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(54) Title: COMPOUNDS AND METHODS

(57) Abstract: The subject matter generally relates to compounds and methods of treatment and/or prophylaxis of CNS diseases, disorders, and/or injuries. In one aspect, the subject matter relates to inhibitors of phosphodiesterase 1 (PDE1) as neuroprotective agents and/or neural regenerative agents. In a further aspect, the subject matter relates to individuals that are at risk for the development of CNS disease or disorder.

COMPOUNDS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

This International Application claims the benefit of earlier filed United States provisional applications US 62/051,735, filed September 17, 2014, and US 62/052,283, filed September 18, 2014, each of which is incorporated herein by reference in their entirities.

FIELD OF THE INVENTION

[0001] The field generally relates to compounds and methods of treatment and/or prophylaxis of central nervous system (CNS) diseases, disorders, and/or injuries. In one aspect, the field relates to inhibitors of phosphodiesterase 1 (PDE1) as neuroprotective agents and/or neural regenerative agents. In a further aspect, the field relates to preventing the development of a CNS disease or disorder in an individual at risk for the development of a CNS disease or disorder.

BACKGROUND OF THE INVENTION

[0002] Cyclic nucleotide phosphodiesterases (PDEs) downregulate intracellular cAMP and cGMP signaling by hydrolyzing these cyclic nucleotides to their respective 5'-monophosphates (5'AMP and 5'GMP). Eleven families of phosphodiesterases have been identified, but only PDEs in Family I, the Ca^{2+}/calmodulin-dependent phosphodiesterases (CaM-PDEs), which are activated by Ca^{2+}-calmodulin, have been shown to mediate the calcium and cyclic nucleotide (e.g. cAMP and cGMP) signaling pathways. The three known CaM-PDE genes, PDE1A, PDE1B, and PDE1C, are all expressed in central nervous system tissue. PDE1A is expressed throughout the brain with higher levels of expression in the CA1 to CA3 layers of the hippocampus and cerebellum and at a lower level in the striatum. PDE1A is also expressed in the lung and heart. PDE1B is predominately expressed in the striatum, dentate gyrus, olfactory tract and cerebellum, and its expression correlates with brain regions having high levels of dopaminergic innervation. Although PDE1B is primarily expressed in the central nervous system, it is also detected in the heart, is present in neutrophils and has been shown to be involved in

inflammatory responses of this cell. PDE1C is expressed in olfactory epithelium, cerebellar granule cells, striatum, heart, and vascular smooth muscle.

[0003] CaM-PDEs play a critical role in mediating signal transduction in brain cells, particularly within an area of the brain known as the basal ganglia or striatum. For example, NMDA-type glutamate receptor activation and/or dopamine D2 receptor activation result in increased intracellular calcium concentrations, leading to activation of effectors such as calmodulin-dependent kinase II (CaMKII) and calcineurin and to activation of CaM-PDEs, resulting in reduced cAMP and cGMP. Dopamine D1 receptor activation, on the other hand, leads to activation of adenylate cyclases, resulting in increased cAMP. This cyclic nucleotide in turn activates protein kinase A (PKA; cAMP-dependent protein kinase). Production of cGMP is known to occur in tissues involved in cognitive function through various stimulations such as nitric oxide production induced by high intra-cellular calcium levels and to subsequently activate protein kinase G (PKG; cGMP-dependent protein kinase). PKG and PKA phosphorylate downstream signal transduction pathway elements such as DARPP-32 (dopamine and cAMP-regulated phosphoprotein) and cAMP responsive element binding protein (CREB). Phosphorylated DARPP-32 in turn inhibits the activity of protein phosphates-1 (PP-1), thereby increasing the state of phosphorylation of substrate proteins such as progesterone receptor (PR), leading to induction of physiologic responses. D1 receptor signaling is disrupted in schizophrenia, contributing to cognitive impairment in the disease. The role of cAMP and cGMP in cognitive function has been well established in animal studies. Studies in rodents also have suggested that inducing cAMP and cGMP synthesis through activation of dopamine D1 or progesterone receptor enhances progesterone signaling associated with various physiological responses, including the lordosis response associated with receptivity to mating in some rodents. See Mani, et al., Science (2000) 287: 1053, the contents of which are incorporated herein by reference.

[0004] CaM-PDEs can therefore affect dopamine-regulated and other intracellular signaling pathways in the basal ganglia (striatum), including but not limited to nitric oxide, noradrenergic, neurotensin, CCK, VIP, serotonin, glutamate (e.g., NMDA

receptor, AMPA receptor), GABA, acetylcholine, adenosine (e.g., A2A receptor), cannabinoid receptor, natriuretic peptide (e.g., ANP, BNP, CNP), DARPP-32, and endorphin intracellular signaling pathways.

[0005] Phosphodiesterase (PDE) activity, in particular, phosphodiesterase 1 (PDE1) activity, functions in brain tissue as a regulator of locomotor activity and learning and memory. PDE1 is a therapeutic target for regulation of intracellular signaling pathways, preferably in the nervous system, including but not limited to a dopamine D1 receptor, dopamine D2 receptor, nitric oxide, noradrenergic, neurotensin, CCK, VIP, serotonin, glutamate (e.g., NMDA receptor, AMPA receptor), GABA, acetylcholine, adenosine (e.g., A2A receptor), cannabinoid receptor, natriuretic peptide (e.g., ANP, BNP, CNP), endorphin intracellular signaling pathway and progesterone signaling pathway. For example, inhibition of PDE1B should act to potentiate the effect of a dopamine D1 agonist by protecting cGMP and cAMP from degradation, and should similarly inhibit dopamine D2 receptor signaling pathways, by inhibiting PDE1 activity that is a consequence of D2 receptor-mediated increases in intra-cellular calcium. Chronic elevation in intracellular calcium levels is linked to cell death in numerous disorders, particularly in neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's Diseases and in disorders of the circulatory system leading to stroke and myocardial infarction. PDE1 inhibitors are therefore potentially useful in diseases characterized by reduced dopamine D1 receptor signaling activity, such as Parkinson's disease, restless leg syndrome, depression, narcolepsy and cognitive impairment such as cognitive impairment associated with schizophrenia. PDE1 inhibitors are also useful in diseases that may be alleviated by the enhancement of progesterone-signaling such as female sexual dysfunction.

[0006] Additionally, neurogenesis is a vital process in the brains of animals and humans, whereby new nerve cells are continuously generated throughout the life span of the organism. The newly formed cells are able to differentiate into functional cells of the central nervous system and integrate into existing neural circuits in the brain. Neurogenesis is known to persist throughout adulthood in two regions of the mammalian

brain: the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus of the hippocampus. In these regions, multipotent neural progenitor cells (NPCs) continue to divide and give rise to new functional neurons and glial cells. It has been shown that a variety of factors can stimulate adult hippocampal neurogenesis, e.g., adrenalectomy, voluntary exercise, enriched environment, hippocampus dependent learning and antidepressants. Other factors, such as adrenal hormones, stress, age and drugs of abuse negatively influence neurogenesis.

