulation of cytokine biosynthetic process	GO:0042108	6.91	8.67E-05
regulation of interleukin-2 production	GO:0032663	6.47	5.06E-03
cellular response to lipopolysaccharide	GO:0071222	5.49	1.53E-07
positive regulation of inflammatory response	GO:0050729	5.27	7.64E-05
positive regulation of tumor necrosis factor production	GO:0032760	5.26	6.81E-03
granulocyte migration	GO:0097530	4.95	3.20E-02
leukocyte chemotaxis	GO:0030595	4.68	4.81E-04
regulation of myeloid leukocyte differentiation	GO:0002761	4.6	6.24E-04
response to calcium ion	GO:0051592	4.28	3.35E-02
positive regulation of leukocyte migration	GO:0002687	4.14	2.99E-03
positive regulation of lymphocyte proliferation	GO:0050671	3.87	1.58E-02
B cell activation	GO:0042113	3.82	2.71E-03
regulation of T cell proliferation	GO:0042129	3.75	3.66E-03
regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	GO:0002822	3.7	2.88E-02
inflammatory response	GO:0006954	3.67	1.65E-11
glycerolipid metabolic process	GO:0046486	3.3	3.75E-04
regulation of I-kappaB kinase/NF-kappaB signaling	GO:0043122	3.28	9.84E-03
response to virus	GO:0009615	3.24	2.08E-02
regulation of peptidyl-tyrosine phosphorylation	GO:0050730	3.01	1.57E-02
regulation of angiogenesis	GO:0045765	2.94	3.71E-02
cytokine-mediated signaling pathway	GO:0019221	2.91	1.80E-02
negative regulation of cell adhesion	GO:0007162	2.89	3.19E-02
phospholipid metabolic process	GO:0006644	2.79	3.78E-02
regulation of MAP kinase activity	GO:0043405	2.73	3.61E-02
positive regulation of apoptotic process	GO:0043065	2.69	7.61E-07
negative regulation of immune system process	GO:0002683	2.64	1.47E-03
leukocyte differentiation	GO:0002521	2.56	2.74E-02

immune effector process	GO:0002252	2.55	9.04E-05
negative regulation of protein phosphorylation	GO:0001933	2.42	3.00E-02
innate immune response	GO:0045087	2.36	3.26E-04
negative regulation of intracellular signal transduction	GO:1902532	2.31	1.29E-02
defense response to other organism	GO:0098542	2.21	2.19E-02
vasculature development	GO:0001944	2.19	4.69E-02
positive regulation of transferase activity	GO:0051347	2.18	4.90E-02
regulation of catabolic process	GO:0009894	2.07	9.69E-03
intracellular signal transduction	GO:0035556	2.06	8.20E-07
apoptotic process	GO:0006915	2.04	5.92E-03

Vehicle v. LPS + Compound 214 (excluding differentially expressed genes also found in Vehicle vs LPS or Vehicle vs. Compound 214)

Biological Process	**Pathway ID**	**Fold Enrichment**	**p-value**
pseudouridine synthesis	GO:0001522	10.45	1.23E-02
pyrimidine nucleotide metabolic process	GO:0006220	7.3	4.98E-02
DNA-dependent DNA replication	GO:0006261	5	7.52E-05
mitotic sister chromatid segregation	GO:0000070	4.05	3.36E-02
tRNA metabolic process	GO:0006399	3.57	1.38E-03
DNA repair	GO:0006281	2.52	5.78E-04
organophosphate biosynthetic process	GO:0090407	2.51	1.05E-02
regulation of chromosome organization	GO:0033044	2.45	4.74E-02
regulation of cell cycle process	GO:0010564	2.35	3.32E-04
regulation of mitotic cell cycle	GO:0007346	2.33	1.00E-03
cell division	GO:0051301	2.23	1.01E-02

Response of selected genes to ITI-214

[00115] In a further experiment a group of genes is selected for further analysis by RT-qPCR. For comparison, genes are analyzed which have been implicated in the scientific literature in microglial and astrocyte activation, including genes in the complement pathway.

[00116] In this select panel which can be derived from transcriptional response experiments, generally speaking, most of the genes fall into three general categories:

those strongly induced by LPS and attenuated or reversed upon inhibition of PDE1, those predominantly responsive to Compound 214, and those strongly induced by LPS and weakly responsive to Compound 214. These patterns are displayed on parallel coordinates graphs in Figure 9.

[00117] In one experiment, Figure 10 demonstrates quantitated changes in target gene expression after treating BV2 cells with varying doses of ITI-214. A notable observation is that modulation of transcriptional responses to LPS by ITI-214 is dose dependent (Figure 10a). Figure 10a shows the change in expression levels of each gene in BV2 cells with 50 ng/ml LPS and the indicated dose of ITI-214. Dose dependent effects of ITI-214 in the absence of LPS stimulation are shown in Figure 10b. All sample values are normalized to an average of 3 reference genes and to vehicle control ($\Delta\Delta$Ct). The error bars represent the mean +/- SEM (n = 4). From left to right, for each cytokine grouping, the bars indicate samples treated with: LPS, 0.1 μM 214 + LPS, 0.4 μM 214 + LPS, 1.1 μM 214 + LPS, 3.3 μM 214 + LPS, 10 μM 214 + LPS, for each particular gene shown on the X-axis. Significant changes between LPS and 10 μM ITI-214 + LPS (**A**) and Vehicle and 10 μM ITI-214 (**B**) are marked on the X-axis gene names and are calculated using a one-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Effect of cAMP and cGMP on ITI-214 dependent inhibition of LPS; activators

[00118] To investigate cGMP-dependent activity of the PDE1 inhibitor of the present invention (Compound 214), particulate guanylyl cyclase activity with atrial natriuretic peptide (ANP) or soluble guanylyl cyclase activity, with nitric oxide donor DEANO, can be stimulated. In one particular experiment, BV2 cells are treated with 10 μM forskolin (FSK) to activate adenylyl cyclases, 5 μM DEANO to activate soluble guanylyl cyclase, or 100 nM atrial natriuretic peptide (ANP) to activate particulate guanylyl cyclase. Alone, these activators are believed to have little or no effect on LPS induction of the selected genes. Upon combining forskolin with ITI-214 some of the effects of ITI-214 inhibition can be increased. For example, the following ITI-214 responsive transcripts are further down- or up-modulated by concomitant forskolin: chemokine receptor Cx3cr1, cytokines Csf2 and TNF, chemokine Ccl4, kinase MAPKAPK2, phosphatase PtpN6,

chemokine receptor Ccr1. Cytokine LIF, demonstrates increased expression with LPS, and is further increased with ITI-214. However, LIF reverts to LPS-level expression when forskolin is added. Data is demonstrated in Fig. 11a.

[00119] Responses to DEANO treatment together with ITI-214 closely matches the responses seen with forskolin and ITI-214, and indicates that ITI-214 enhancement of either cGMP or cAMP signaling can reverse LPS-induction. There is not believed to be equivalent responses upon ANP and ITI-214 treatment. This possibly indicates that distinct pools of cGMP (cytosolic versus membrane associated) govern certain of the transcriptional responses to inhibition of PDE1. There are believed to be equivalent responses upon addition of DEANO, FSK, or ANP to ITI-214 for the following cytokines: Cx3cr1, Csf2, PtpN6, and Ccr1 and LIF. Ccl4, TNF, and MAPKAPK2, show weak or no response to the addition of ANP to the PDE1 inhibitor. Finally, mRNA responses to ITI-214 for C-type lectin CD72 and transcription factor Ddit3 are enhanced selectively by ANP. Data is demonstrated in Fig. 11a.

[00120] Two transcripts in the panel, Cx3cr1 and Tgm2, have the most dramatic change upon ITI-214 treatment. Induction of Cx3cr1 by LPS was significantly reduced by ITI-214 treatment, as was basal expression of this marker in the absence of LPS.

Effect of cAMP and cGMP on ITI-214 dependent inhibition of LPS; inhibitors

[00121] The influence of each cyclic nucleotide (cAMP or cGMP) on the ITI-214 response by combining LPS stimulation, ITI-214 inhibition of PDE1, and either PKA inhibitor cAMPS-Rp (100 μM) or PKG inhibitor -8-Br-PET-cGMPS (100 μM). Binding to cyclic nucleotide-dependent signaling molecules, these analogs do not provide activation, and prevent activation by authentic cyclic nucleotides. Moderate reduction of the impact of ITI-214 is the norm for these inhibitors (Figure 11b). Note that gene expression levels upon addition of PKA or PKG inhibitor to 10 μM ITI-214 are on the order of those relative to LPS treatment alone were reduced to levels equivalent to 3.3 μM ITI-214. Data is demonstrated in Fig. 11b.

EXAMPLE 9 - *Effect on stress-induced inflammation*

[00122] This experiment compares the impact of chronic stress on the brain cytokine/chemokine profile in wild type mice vs PDE1B conditional knockout mice. Assessing the effect of a PDE1 inhibitor of the present invention (Compound 214) on stress-induced cytokines changes in this model in the wild type and PDE1B knockout mice allows us to confirm that the effect of the PDE1 inhibitor is specific for PDE1B.

[00123] PDE1B conditional knockout mice are described in Siuciak JA, et al. "*Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-1B (PDE1B) enzyme*." Neuropharmacology. 2007 Jul; 53(1):113-24. Protocols for CUS and other models of chronic stress are described in Monteiro, et al., "*An Efficient Chronic Unpredictable Stress Protocol to Induce Stress-Related Responses in C57BL/6 Mice*," Front Psychiatry. 2015; 6: 6 and in Mann et al., *"Chronic social defeat, but not restrain stress, alters bladder function in mice"* Physiol. Behav. 2015; Oct 15; 150: 83-92.

[00124] Generally, wild type (control) mice and PDE1B conditional knockout mice, as appropriate, are exposed to the chronic stress using restraint stress in shallow water model (RSSW) as described by Mann et al (2015), above. For RSSW (21 days) mice are treated daily with either vehicle or Compound 214 (10 mg/kg, i.p. or p.o.) then put in a perforated conical tube with feet immersed in water for 4h, then returned to single housing cages. Control mice for these experiments are also maintained in single housing cages. In accordance the stress protocol, the mice are monitored for the depression-like behavior profile. Mice are then sacrificed and tissue samples are collected. Brain are rapidly dissected (under conditions to preserve mRNA) and frozen at -80°C for shipment to ITI. Blood is collected at sacrifice, prepared for serum. Brain tissue and serum is analyzed for a panel of markers, including pro-inflammatory and anti-inflammatory cytokines and chemokines.

A) Effect of the CUS protocol on neuroinflammatory markers:

[00125] C57Bl/6 mice are subjected to the CUS protocol (14d, as described) or sham treatment, evaluated for depression-like behaviors at a specified time points, and sacrificed for collection of blood (serum preparation) and brain collection. Serum is analyzed for corticosterone (CORT) levels and other inflammatory markers. The brain is analyzed for a panel of inflammatory markers by qPCR (mRNA) and MSD (protein). The

CUS protocol significantly elevates CNS and serum levels of proinflammatory cytokines/chemokines in parallel with induction of depression-like behaviors in normal mice.

B) Effect of a PDE1 inhibitor on stress-induced brain and serum inflammatory markers:

[00126] Cohorts of C57Bl/6 mice (WT for the PDE1B KO) are subjected to the CUS protocol (14d, as described) or sham treatment will receive either daily dosing with Compound 214 (10 mg/kg, i.p.) or vehicle. Mice are evaluated for depression-like behaviors at a specified time point to test the impact of the CUS protocol and of the PDE1 inhibitor treatment on the depression-like phenotype. Mice are immediately sacrificed for collection of blood (serum preparation) and brain collection. Serum is analyzed for CORT levels and other inflammatory markers. Brain samples are analyzed for a panel for a panel of inflammatory markers by qPCR (mRNA) and MSD (protein). The PDE1 inhibitor significantly suppresses CUS-induced CNS and serum markers (proinflammatory) and expression of depression-like behaviors in normal mice.

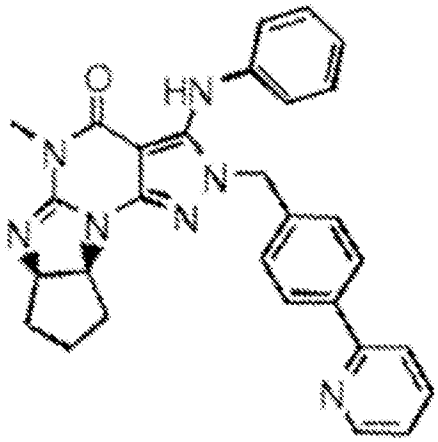
C) Effect of PDE1B KO on stress-induced brain and serum markers:

[00127] Cohorts of WT and PDE1B KO mice are treated and subjected to either CUS protocol or sham treatment, with either daily dosing with Compound 214 (10 mg/kg, i.p.) or vehicle. Mice are evaluated for depression-like behaviors at a specified time point to test the impact of the CUS protocol and the treatment of the PDE1 inhibitor on the depression-like phenotype. Mice are immediately killed for collection of blood (serum preparation) and brain collection. Serum is analyzed for CORT levels and other inflammatory markers, and brain samples are also analyzed for a panel of inflammatory markers by qPCR (mRNA) and MSD (protein). PDE1B KO mice exhibit significantly suppressed CUS-induced CNS and serum markers (proinflammatory). Moreover, expression of depression-like behaviors which are seen in normal mice (i.e., having been subject to CUS protocol) are reduced in the KO. The PDE1 inhibitor on these tissue and serum markers and behaviors has no significant effect in the PDE1B KO mice.

EXAMPLE 10 – *Effects in the Optic Nerve Injury Model*

[00128] A PDE1 inhibitory compound is tested in an optic nerve injury model (i.e., the

"optic crush" model). The studies described below are carried out using the selective PDE1 inhibitor IC200041, which has the following structure:



[00129] In this model, optic nerve surgeries are carried out on male mice 8 wk of age (average body weight, 20-26 g) under general anesthesia, as described previously (Yin Y, *et al.*, Oncomodulin links inflammation to optic nerve regeneration. Proc Natl Acad Sci U S A. 2009; 106:19587–19592). Following nerve injury, 3 μl of fluid is removed from the eye and a solution containing the PDE1 inhibitory compound is injected intraocularly.

[00130] A total of 4 mouse retinas are treated with the PDE1 inhibitor, and phosphate buffered saline (PBS) is administered to a total of 10 mouse retinas as a control. Mice are typically euthanized with an overdose of anesthesia 14 days after optic nerve injury and are perfused with saline and 4% paraformaldehyde (PFA). These mice are 10 weeks old when killed. Optic nerves and eyes are dissected and postfixed in PFA. Nerves are impregnated with 10% and then 30% sucrose, embedded in OCT Tissue Tek Medium (Sakura Finetek), frozen, cut in the longitudinal plane at a thickness of 14 μm, and mounted on coated slides. Regenerating axons are visualized by staining with a sheep antibody to GAP-43 followed by a fluorescently labeled secondary antibody. Axons are counted manually in at least 8 longitudinal sections per case at pre-specified distances from the injury site, and these numbers were converted into the number of regenerating axons at various distances.

[00131] After retinal insult, quantitative analysis shows that PDE expression in retinal ganglion cells is greatly increased. Messenger RNA (mRNA) for PDE1B in particular is

upregulated about fourfold after injury. RNA is extracted from tissue using standard methods, and mRNA was quantitated using quantitative RNAseq sequencing methods. Standard mRNA for beta actin, and four glutamate receptors, namely NMDA R1 and AMPAK R1, R2 and R3, are evaluated in parallel and show no significant changes.

[00132] IC200041 is highly effective in increasing the survival of retinal ganglion cells when compared with the PBS control. Further, the difference in control retinal ganglion cells and those treated with IC200041 is statistically significant. Data is demonstrated within Figure 12.

CLAIMS

We claim:

1. A method of treatment or prophylaxis of inflammation and/or an inflammatory disease or disorder (e.g., neuroinflammation) comprising administration of a PDE1 inhibitor to a patient in need thereof.

2. A method according to claim 1 wherein the PDE1 inhibitor inhibits the activity of PDE1 (e.g., in the assay described in Example 1) with an IC_{50} of less than 10 nM, e.g., wherein the PDE1 inhibitor does not inhibit the activity of PDE types other than PDE1, e.g., has an IC_{50} at least 1000 times greater for PDE types other than PDE1.

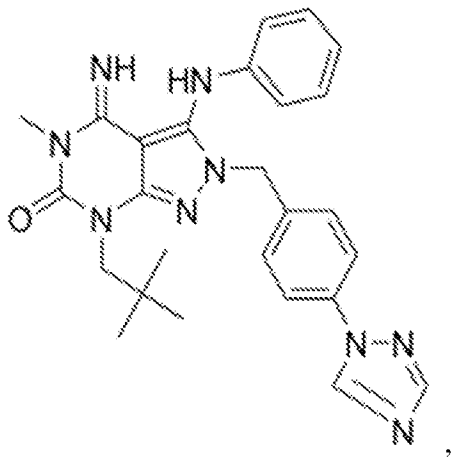
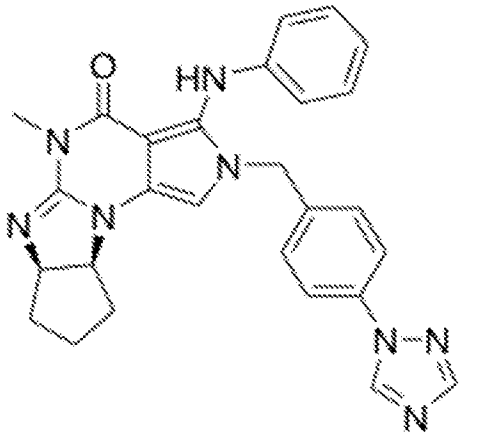
3. A method according to claim 1 or 2 wherein the PDE1 inhibitor is of Formula I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and/or XII as hereinbefore described.

4. A method according to any preceding claim for treatment or prophylaxis of neuroinflammation and/or diseases or disorders associated with neuroinflammation and/or microglial function, e.g., selected from:
    a. neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and demyelinating conditions, e.g., multiple sclerosis (MS), and prion diseases;
    b. stroke, cardiac arrest, hypoxia, intracerebral hemorrhage or traumatic brain injury;
    c. conditions characterized by abnormal neurotransmitter production and/or response, including depression, schizophrenia, post-traumatic stress disorder, anxiety, attention deficit disorder, and bipolar disease; e.g., wherein any of the foregoing are associated with neuroinflammation; and
    d. chronic CNS infections, e.g., Lyme disease or CNS infection consequent to an immunosuppressive condition, e.g., HIV dementia;

e. neuroinflammation consequent to chemotherapy.

5. The method of any preceding claim wherein the patient has
   a. elevated levels of one or more pro-inflammatory cytokines (e.g., selected from IL1β, TNFα, Ccl2, IL-6, and combinations thereof);
   b. reduced levels of one or more anti-inflammatory cytokines (e.g., IL-10);
   c. elevated levels of microglial M1 phenotype compared to microglial M2 phenotype.