[0007] While the importance of neurogenesis cannot be overstated, the failure of axons to regenerate after spinal cord injury still remains one of the greatest challenges facing both medicine and neuroscience. Unlike the myelinated axons of the peripheral nervous system, myelinated axons of the central nervous system do not regenerate after being severed. An important development, however, has been the identification of inhibitory proteins in the myelin sheaths that surround CNS axons. Certain bioactive molecules appear to inhibit neurite outgrowth, leading to failure of CNS neuron regeneration. Myelin contains a number of proteins that have been shown to inhibit neurite process outgrowth. NogoA, a member of the reticulon family, was the first protein identified as a neurite outgrowth inhibitor. It is expressed by oligodendrocytes and some neurons, and can be found both intracellularly and on the cell surface (particuarly on the myelin sheaths of axons). Other proteins that can contribute to inhibition of axon regeneration include myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp) and the proteoglycan versican.

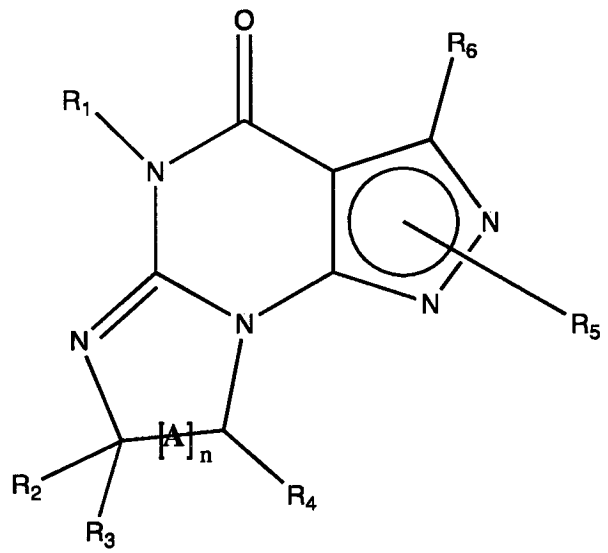
[0008] Thus, it appears that the CNS environment limits axonal regeneration after injury. Indeed, CNS myelin has been identified as a major factor contributing to regenerative failure. Evidence exists that shows that CNS proteins present in the myelin sheath inhibit axonal growth and regeneration.

[0009] Various strategies have been proposed for overcoming the inhibition of axonal regeneration. One strategy that has been effective has been to elevate the levels of intracellular cAMP. This can be accomplished in several ways, such as: a peripheral

conditioning lesion, administration of cAMP analogues, priming with neurotrophins or treatment with the phosphodiesterase inhibitor rolipram (PDE4 inhibitor). The effects of cAMP may be transcription dependent, and cAMP-mediated activation of CREB may lead to upregulation and expression of genes such as arginase I and interleukin-6. The products of these genes are believed to promote axonal regeneration, which raises the possibility that other cAMP-regulated genes could yield additional agents that would be beneficial in the treatment of spinal cord injury. However, with regard to increasing the expression of IL-6, one significant disadvantage to this mechanism of action may be that IL-6 is a potentially harmful pro-inflammatory cytokine, meaning, it is possible that high levels of IL-6 could actually exacerbate the inflammation that occurs after spinal cord injury which could then lead to increase in cell death. Indeed, a factor supporting this concern is that IL-6 transgenic mice have been observed to have extensive astrogliosis, neurodegeneration, and breakdown of the blood brain barrier.

SUMMARY OF THE INVENTION

[00010] The invention provides for a compound of Formula V:



Formula V

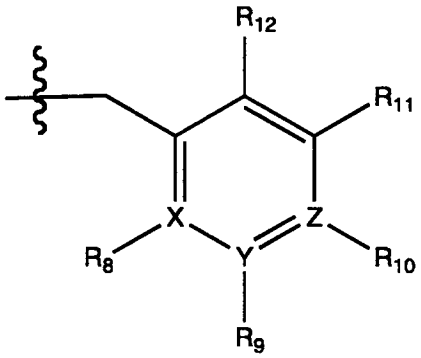
wherein

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

(ii) R_4 is H 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);

(iii) 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 C, and R_8, R_9, R_{11} and R_{12} are H, and R_{10} is halogen (e.g. chloro), or heteroaryl optionally substituted with halogen, alkyl, haloalkyl, hydroxy or carboxy (e.g., pyridyl or 2-halopyridyl, (for example, pyrid-2-yl, 5-fluoropyrid-2-yl or 6-fluoropyrid-2-yl)); and

(iv) R_6 is H, C_{1-4}alkyl (e.g. methyl, ethyl or propyl), arylamino optionally substituted with C_{1-4}alkyl or halogen (e.g., phenylamino or 4-fluorophenylamino), or thioC_{1-4}alkyl (e.g., thioethyl); and

(v) n=0;

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

[00011] In a further aspect, the invention contemplates that the PDE1 inhibitors (e.g., Formula V) are compounds of Formula V according to any of the following formulae:

1.1 The compound of Formula V, wherein R_1 is methyl;

1.2 The compound of Formula V or 1.1, wherein R_2 and R_3 are C_{1-4} alkyl;

1.3 The compound of Formula V or any of 1.1-1.2, wherein R_2 and R_3 are both methyl;

1.4 The compound of Formula V or any of 1.1-1.3, wherein R_{10} is heteroaryl optionally substituted with halogen;

1.5 The compound of Formula V or any of 1.1-1.4, wherein R_{10} is pyrid-2-yl;

1.6 The compound of Formula V or any of 1.1-1.4, wherein R_{10} is 5-fluoro-pyrid-2-yl;

1.7 The compound of Formula V or any of 1.1-1.4, wherein R_{10} is 6-fluoro-pyrid-2-yl;

1.8 The compound of Formula V or any of 1.1-1.7, wherein R_6 is C_{1-4}alkyl;

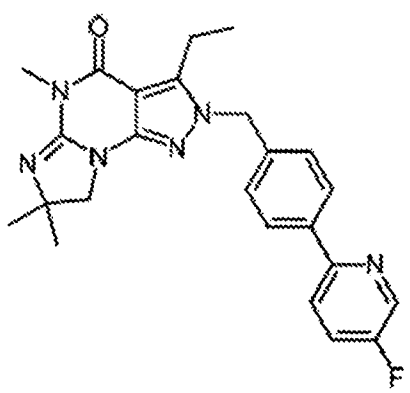
1.9 The compound of Formula V or any of 1.1-1.8, wherein R_6 is ethyl;

1.10 The compound of Formula V or any of 1.1-1.8, wherein R_6 is propyl;

1.11 The compound of Formula V or any of 1.1-1.7, wherein R_6 is arylamino optionally substituted with C_{1-4}alkyl or halogen;