6. Any of the preceding claims, wherein the PDE1 inhibitor is selected from any of the following:


,

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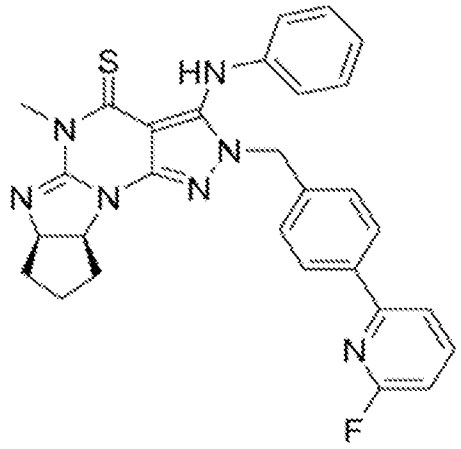
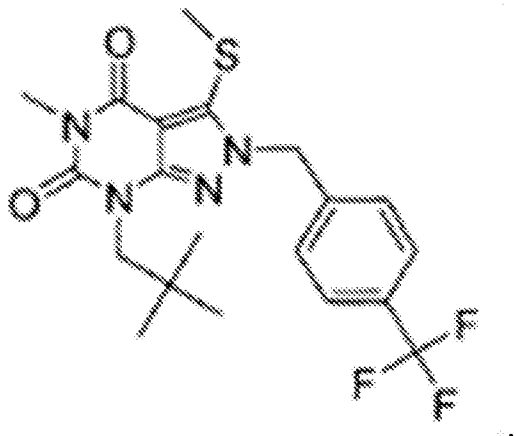
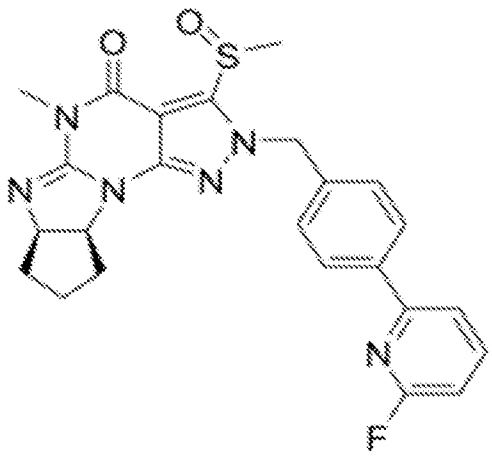
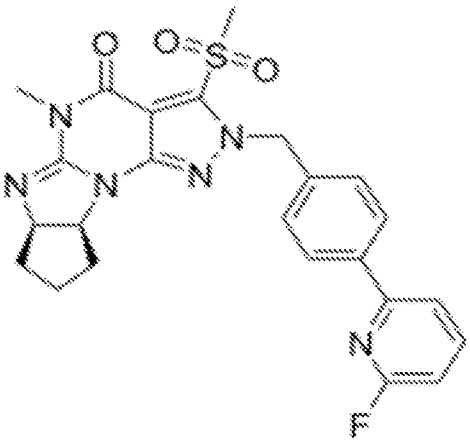
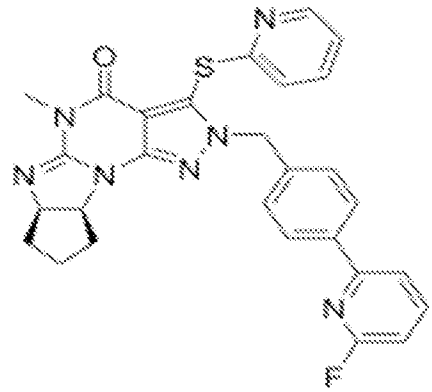
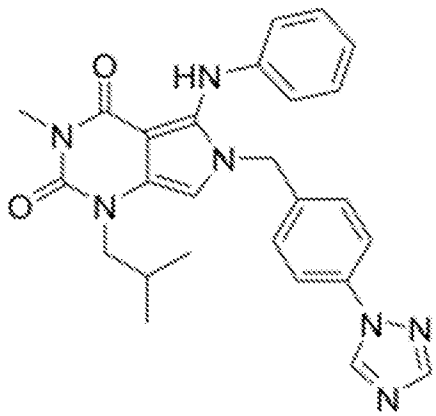

,

,


,

,

,

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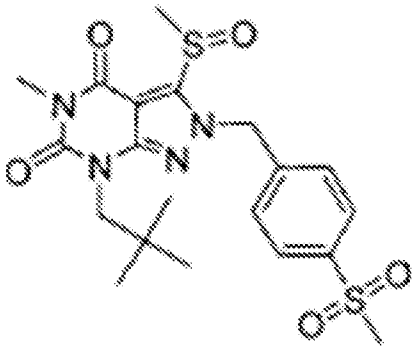
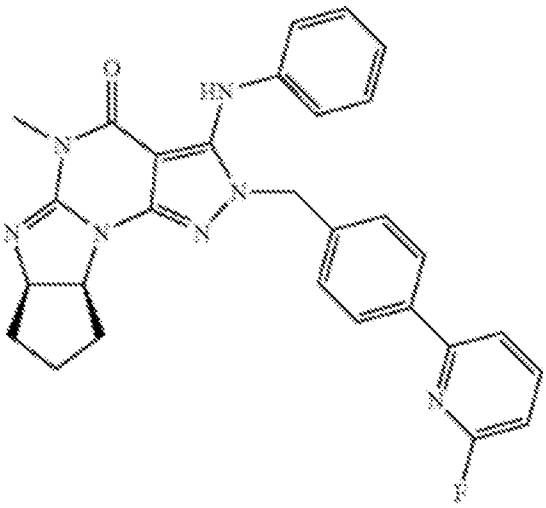
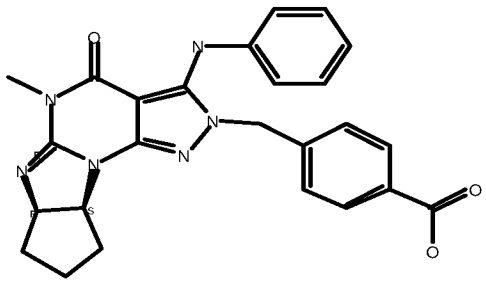
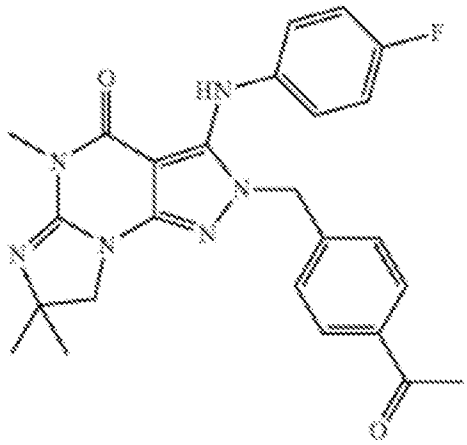
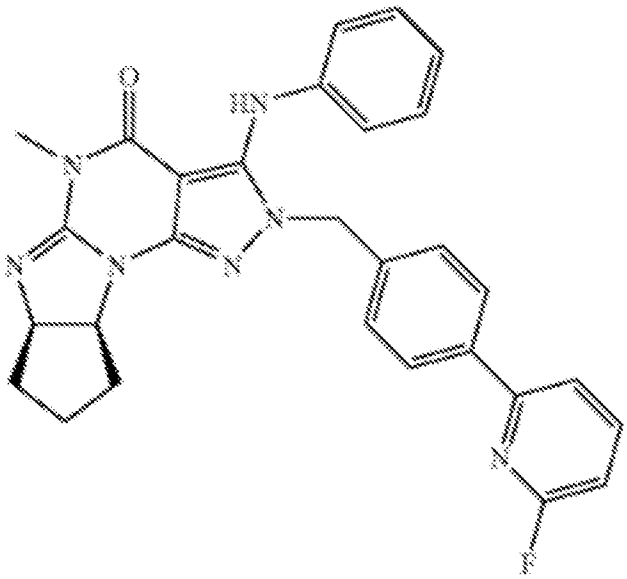




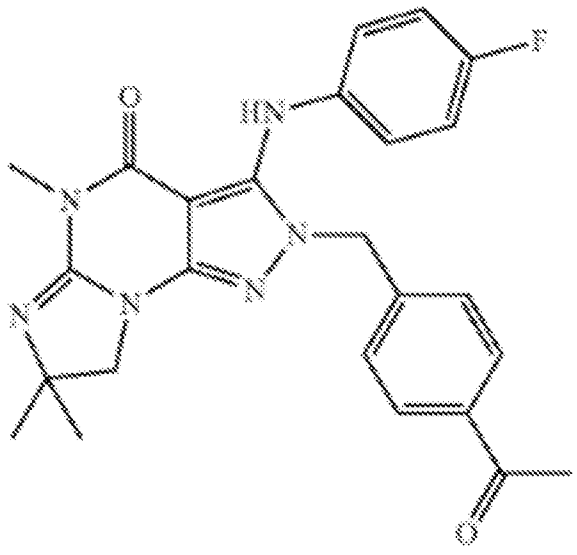




7. The method of claim 6, wherein the PDE1 inhibitor is the following:



8. The method of claim 6, wherein the PDE1 inhibitor is the following:



9. The method of any of the preceding claims, wherein a PDE1 inhibitor is administered in combination with a PDE4 inhibitor (e.g., rolipram).

10. The method of any of the preceding claims wherein a PDE1 inhibitor is administered in combination with one or more antidepressant agents, in free or pharmaceutically acceptable salt form, selected from selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and atypical antipsychotic agents; e.g., an atypical antipsychotic agent, e.g., lumateperone, in free or pharmaceutically acceptable salt form, or an SSRI, e.g., fluoxetine, in free or pharmaceutically acceptable salt form.

11. A PDE1 inhibitor or a pharmaceutical composition comprising a PDE1 inhibitor for use in any of the methods of any preceding claim.

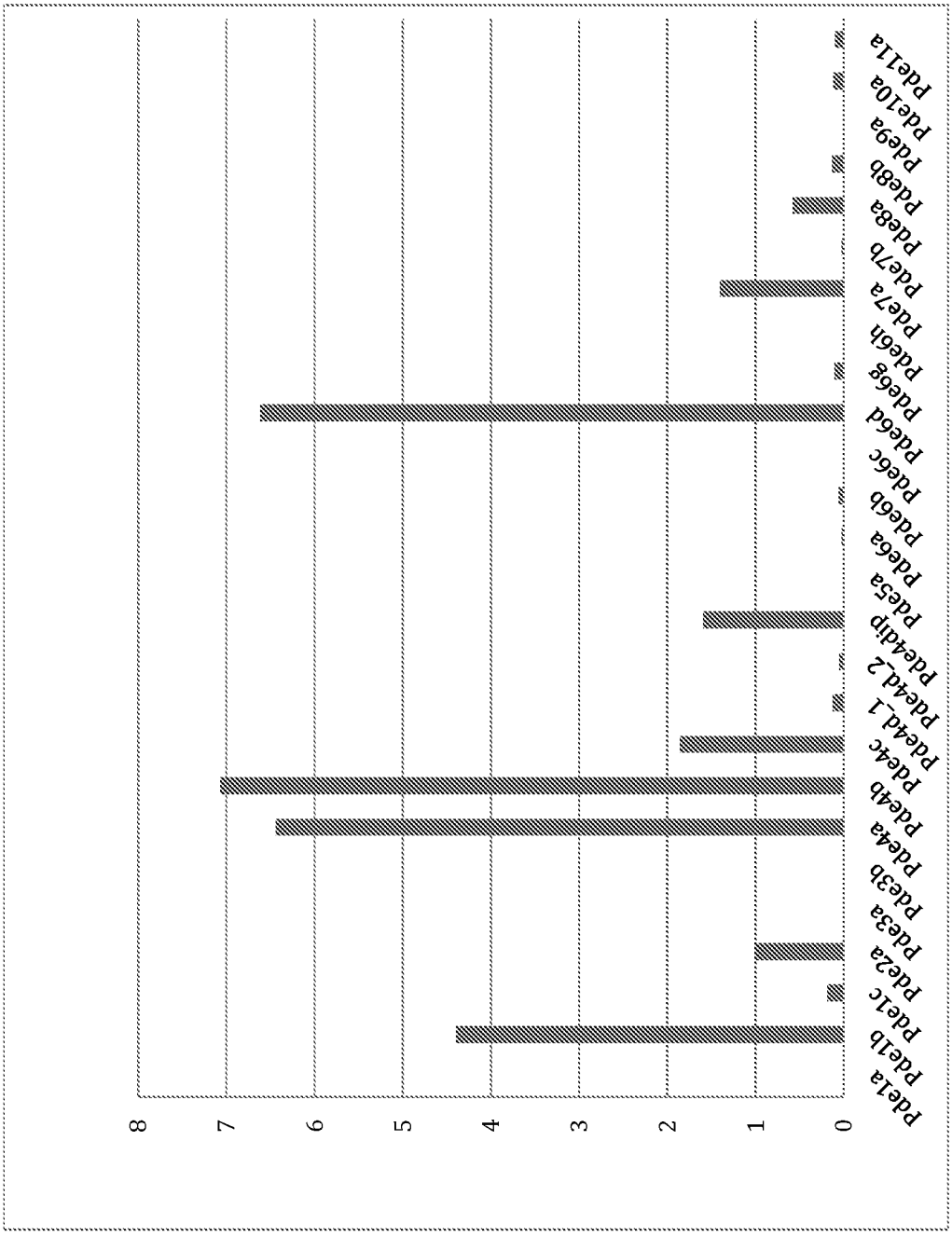

0
1
2
3
4
5
6
7
8
pde1a
pde1b
pde1c
pde2a
pde3a
pde3b
pde4a
pde4b
pde4c
pde4d_1
pde4d_2
pde4dip
pde5a
pde6a
pde6b
pde6c
pde6d
pde6g
pde6h
pde7a
pde7b
pde8a
pde8b
pde9a
pde10a
pde11a

FIG. 1

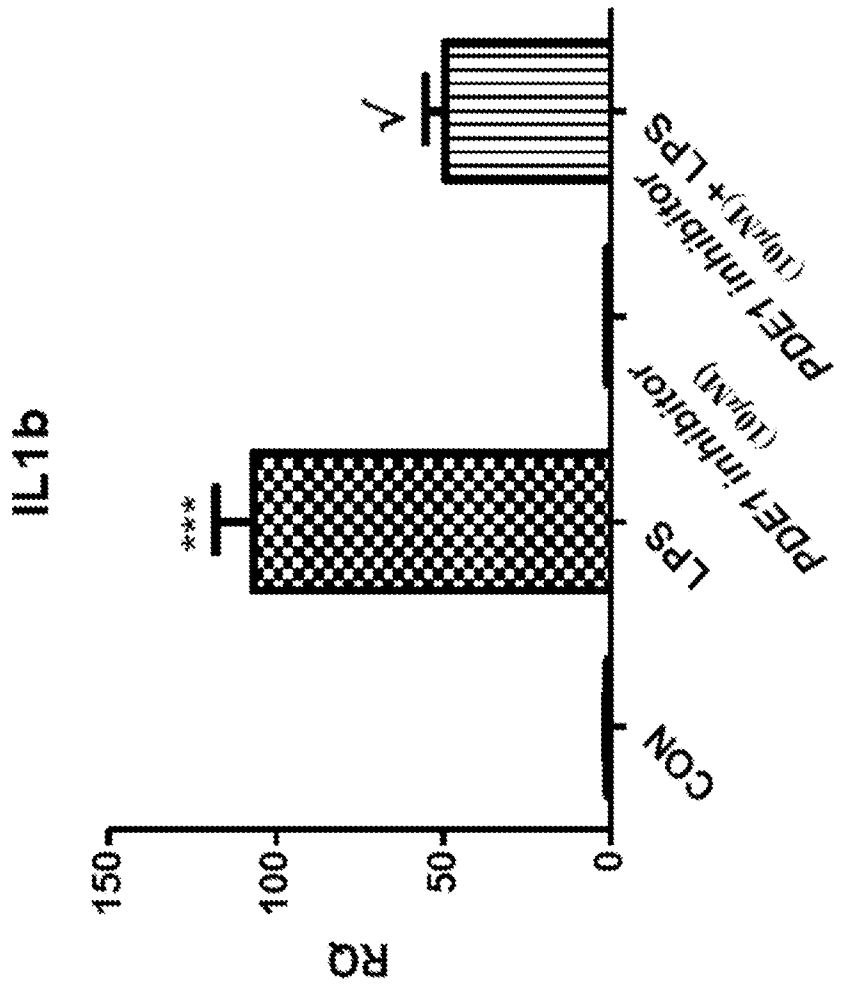

IL1b
RQ
150
100
50
0
CON
LPS
PDE4 inhibitor (10μM)
PDE4 inhibitor (10μM)+ LPS
***

FIG. 2

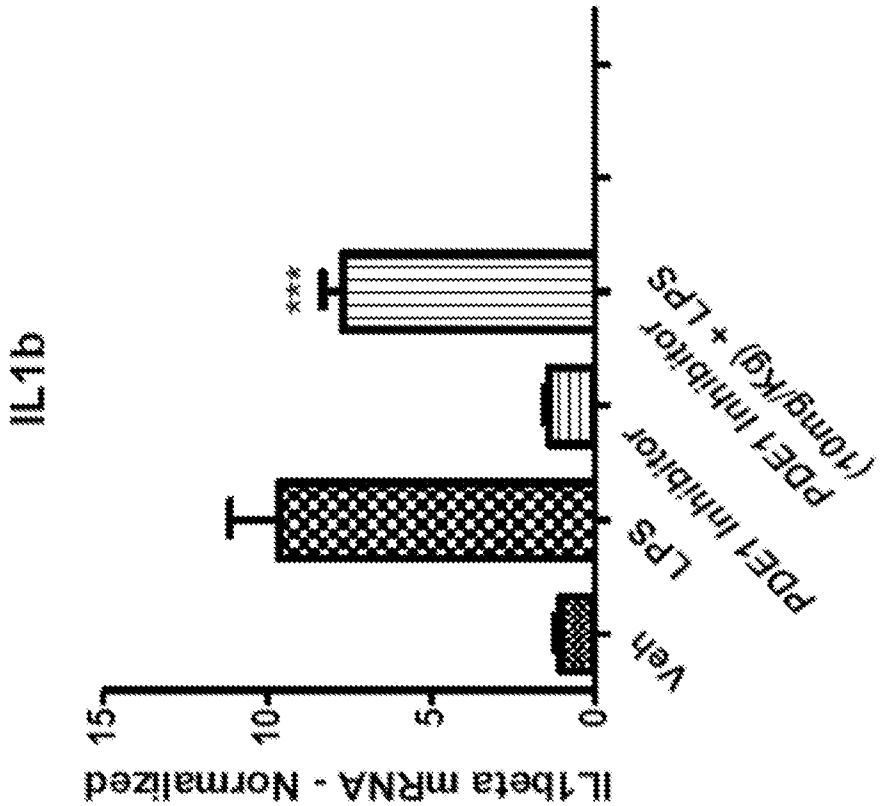

IL1b
IL.1beta mRNA - Normalized
15
10
5
0
***
Veh
LPS
PDE1 Inhibitor
PDE1 Inhibitor (10mg/kg) + LPS

FIG. 3

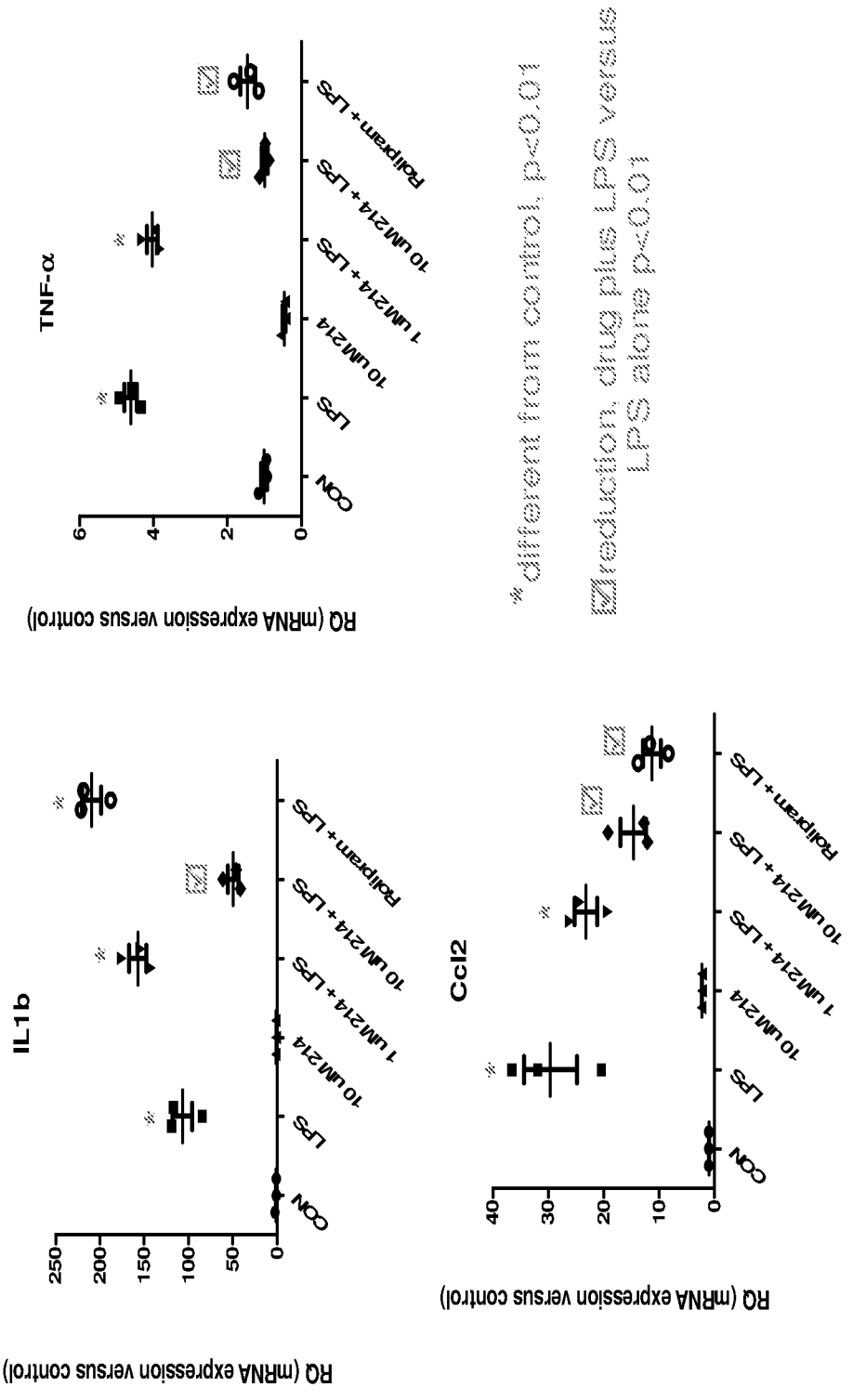

TNF-α
IL1b
Ccl2
CON
LPS
10 uM 214
1 uM 214 + LPS
10 uM 214 + LPS
Rolipram + LPS
RQ (mRNA expression versus control)
* different from control, p<0.01
Reduction, drug plus LPS versus LPS alone p<0.01