1.12 The compound of Formula V or any of 1.1-1.7, wherein R_6 is 4-fluorophenylamino;

1.13 Any of the preceding formulae wherein the compound is selected from the group consisting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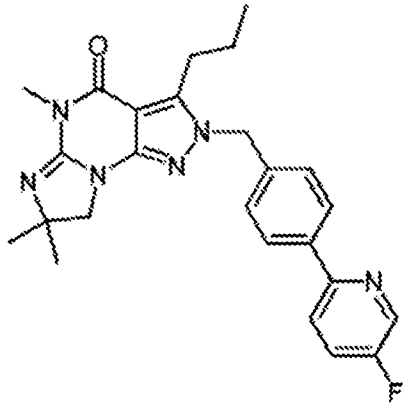
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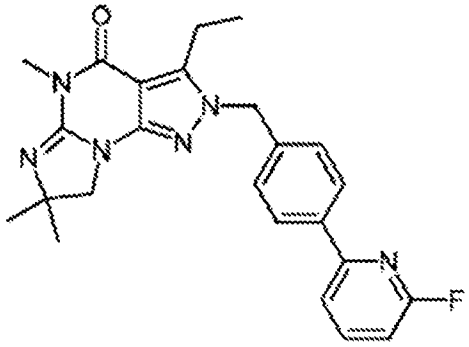
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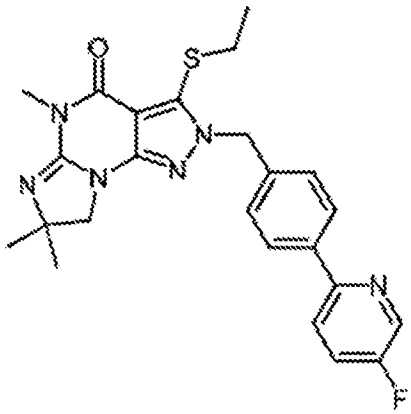
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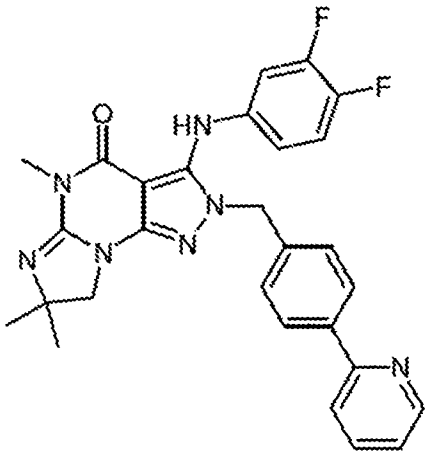
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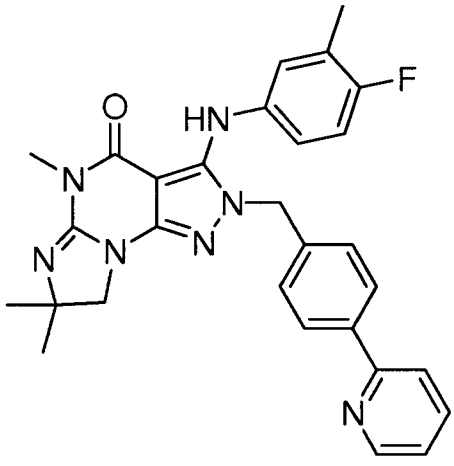
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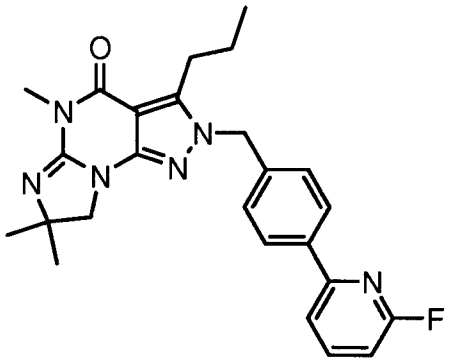
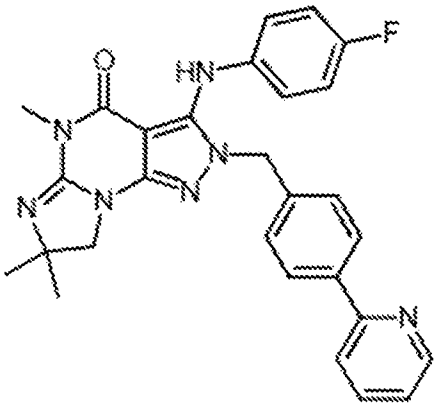
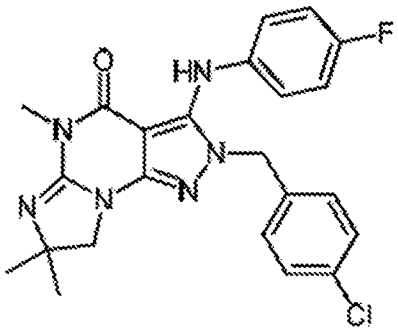


or

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

1.14 Any of the preceding formulae wherein the compound is selected from a group consisting of:





, or

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

[00012] In one aspect, selective PDE1 inhibitors of the any of the preceding formulae (e.g., Formula V or 1.1 – 1.14) are compounds that inhibit phosphodiesterase-mediated (e.g., PDE1-mediated, especially PDE1A or PDE1C-mediated) hydrolysis of cGMP, e.g., the preferred compounds have an IC50 of less than 1 M, preferably less than 500 nM, and more preferably less than 50 nM, in an immobilized-metal affinity particle reagent PDE assay, in free or salt form.

[00013] It is one advantage of the present invention that a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) may act as a neuroprotective agent and/or

neuroregenerative agent. In the event of a CNS injury (e.g., spinal cord injury), disease, or disorder, the compounds and methods disclosed herein may be employed to aid or enhance neurite outgrowth and axonal regeneration even in the presence of inhibitors of axonal regeneration.

[00014] Without being bound by any particular theory, it is believed that at least one advantage of the present invention is that the administration of a PDE1 inhibitor (e.g., any compound of Formula V or 1.1-1.14) may act to increase levels of intracellular cAMP and initiate the transcription of genes that are necessary for overcoming the inhibition of axonal regeneration and promoting neurite outgrowth and/or axonal regeneration in the case of a CNS disease, disorder, or injury. For instance, increased intracellular cAMP, such as would result from PDE1 inhibition, would lead to increased activity of cAMP-dependent proteins, such as protein kinase C (PKC).

[00015] Furthermore, it is believed that the administration of a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) may elevate the intracellular levels of both cAMP and cGMP. Without being bound by theory, this rise in both cAMP and cGMP may serve to counterbalance the potentially detrimental effects that may be associated with chronically elevated levels of intracellular calcium. It has been observed that elevated levels of intracellular calcium may be associated with the development of various degenerative diseases. For instance, one possible explanation is that elevated levels of intracellular calcium (e.g., chronically elevated levels of intracellular calcium) leads to the activation of PDE1, which then stimulates cAMP hydrolysis. The decreased concentration of cAMP would then deactivate cAMP-dependent proteins such as protein kinase C (PKC).

[00016] However, without being bound by any theory, it is believed that another potential benefit of the administration of a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) is an increase in intracellular cGMP. This increase in intracellular cGMP may lead to an increase in the activity of PKG, preventing a further rise in intracellular calcium levels. Thus, without being bound by any theory, the administration of a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) could have the

dual benefit of, for example, playing a beneficial role in axonal regeneration (and/or neuroprotection) while simultaneously decreasing the deleterious effects that may be associated with elevated intracellular calcium levels.

[00017] In one embodiment the invention comprises compositions and methods to treat or prevent a CNS disease, disorder, or injury (e.g., spinal cord injury, e.g., spinal muscular atrophy, e.g., motor neuron injury), wherein the method comprises administration of an effective amount of a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) to modulate intracellular levels of cAMP and/or cGMP. In one embodiment, this increase in intracellular cAMP is neuroprotective and/or aids in the increase or stimulation of neurogenesis (e.g., the PDE1 inhibitor increases neurite outgrowth and/or axonal regeneration).