FIG. 4a

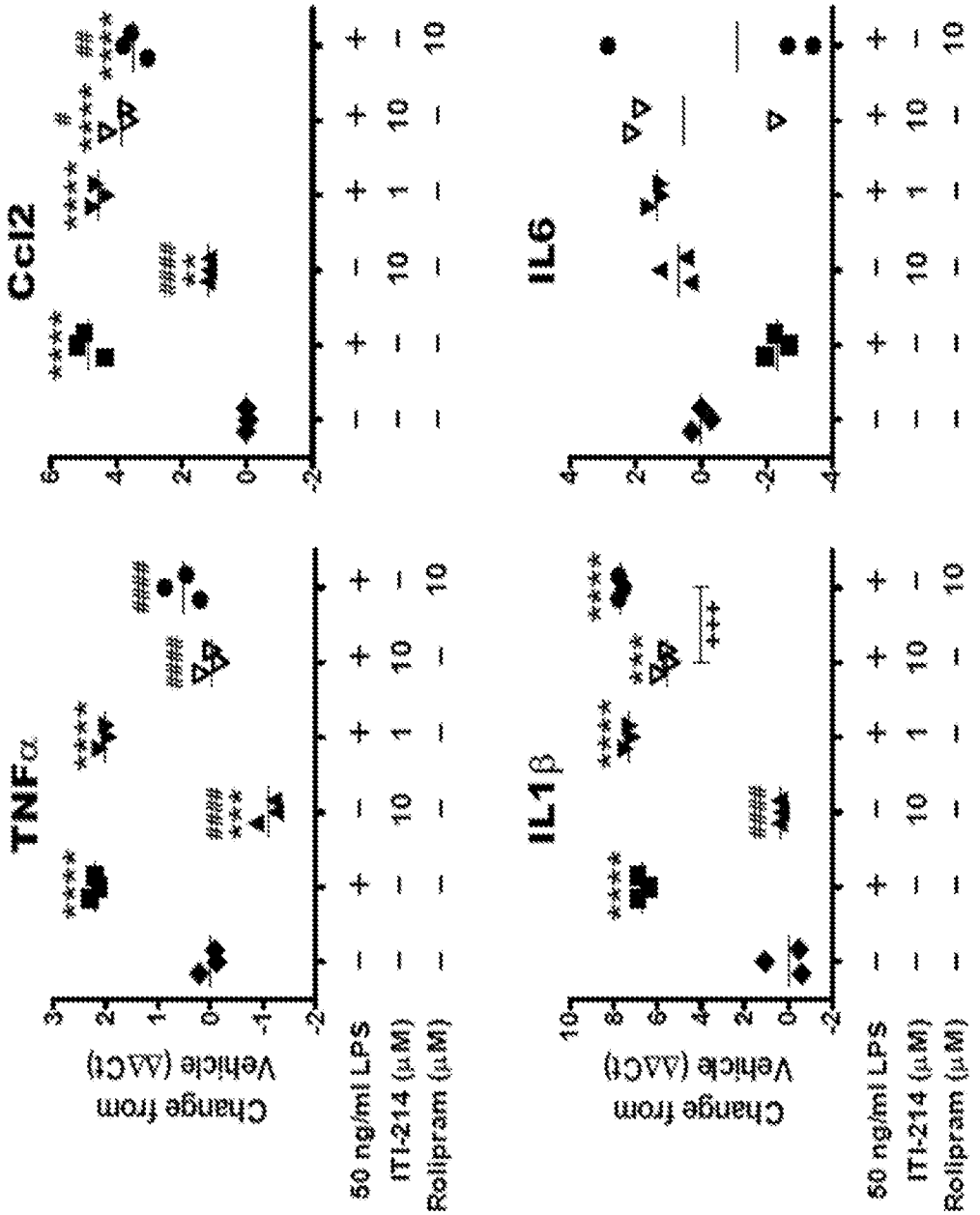

TNFα
Ccl2
IL1β
IL6
Change from Vehicle (ΔΔCt)
50 ng/ml LPS
ITI-214 (μM)
Rolipram (μM)

Fig. 4b

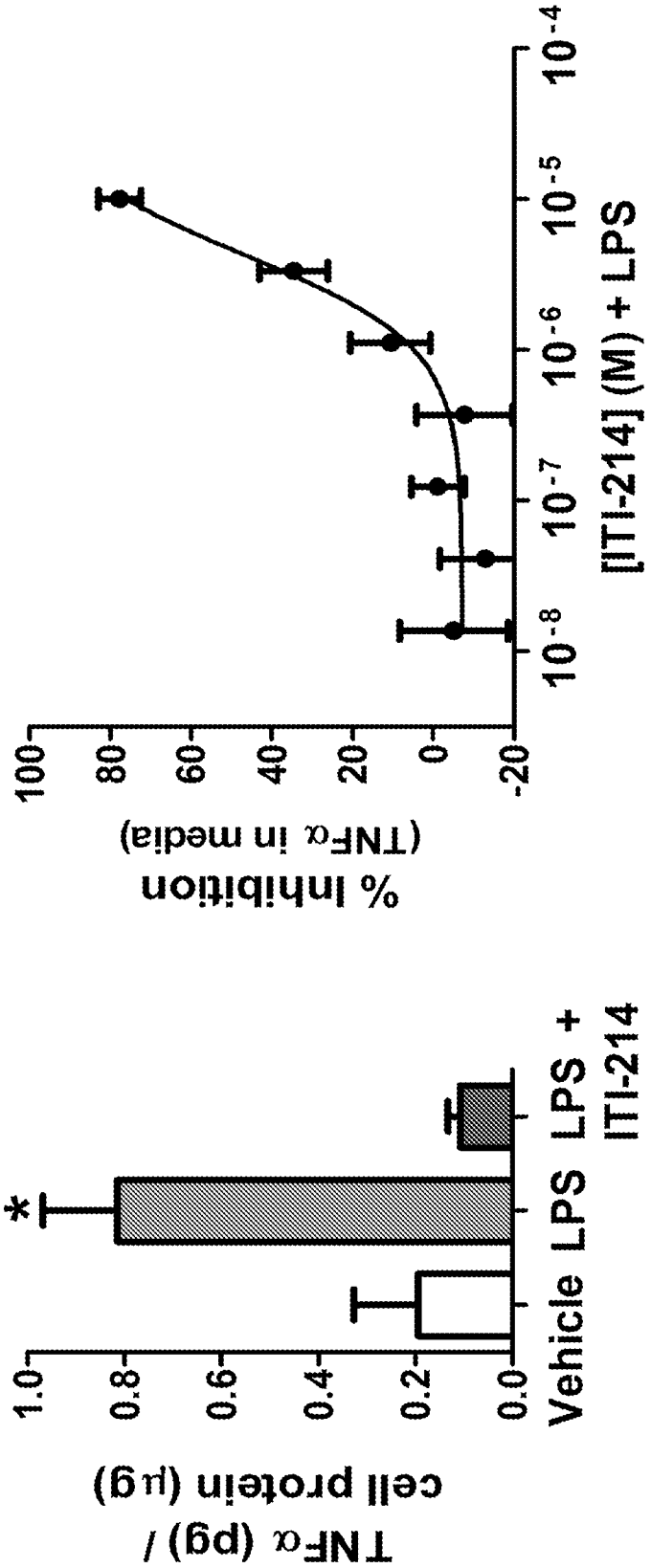

TNFα (pg) / cell protein (μg)
1.0
0.8
0.6
0.4
0.2
0.0
*
Vehicle
LPS
LPS + ITI-214
% Inhibition (TNFα in media)
100
80
60
40
20
0
-20
10-8
10-7
10-6
10-5
10-4
[ITI-214] (M) + LPS

FIG. 5

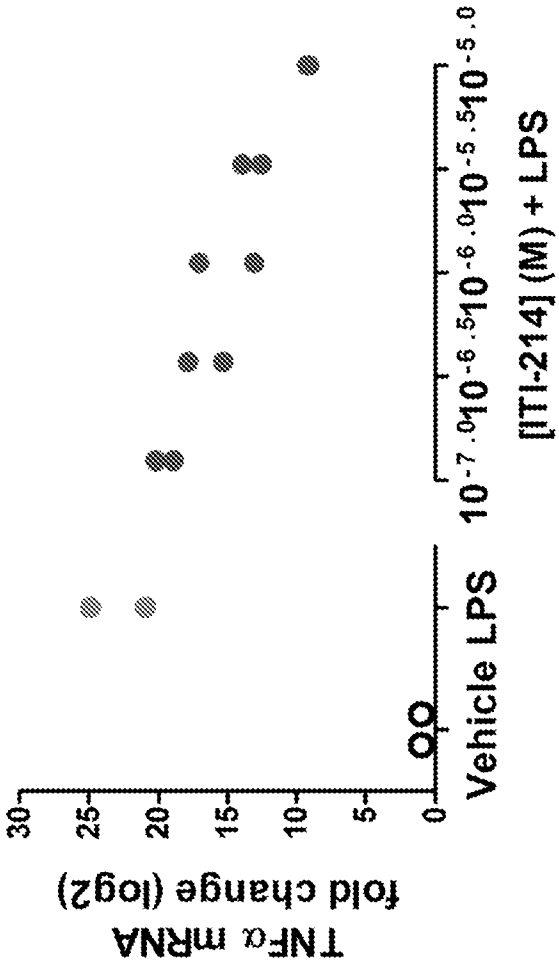

TNFα mRNA
fold change (log2)
30
25
20
15
10
5
0
Vehicle LPS
10-7.0
10-6.5
10-6.0
10-5.5
10-5.0
[ITI-214] (M) + LPS