[00018] In still a further embodiment, the invention comprises compositions and methods to treat or prevent injuries to the peripheral nervous system (PNS) wherein the method comprises administration of a PDE1 inhibitor to increase intracellular levels of cAMP and/or cGMP which, either directly or indirectly, increases nerve regeneration and/or is protective against further nerve damage.

[00019] In one embodiment the invention comprises compositions and methods to prevent a CNS disease or disorder in a subject that is at risk for developing said disease or disorder, wherein the method comprises:

1.) Obtaining a CNS sample from the subject;

2.) Measuring the levels of intracellular calcium from the sample;

3.) Comparing the levels of intracellular calcium in the biological sample to a reference standard;

4.) Determining whether a patient is at risk for developing a CNS disease or disorder based upon the level of intracellular calcium compared to the reference standard;

5.) Administering a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) to a subject based upon the subject's levels of intracellular calcium (e.g., administration of a PDE1 inhibitor to a subject because they have elevated intracellular calcium levels compared to the reference standard).

[00020] If not otherwise specified or clear from context, the following terms herein have the following meanings:

(a) "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.

(b) "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. Where the cycloalkyl optionally contains one or more atoms selected from N and O and/or S, said cycloalkyl may also be a heterocycloalkyl.

(c) "Heterocycloalkyl" is, unless otherwise indicated, 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, 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.

(d) "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).

(e) "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.

(f) 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, for example, $-C_6H_4-CH_2-$ or $-CH_2-C_6H_4-$.

[00021] 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

[00022] Compounds of the Invention, encompassing any of the compounds disclosed herein, 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.

[00023] Compounds of the Invention may in some cases also exist in prodrug form. A prodrug form is a 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 carboxylic 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 alcohol HO-C_{1-4}alkyl. As will be appreciated the term thus embraces conventional pharmaceutical prodrug forms.

[00024] 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.

Methods of Making Compounds of the Invention

[00025] 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 are 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.

[00026] 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, US 2014/0194396, PCT/US14/30412, and each reference is herein incorporated by reference in its entirety.

[00027] 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.

[00028] It is also intended that the Compounds of the Invention encompass their stable and unstable isotopes. That is, the Compounds of the Invention embrace the replacement or enrichment of any atom, or more than one atom, of the structure by any stable or unstable isotopic variant of that atom. Isotopes are atoms of the same element that contain varying numbers of neutrons. An isotopic variant is any isotope of any element other than its naturally most abundant isotope. An isotopic variant will contain one or more additional, or one or more fewer, neutrons compared to the most naturally abundant nuclide of the same element. Isotopes may either be stable (non-radioactive) or unstable (radioactive). For example, the most naturally abundant nuclide of carbon is ^{12}C, and one known stable isotope of carbon is ^{13}C. Isotopes of an element generally share the same characteristic electronic and chemical properties. 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 one or more atomic positions of the Compounds of the Invention may be replaced with (or enriched in) deuterium. Examples of known stable isotopes include, but are not limited to, deuterium (^{2}H), ^{13}C, ^{15}N, and ^{18}O. Examples of known unstable isotopes include ^{3}H, ^{123}I, ^{131}I, ^{125}I, ^{11}C, ^{18}F. Unstable isotopes may be useful for radio-imaging and/or pharmacokinetic studies of the compounds of the invention. One or more atomic positions in a Compound of the Invention may be replaced with or enriched

in any known isotopic variant. Natural sources of chemicals and reagents are not generally isotopically pure, so that Compounds of the Invention made by traditional chemical methods will generally have some normal, natural variation in isotopic abundance. For example, the natural abundance of the element carbon consists approximately of 98.93% ^{12}C and 1.07% ^{13}C. Therefore, Compounds of the Invention made by traditional chemical means will typically consist of about 98.93% ^{12}C and 1.07% ^{13}C at each carbon atom of the structure. Enrichment refers to the presence of more than the natural abundance of a minor isotope in a chemical structure. Thus, for example, a Compound of the Invention may be enriched for the presence of ^{13}C at one or more carbon atom positions. As used herein, "replacement" refers to enrichment of an isotopic variant of greater than about 95%.

[00029] Melting points are uncorrected and "dec" indicates decomposition. Temperatures 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 presented using delta values of the 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.

[00030] Terms and abbreviations:

BOC = *tert*-butoxycarbonyl

BOP = Benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate

BuLi = n-butyllithium

Bu^{t}OH = *tert*-butyl alcohol,

CAN = ammonium cerium (IV) nitrate,

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene,

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,

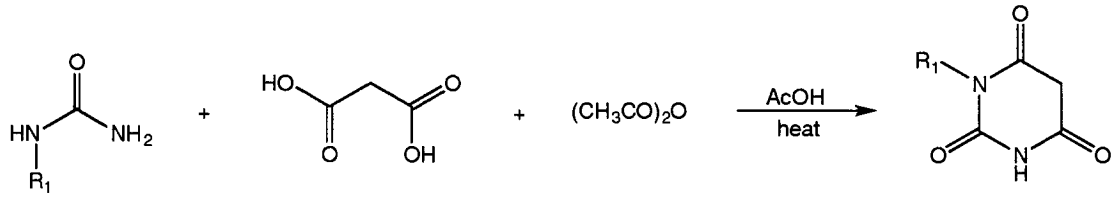
TFA = trifluoroacetic acid,

TFMSA = trifluoromethanesulfonic acid

THF = tetrahedrofuran.

[00031] The synthetic methods useful in this invention are illustrated below. The definitions for the R groups are as set forth above for any of Formulae V or 1.1-1.14, unless otherwise indicated.

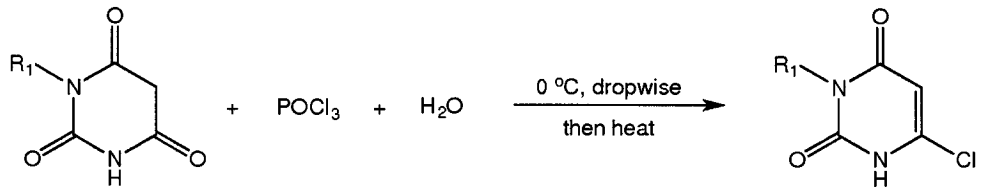
[00032] Intermediate compounds of formula IIb can be prepared by reacting a compound of formula IIa with malonic acid and acetic anhydride in acetic acid, optionally with heating (e.g., to about 90°C for about 3 hours):



(IIa) (IIb)

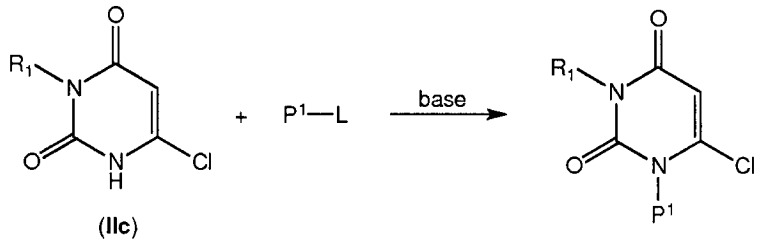
wherein R_1 is C_{1-4} alkyl, e.g., methyl.