FIG. 6

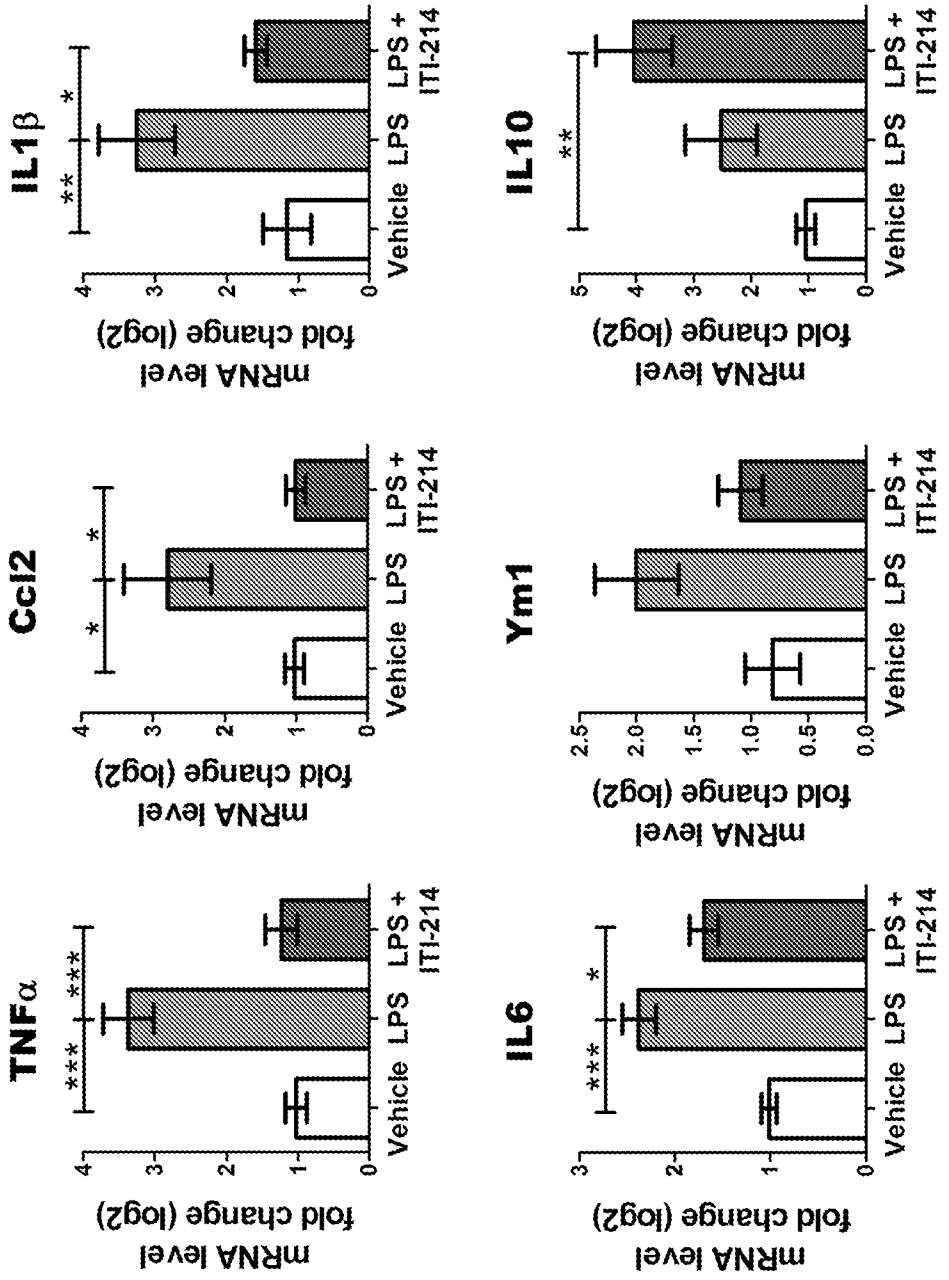

TNFα
IL1β
Ccl2
IL10
IL6
Ym1
mRNA level
fold change (log2)
Vehicle
LPS
LPS + ITI-214
*
**
***

FIG. 7

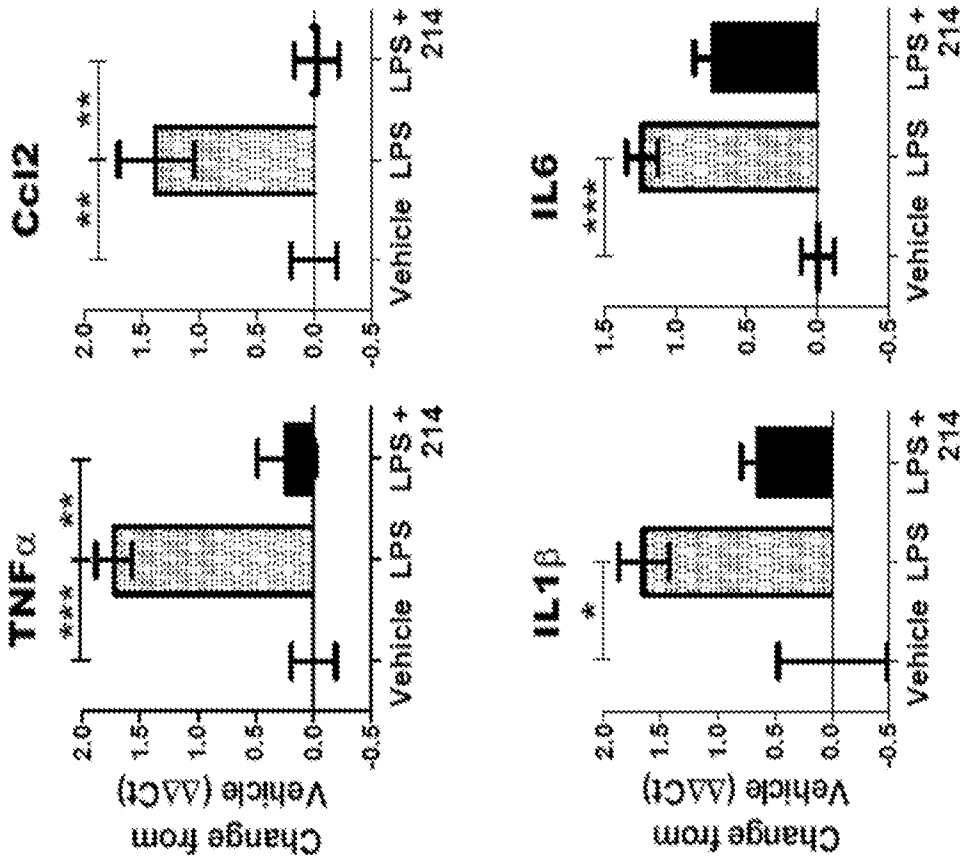

Ccl2
TNFα
IL6
IL1β
Change from Vehicle (ΔΔCt)
2.0
1.5
1.0
0.5
0.0
-0.5
Vehicle
LPS
LPS + 214
*
**
***

Fig. 8

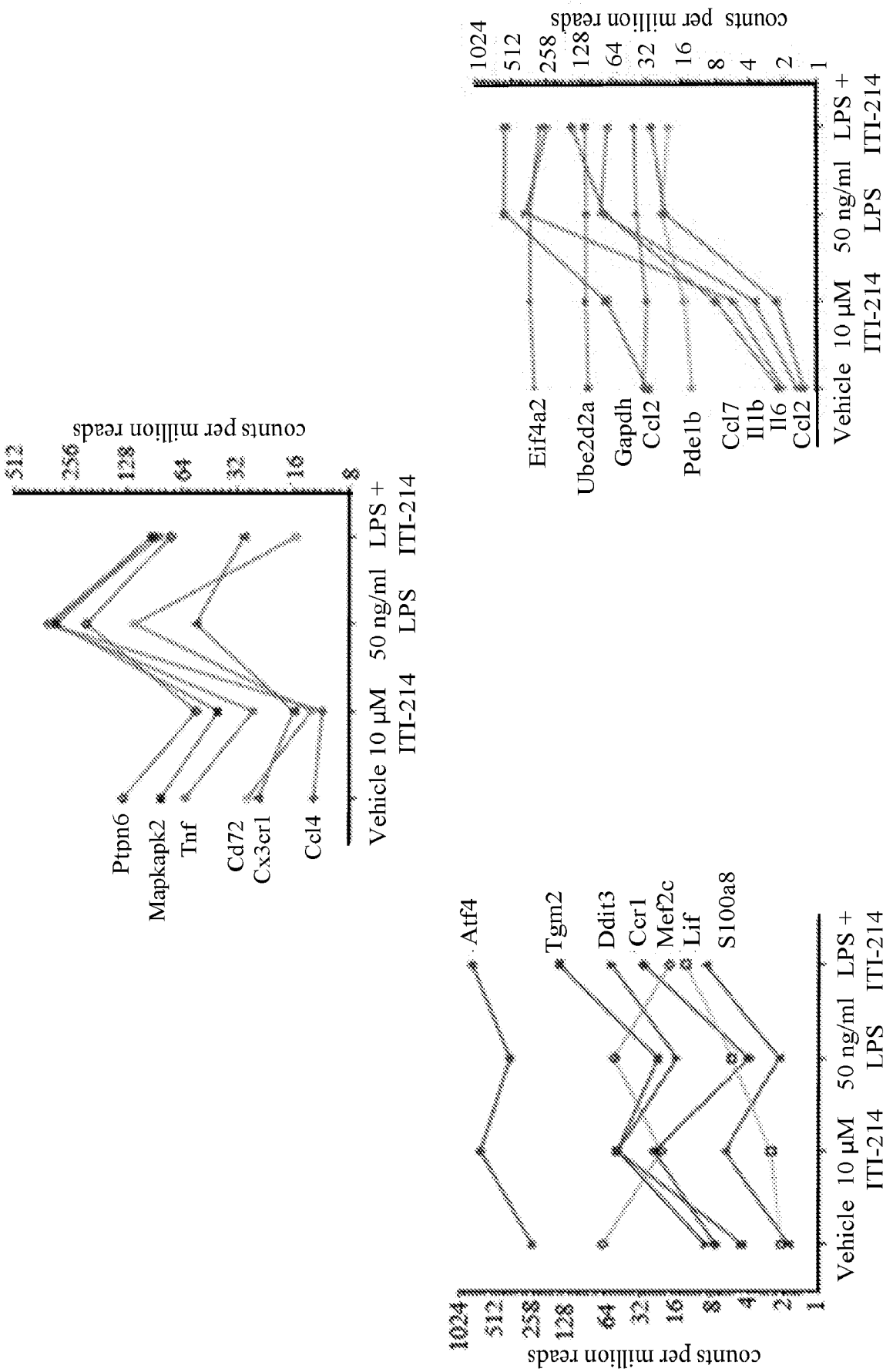

counts per million reads
1024
512
258
128
64
32
16
8
4
2
1
Atf4
Tgm2
Ddit3
Ccr1
Mef2c
Lif
S100a8
Vehicle 10 µM ITI-214
50 ng/ml LPS
LPS + ITI-214
counts per million reads
1024
512
258
128
64
32
16
8
4
2
1
Eif4a2
Ube2d2a
Gapdh
Ccl2
Pde1b
Ccl7
Il1b
Il6
Ccl2
Vehicle 10 µM ITI-214
50 ng/ml LPS
LPS + ITI-214
counts per million reads
512
256
128
64
32
16
8
Ptpn6
Mapkapk2
Tnf
Cd72
Cx3cr1
Ccl4
Vehicle 10 µM ITI-214
50 ng/ml LPS
LPS + ITI-214