[00033] Intermediates of formula IIc can be prepared by reacting a compound of formula IIb with a chlorinating compound such as $POCl_3$, optionally with small amounts of water and/or heating (e.g., heating to about 80°C for about 4 hours):



(IIb) (IIc)

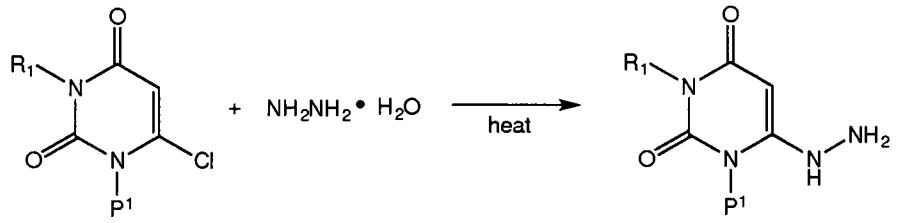
[00034] Intermediates of formula IId may be prepared by reacting compounds of formula IIc with, for example, a reagent P^1-L in a solvent such as DMF, with a base such as potassium carbonate, sodium bicarbonate, cesium carbonate, sodium hydroxide, triethylamine, diisopropylethylamine or the like, at room temperature or with heating:



(IIc) (IId)

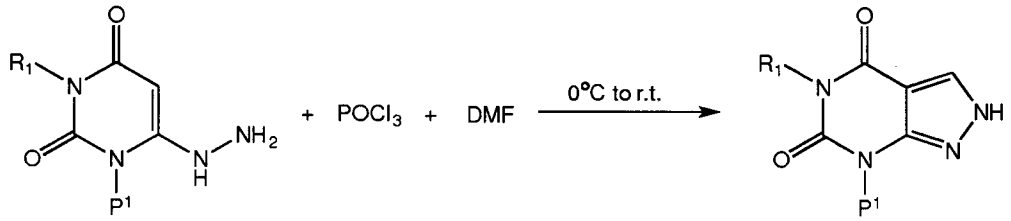
wherein P^1 is a protective group (e.g., PMB or BOC); and L is a leaving group such as a halogen, mesylate, or tosylate. Preferably, P^1 is PMB and the base is potassium carbonate.

[00035] Intermediates of formula IIe may be prepared by reacting compounds of formula IId with hydrazine or hydrazine hydrate in a solvent such as methanol, preferaby with heating (e.g. reflux for about 4 hours):



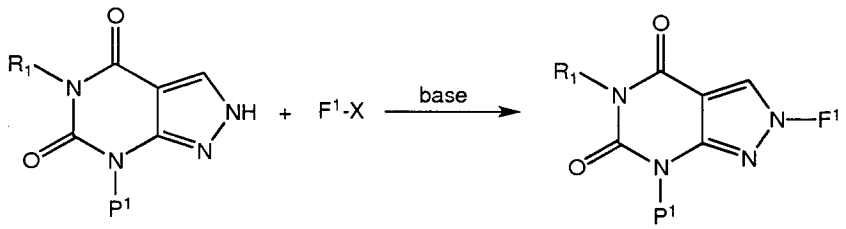
(IId) (IIe)

[00036] Intermediates of formula IVa may be prepared by reacting compound of formula IIe with $POCl_3$ and DMF:



(IIe) (IVa)

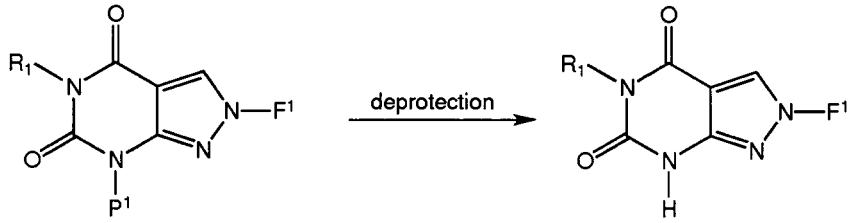
[00037] Intermediates of formula IVb may be prepared by reacting a compound of formula IVa with a reagent of formula F^1–X in a solvent such as DMF with a base such as potassium carbonate at room temperature:



(IVa) (IVb)

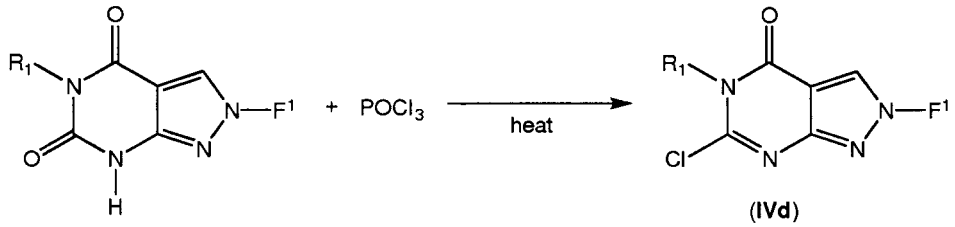
wherein F^1 is a protecting group (e.g., a substituted benzyl group, such as 4-bromobenzyl), and X is a halogen (e.g., Br).

[00038] Intermediates of formula IVc may be prepared from compounds of formula IVb by removing the protective group P^1 using an appropriate method. For example, if P^1 is a PMB group, then it can be removed with TFA/TFMSA at ambient or elevated temperature, whereas if P1 is BOC, then it can be removed using an acid such as TFA or aqueous hydrochloric acid:



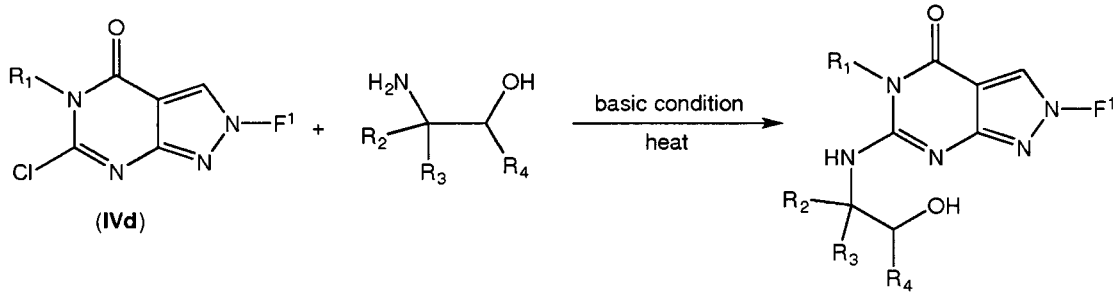
(IVb) (IVc)

[00039] Intermediates of formula IVd can be prepared by reacting a compound of formula IVc with a chlorinating compound such as $POCl_3$, optionally with heating (e.g., reflux for 2 days or more, or microwave irradiation at 150-200°C for 5-10 minutes in a sealed vial):



(IVc) (IVd)

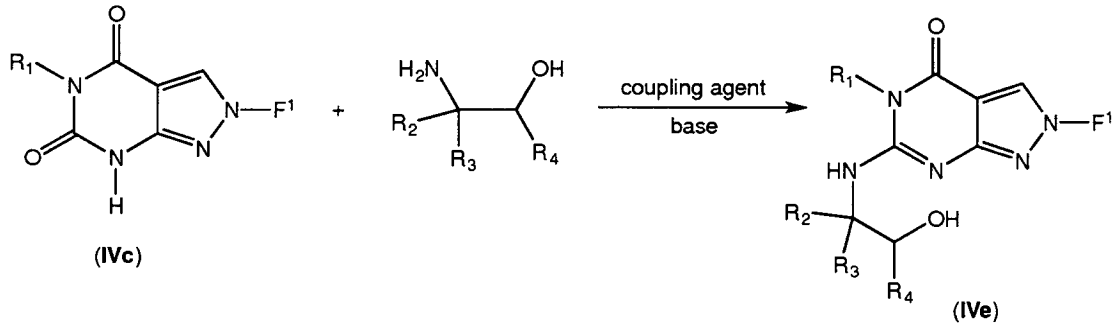
[00040] Intermediates of formula IVe can be prepared by reacting a compound of formula IVd with an amino alcohol under basic condition in a solvent such as DMF, optionally with heating:



(IVd) (IVe)

wherein R_1, R_2, R_3, and R_4 are as defined previously for any of Formulae V or 1.1-1.14.

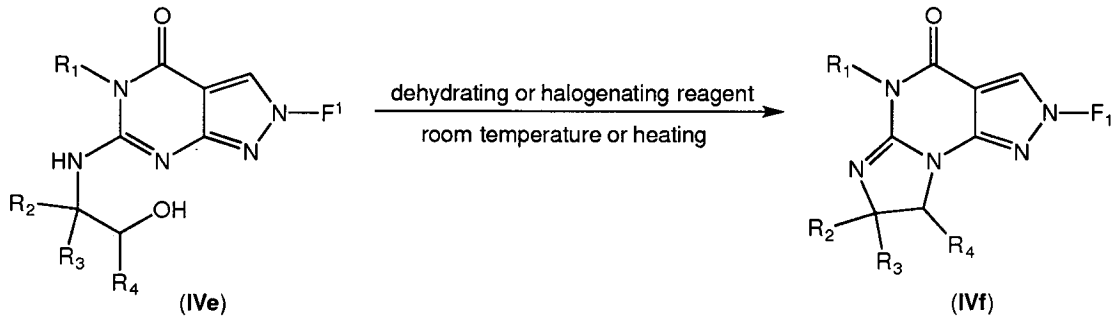
[00041] Alternatively, intermediates IVe can be prepared directly from compounds of formula IVc by reacting with an amino alcohol and a coupling reagent such as BOP in the presence of a base such as DBU:



(IVc) (IVe)

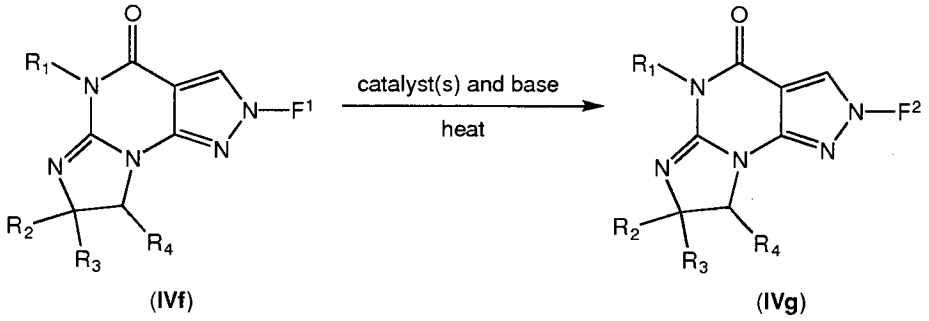
wherein R_1, R_2, R_3, and R_4 are as defined previously for any of Formulae V or 1.1-1.14.

[00042] Intermediates of formula IVf may be prepared by reacting a compound of formula IVe with a dehydrating/halogenating agent such as $SOCl_2$ in a solvent such as dichloromethane at room temperature or with heating at 35 °C:



(IVe) (IVf)

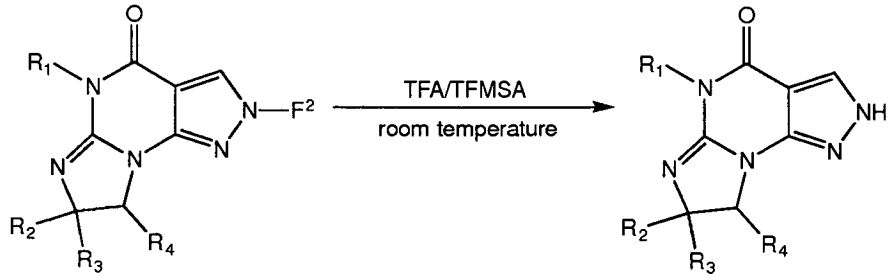
[00043] Intermediates of formula IVg may be prepared by reacting a compound of formula IVf with, catalysts such as a copper salt and 2,2,6,6-tetramethylheptane-3,5-dione and a base such as cesium carbonate in a solvent such as NMP with heating:



(IVf) (IVg)

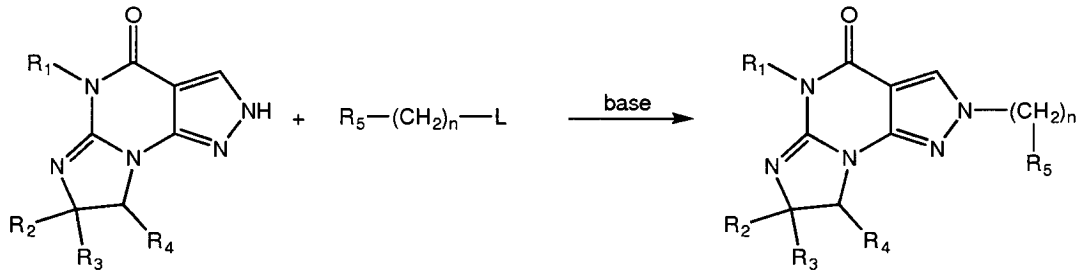
wherein, F^2 is a diaryl ether.

[00044] Intermediates of formula IVh may be prepared by reacting a compound of formula IVg with an acidici system, such as TFA and TFMSA in a solvent such as dichloromethane, at room temperature:



(IVg) (IVh)

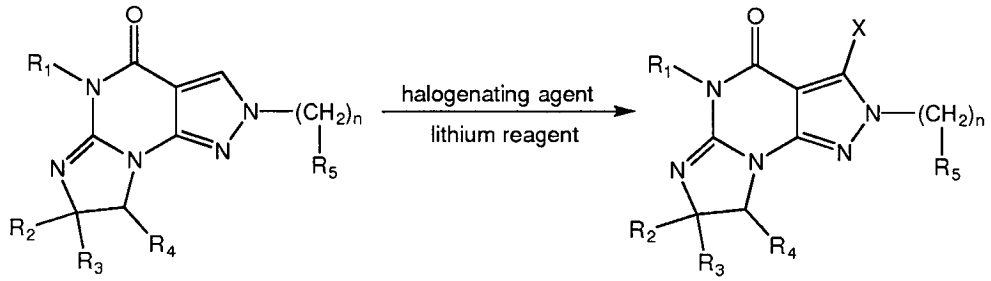
[00045] Intermediates of formula IVi may be prepared by reacting a compound of formula IVh with a reagent of formula R_5–$(CH_2)_n$–L in the presence of a base such as potassium carbonate, in a solvent such as DMF at room temperature:



(IVh) (IVi)

wherein n is 0, and R_5 is a moiety of Formula A, as defined previously for any of Formulae V or 1.1-1.14, and L is a leaving group such as a halogen (e.g., Br).