Fig. 9

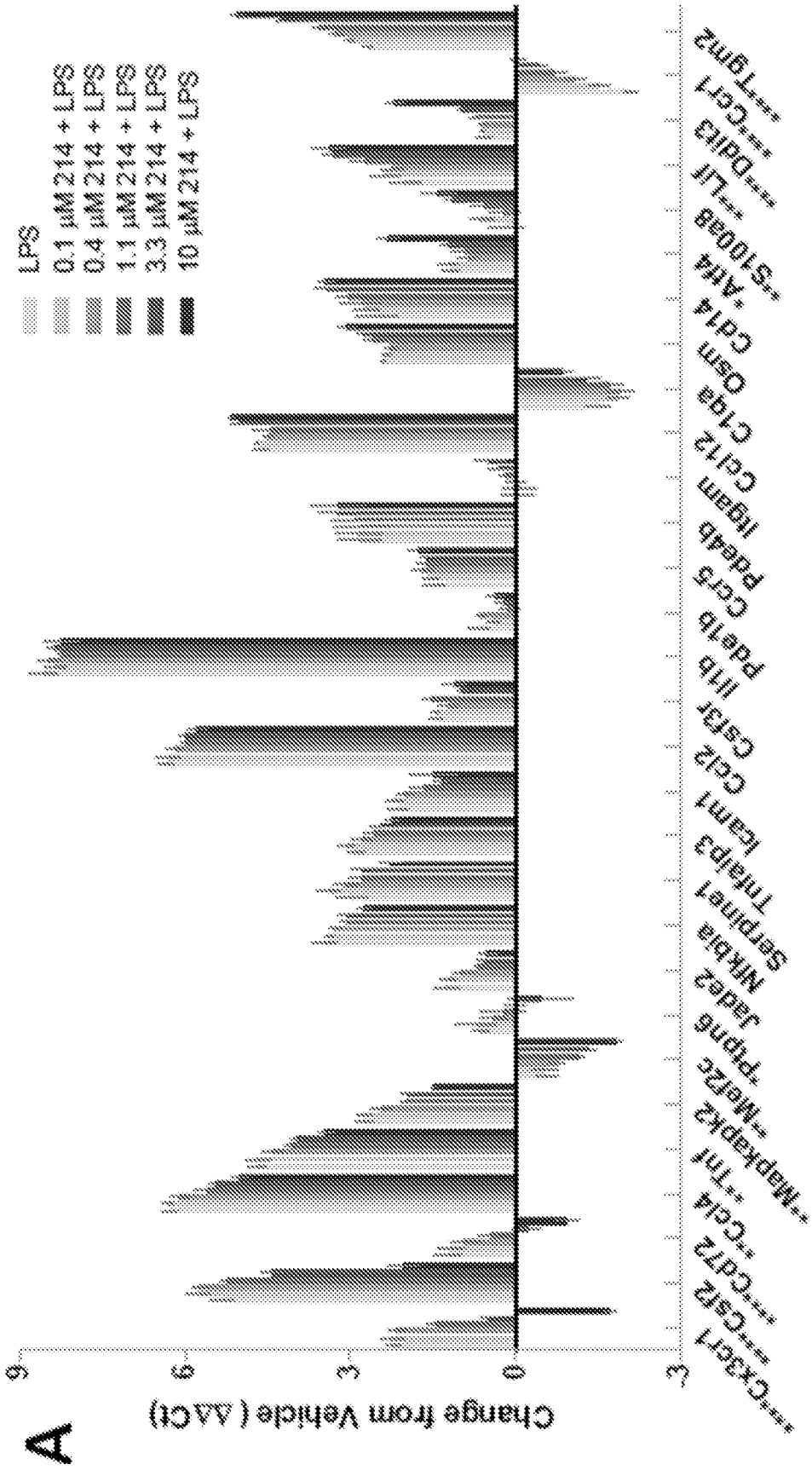

A
Change from Vehicle (ΔΔCt)
9
6
3
0
-3
LPS
0.1 μM 214 + LPS
0.4 μM 214 + LPS
1.1 μM 214 + LPS
3.3 μM 214 + LPS
10 μM 214 + LPS
***Cx3cr1
***Csf2
***Cd72
***Ccl4
***Tnf
***Mapkapk2
***Mef2c
*Pspn6
Jade2
Nfkbia
Serpine1
Tnfaip3
Icam1
Ccl2
Cstar
Il1b
Pde1b
Ccr5
Pdeab
Ibgam
Cd12
C1qa
Osm
Cd14
*Ahr
***S100a9
***Ltf
***Dclk3
***Ccr1
***Tgm2

Fig. 10a

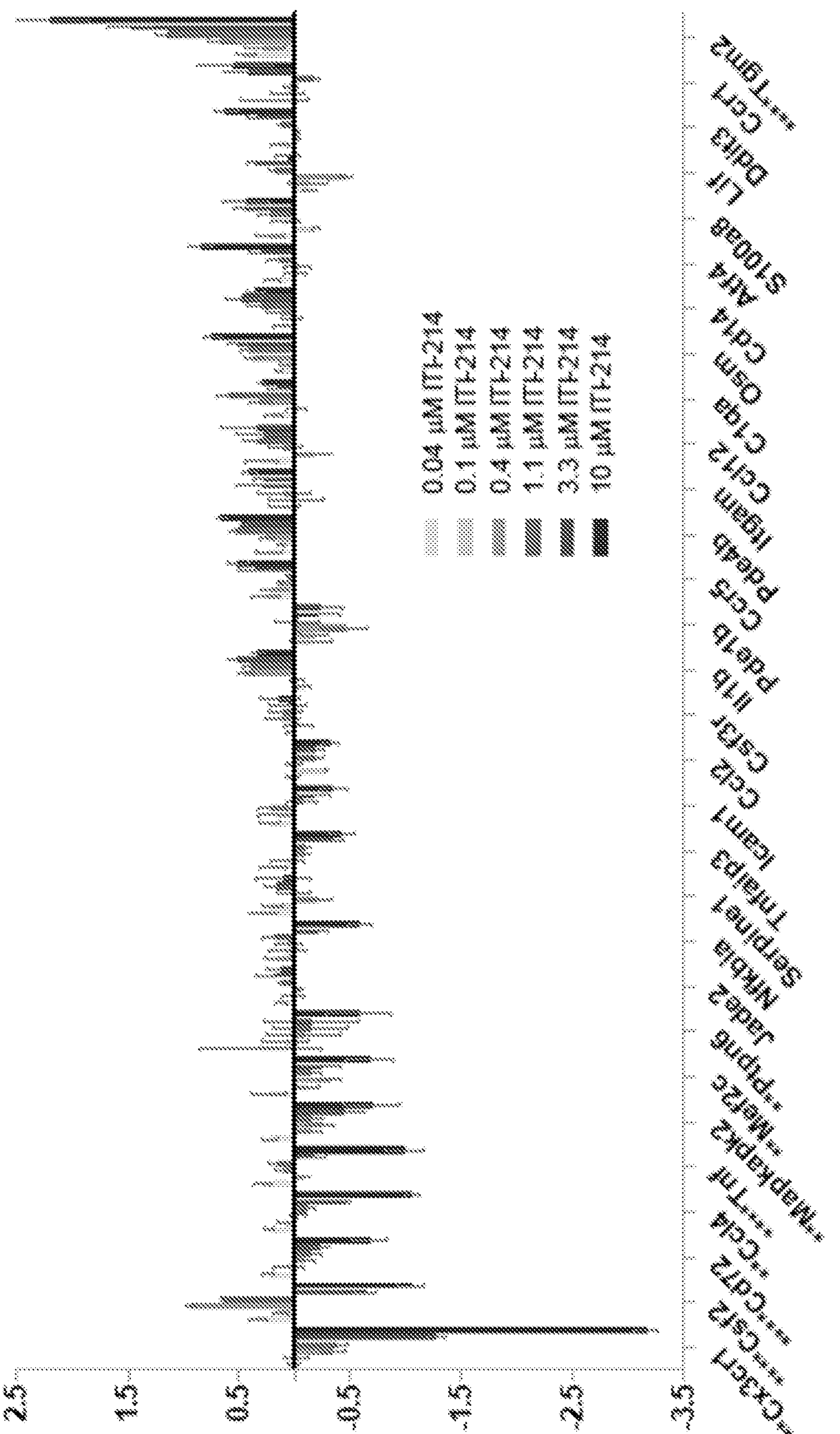

B
Change from Vehicle (ΔΔCt)
2.5
1.5
0.5
-0.5
-1.5
-2.5
-3.5
0.04 μM ITI-214
0.1 μM ITI-214
0.4 μM ITI-214
1.1 μM ITI-214
3.3 μM ITI-214
10 μM ITI-214
***Cx3cr1
***Csf2
***Cd72
**Ccl4
***Tnf
**Mapkapk2
**Mef2c
**Ptpn6
Jade2
Nfkbia
Serpine1
Tnfaip3
Icam1
Ccl2
Csf3r
Il1b
Pde1b
Ccr5
Pde4b
Itgam
Ccrl2
C1qa
Osm
Cd14
S100a8
Lif
Ddit3
Ccr1
***Tgm2