[00046] Intermediates of formula IVj, wherein X is halogen (e.g., Cl), may be prepared by reacting compounds of formula IVi with a halogenating agent (e.g. NCS or NBS) and a base such as LiHMDS in a solvent such as THF at low temperature:



(IVi) (IVj)

[00047] Compounds of the Invention, may then be prepared from compounds of Formula IVj by methods known to those skilled in the art. For example, by displacement of the halogen X with an arylamine or an alkylmercaptan.

Methods of Using Compounds of the Invention

[00048] The invention further provides Method I, wherein Method I comprises the prophylaxis and/or treatment of diseases, disorders, and injuries of the central nervous system, wherein the method comprises the administration of an effective amount of a PDE1 inhibitor (e.g., any compound of Formula V or 1.1 – 1.14) to modulate the level of intracellular cAMP.

[00049] For example, Method I also includes:

1.1. Method I, wherein the administration of the PDE1 inhibitor enhances the axonal growth or regeneration, and/or slows or reverses the loss of such cells in a neurodegenerative condition.

1.2. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury, refers to damage that directly or indirectly affects the normal functioning of the CNS.

1.3. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury can be a structural, physical, or mechanical impairment and may be caused by physical impact, e.g., crushing, compression, or stretching of nerve fibers.

1.4. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury is a spinal cord injury.

1.5. Method of 1.4, wherein the PDE1 inhibitor slows or arrests the progression of the spinal cord injury.

1.6. Any of preceding Method-I, et seq., wherein the PDE1 inhibitor slows or arrests axonal filament degradation.

1.7. Any of preceding Method-I, et seq. wherein the CNS disease, disorder, or injury relates to motor neuron trauma.

1.8. Any of preceding Method-I, et seq., wherein the disease, disorder, or injury is selected from the group consisting of: neurological traumas and injuries, surgery related trauma and/or injury, retinal injury and trauma, injury related to epilepsy, spinal cord injury, brain injury, brain surgery, trauma related brain injury, trauma related to spinal cord injury, brain injury related to cancer treatment, spinal cord injury related to cancer treatment, brain injury related to infection, brain injury related to inflammation, spinal cord injury related to infection, spinal cord injury related to inflammation, brain injury related to environmental toxins, and spinal cord injury related to environmental toxins.

1.9. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury includes neuron or nerve fibers destroyed by or degraded by an illness (e.g., Parkinson's Disease), a chemical imbalance, or a physiological malfunction such anoxia (e.g., stroke), aneurysm, or reperfusion injury.

1.10. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury is a neurodegenerative disorder.

1.11. Method of 1.10, wherein the neurodegenerative disease, disorder, or injury is selected from the group consisting of: Alzheimer's disease, Multiple Sclerosis, Spinal Muscular Atrophy, Glaucoma, Frontotemporal dementia, Dementia with Lewy bodies, Corticobasal degeneration, Progressive supranuclear palsy, Prion disorders, Huntington's disease, Multiple system atrophy, Parkinson's disease, Amyotrophic lateral sclerosis, Hereditary spastic paraparesis, Spinocerebellar atrophies, Friedreich's ataxia, Amyloidoses, Metabolic (diabetes) related disorders, Toxin related disorders, chronic CNS inflammation, Charcot Marie Tooth disease, diabetic neuropathy, injury due to cancer chemotherapy (e.g., by vinca alkaloids and doxorubicin), brain damage associated with stroke, ischemia associated with stroke, and neurological disorders including, but not limited to, various peripheral neuropathic and neurological disorders related to neurodegeneration including, but not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed vertebral disk

syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathies such as those caused by e.g., lead, acrylamides, gamma-diketones, carbon disulfide, dapsone, ticks, porphyria, and Gullain-Barre syndrome.

1.12. Any of preceding Method-I, et seq., wherein the CNS disease, disorder, or injury is a CNS lesion, a seizure or injury due to seizures (e.g., epileptic seizures), radiation injury, injury due to chemotherapy and/or stroke or other ischemic injury.

1.13. Any of preceding Method-I, et seq., wherein the administration of the PDE1 inhibitor is used to replenish, replace, and/or supplement neurons and/or glial cells.

1.14. Any of preceding Method-I, et seq., wherein the PDE1 inhibitor is administered to a subject or a patient in need thereof.

1.15. Any of preceding Method-I, et seq., wherein the PDE1 inhibitor elevates the level or expression of intracellular cAMP.

1.16. Any of preceding Method-I, et seq., wherein the PDE1 inhibitor decreases the level or expression of intracellular cAMP.

1.17. Any of preceding Method-I, et seq., wherein the PDE1 modulates activity of PKA or PKG.

1.18. Any of preceding Method-I, et seq., wherein the PDE1inhibitor increases the activity of PKA or PKG.

1.19. Any of preceding Method-I, et seq., wherein the administration of the PDE1 inhibitor increases the level of both cAMP and cGMP.

1.20. Any of preceding Method-I, et seq., wherein the administration of the PDE1 inhibitor elevates the level of intracellular cAMP, and wherein this increased level intracellular cAMP has neuroprotective and/or neuroregenerative effects.

1.21. Any of preceding Method-I, et seq., comprising administration of an effective amount of the PDE1 inhibitor to a patient that suffers from a disease or disorder related to elevated (e.g., chronically elevated) intracellular calcium

levels, and wherein the PDE1 inhibitor prevents a further rise in said calcium levels.

1.22. Any of preceding Method-I, et seq., wherein the PDE1 inhibitor is administered either alone or in combination with another active agent.

1.23. Any of preceding Method-I, et seq., wherein the disease, disorder, or injury is related to motor neurons, and wherein the motor neuron disease, disorder, or injury is Multiple Sclerosis.

1.24. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor is administered in combination with another active agent in order to treat Multiple Sclerosis.

1.25. The method of 2.11, wherein the active agent is selected from the group consisting of: Interferon, Glatiramer acetate, Natalizumab, Gilenya® (fingolimod), Fampyra®, immunosuppresents, and corticoids.

[0050] In another embodiment the invention provides for Method II, wherein Method II comprises compositions and methods of treatment or prophylaxis of a peripheral nervous system (PNS) disease, disorder, or injury, wherein the method comprises administration of an effective amount of a PDE1 inhibitor (e.g., any compound of Formula V or 1.1-1.14) to increase intracellular levels of cAMP.

For example, Method II also includes:

2.1. Method II, wherein the PNS disease, disorder, or injury, refers to damage that directly or indirectly affects the normal functioning of the CNS.

2.2. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor is administered to a subject or a patient in need thereof.

2.3. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor elevates the level or expression of intracellular cAMP.

2.4. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor (e.g., directly or indirectly) modulates activity of PKA and/or PKG.

2.5. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor (e.g., directly or indirectly) increases the activity of PKA and/or PKG.

2.6. Any of preceding Method-II, et seq., wherein the administration of the PDE1 inhibitor increases the level of cAMP and/or cGMP.

2.7. Any of preceding Method-II, et seq., wherein the administration of the PDE1 inhibitor elevates the level of intracellular cAMP, and wherein this increased level intracellular cAMP levels protects nerve fibers, regenerates nerve fibers, or promotes nerve fiber growth (e.g., axonal regeneration).