Fig. 10b

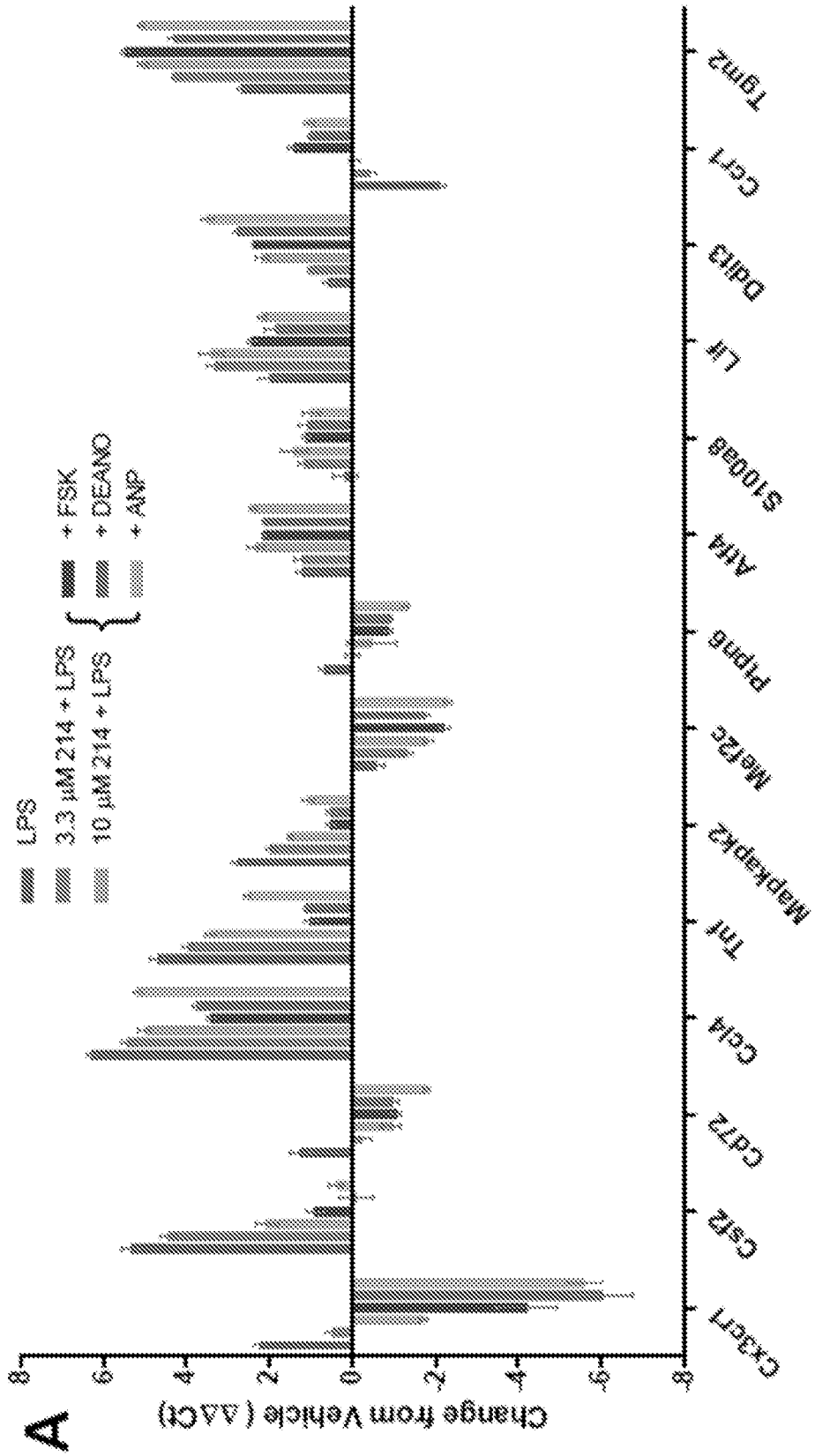

A
Change from Vehicle (ΔΔCt)
8
6
4
2
0
-2
-4
-6
-8
LPS
3.3 μM 214 + LPS
10 μM 214 + LPS
+ FSK
+ DEANO
+ ANP
Cx3cr1
Csf2
Ccl2
Ccl4
Tnf
Mapkapk2
Mef2c
Pdpn
Atf4
S100a9
Il1
Ddit3
Ccl1
Tgm2

Fig. 11a

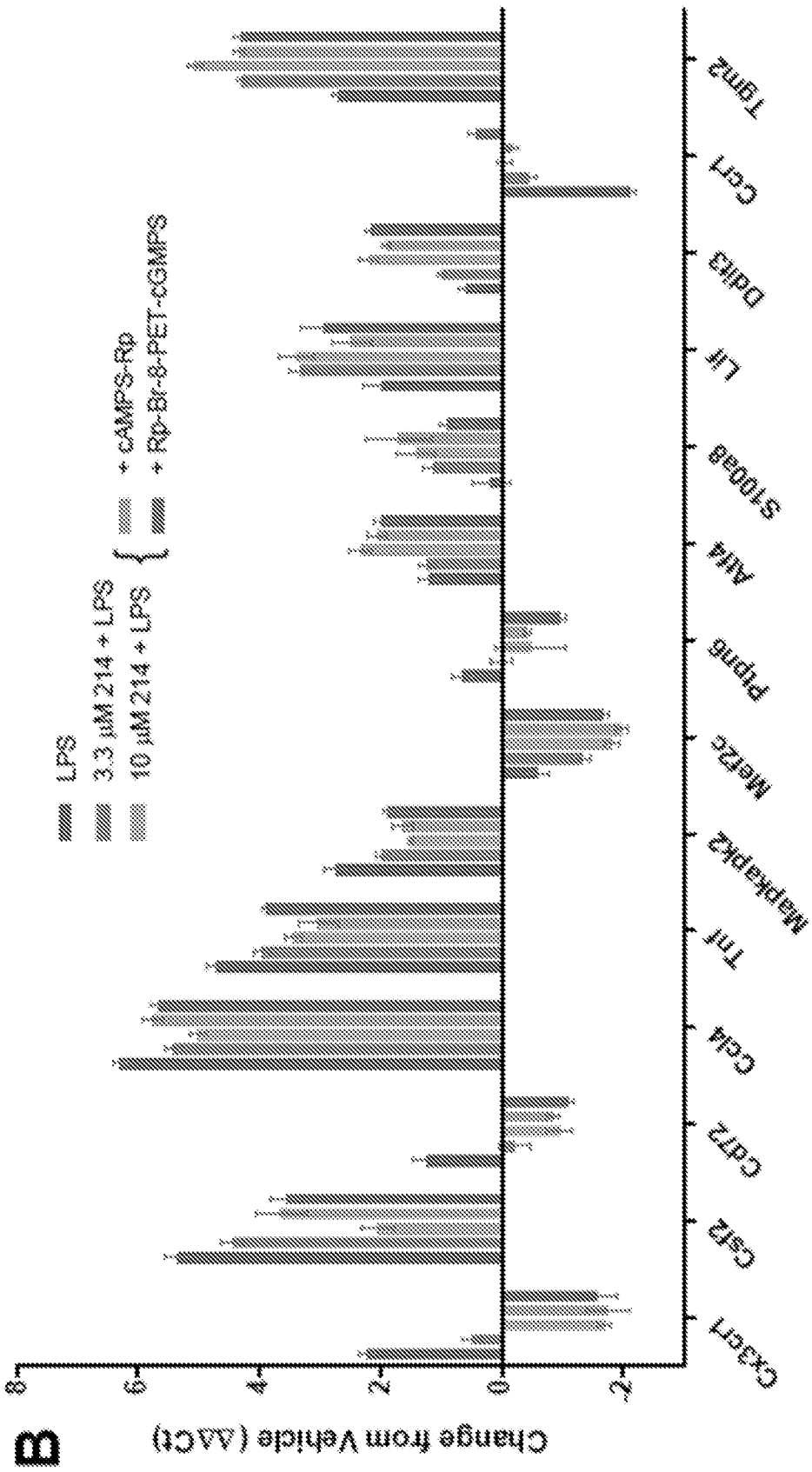
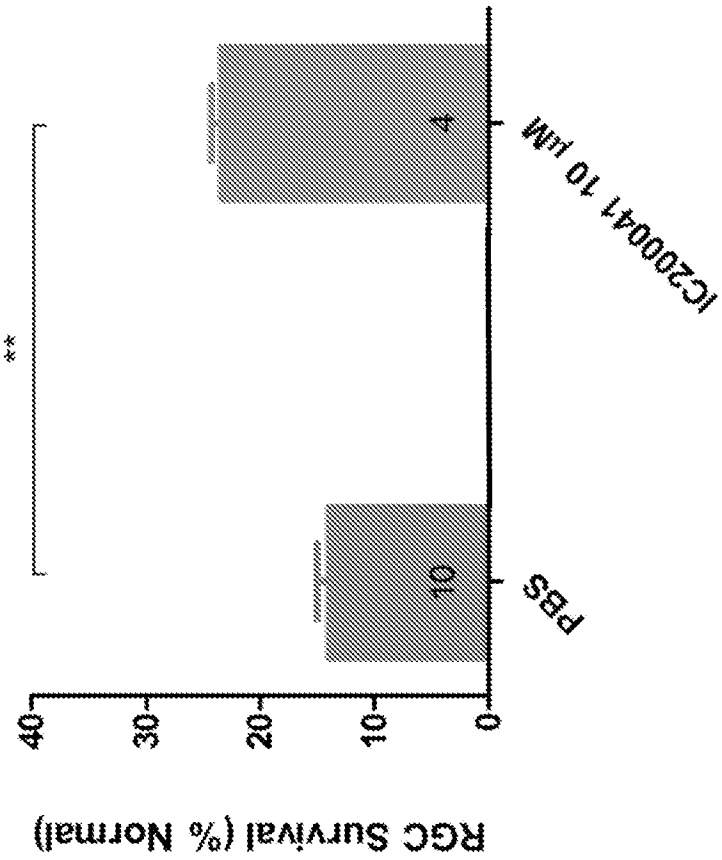

B
Change from Vehicle (ΔΔCt)
8
6
4
2
0
-2
LPS
3.3 μM 214 + LPS
10 μM 214 + LPS
+ cAMPS-Rp
+ Rp-8-Br-8-PET-cGMPS
Cx3cr1
Csf2
Ccl2
Ccl4
Tnf
Mapkapk2
Mefc
Ptpnb
Aim
S100a9
Lif
Ddit3
Ccr1
Tgm2

Fig. 11b



** p < 0.001 One-way ANOVA with Bonferroni post-hoc test

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51220

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/404, A61K 31/4709 (2017.01)
CPC - A61K 9/0043, A61 K45/06, A61K 31/405

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)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0148421 A1 (Li et al.) 29 May 2014 (29.05.2014) para [0002], [0007], [0008], [0526], [0630]	1-3
A	WO 2016/090380 A1 (Intra-Cellular Therapies, Inc.) 09 June 2016 (09.06.2016) entire document	1-3
A	US 2011/0015193 A1 (Eickmeier et al.) 20 January 2011 (20.01.2011) entire document	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2017	22 NOV 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/51220

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-11
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.