2.8. Any of preceding Method-II, et seq., comprising administration of an effective amount of the PDE1 inhibitor to a patient that suffers from a disease or disorder related to elevated (e.g., chronically elevated) intracellular calcium levels.

2.9. Any of preceding Method-II, et seq., wherein the PDE1 inhibitor is administered either alone or in combination with another active agent.

2.10. The method of 2.9, wherein the active agent is selected from the IGF (e.g., IGF-1) or a steroid.

2.11. Any of preceding Method-II, et seq. wherein the PNS disease, disorder, or injury is selected from the group consisting of: neuropathy (e.g., peripheral neuropathy, autonomic neuropathy, and mononeuropathy), sciatica, carpal tunnel syndrome, polyneuropathy, diabetic neuropathy, postherpetic neuralgia, and thoracic outlet syndrome.

[0051] In another embodiment the invention provides for Method III, wherein Method III comprises compositions and methods to prevent a CNS disease or disorder in a subject that is at risk for developing said disease or disorder, wherein the method comprises:

1.) Obtaining a sample from the subject;

2.) Measuring the levels of intracellular calcium from the sample;

3.) Comparing the levels of intracellular calcium in the biological sample to a reference standard;

4.) Determining whether a patient is at risk for developing a CNS disease or disorder based upon the level of intracellular calcium compared to the reference standard;

5.) Administering a PDE1 inhibitor (e.g., a compound of any of Formula V or 1.1-1.14) to a subject based upon the subject's levels of intracellular calcium (e.g., administration of a PDE1 inhibitor to a subject because they have elevated intracellular calcium levels compared to the reference standard).

For example, Method III also includes:

3.1. Method III, wherein the sample is a biological sample.

3.2. Any of preceding Method-III, et seq., wherein the patient's intracellular calcium levels are measured using a chemical fluorescent probe.

3.3. Any of preceding Method-III, et seq., wherein the patient's intracellular calcium levels are elevated compared to a control (e.g., reference standard).

3.4. Any of preceding Method-III, et seq., wherein a PDE1 inhibitor is administered to a patient that is shown to have elevated intracellular calcium levels compared to a control (e.g., reference standard).

3.5. Any of preceding Method-III, et seq., wherein the administration of a PDE1 inhibitor slows or prevents the development of a CNS and/or PNS disease or disorder, wherein the CNS disease or disorder is one that correlates to elevated (e.g., chronically elevated) levels of intracellular calcium.

3.6. Any of preceding Method-III, et seq., wherein the administration of a PDE1 inhibitor decreases the likelihood that an individual will develop a CNS and/or PNS disease or disorder, wherein the CNS and/or PNS disease or disorder is one that correlates with elevated (e.g., chronically elevated) levels of intracellular calcium (e.g., any of the diseases, disorders or injuries listed in Method I, et seq., and Method II, et seq.).

3.7. Any of preceding Method-III, et seq., wherein the method optionally comprises measuring the patient's intracellular levels of cAMP or cGMP.

3.8. Any of preceding Method-III, et seq., wherein the PDE1 inhibitor is administered either alone or in combination with another active agent.

3.9. Any of preceding Method-III, et seq., wherein the PDE1 inhibitor is administered because a patient has low levels of cAMP and/or cGMP compared to a control subject.

[0052] The Compounds of the Invention are useful in the treatment of diseases characterized by disruption of or damage to cAMP and cGMP mediated pathways, e.g., as a result of increased expression of PDE1 or decreased expression of cAMP and cGMP due to inhibition or reduced levels of inducers of cyclic nucleotide synthesis, such as dopamine and nitric oxide (NO). By preventing the degradation of cAMP and cGMP by PDE1, thereby increasing intracellular levels of cAMP and cGMP, the Compounds of the Invention potentiate the activity of cyclic nucleotide synthesis inducers.

[0053] In another embodiment, the invention also provides methods of treatment, wherein the method comprises administering an effective amount of a PDE1 inhibitor (e.g., any compound of Formula V or 1.1-1.14) to treat any one or more of the following conditions:

(i) Neurodegenerative diseases, including Parkinson's disease, restless leg, tremors, dyskinesias, Huntington's disease, Alzheimer's disease, and drug-induced movement disorders;

(ii) Mental disorders, including depression, attention deficit disorder, attention deficit hyperactivity disorder, bipolar illness, anxiety, sleep disorders, e.g., narcolepsy, cognitive impairment, e.g., cognitive impairment of schizophrenia, dementia, Tourette's syndrome, autism, fragile X syndrome, psychostimulant withdrawal, and drug addiction;

(iii) Circulatory and cardiovascular disorders, including cerebrovascular disease, stroke, congestive heart disease, hypertension, pulmonary hypertension, e.g., pulmonary arterial hypertension, and sexual dysfunction, including cardiovascular diseases and related disorders as described in International Application No. PCT/US2014/16741, the contents of which are incorporated herein by reference;

(iv) Respiratory and inflammatory disorders, including asthma, chronic obstructive pulmonary disease, and allergic rhinitis, as well as autoimmune and inflammatory diseases;

(v) Diseases that may be alleviated by the enhancement of progesterone-signaling such as female sexual dysfunction;

(vi) A disease or disorder such as psychosis, glaucoma, or elevated intraocular pressure;

(vii) Traumatic brain injury;

(viii) Any disease or condition characterized by low levels of cAMP and/or cGMP (or inhibition of cAMP and/or cGMP signaling pathways) in cells expressing PDE1; and/or

(ix) Any disease or condition characterized by reduced dopamine D1 receptor signaling activity,

comprising administering an effective amount of a Compound of the Invention, e.g., a compound according to any of (e.g., any compound of Formula V or 1.1-1.14), in free or pharmaceutically acceptable salt or prodrug form, to a human or animal patient in need thereof.

[0054] In one aspect, the invention provides methods of treatment or prophylaxis for narcolepsy. In this embodiment, PDE1 Inhibitors (e.g., any compound of Formula V or 1.1-1.14) may be used as a sole therapeutic agent, but may also be used in combination or for co-administration with other active agents. Thus, the invention further comprises a method of treating narcolepsy comprising administering simultaneously, sequentially, or contemporaneously therapeutically effective amounts of

(i) a PDE1 Inhibitor, e.g., a compound according to any of (e.g., any compound of Formula V or 1.1-1.14), and

(ii) a compound to promote wakefulness or regulate sleep, e.g., selected from (a) central nervous system stimulants-amphetamines and amphetamine like compounds, e.g., methylphenidate, dextroamphetamine, methamphetamine, and pemoline; (b) modafinil, (c) antidepressants, e.g., tricyclics (including imipramine, desipramine, clomipramine, and protriptyline) and selective serotonin reuptake inhibitors (including fluoxetine and sertraline); and/or (d) gamma hydroxybutyrate (GHB),

in free or pharmaceutically acceptable salt or prodrug form, to a human or animal patient in need thereof.

[0055] In another aspect, the invention further provides methods of treatment or prophylaxis of a condition which may be alleviated by the enhancement of the progesterone signaling comprising administering an effective amount of a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt or prodrug form, to a human or animal patient in need thereof. Diseases or conditions that may be ameliorated by enhancement of progesterone signaling include, but are not limited to, female sexual dysfunction, secondary amenorrhea (e.g., exercise amenorrhoea, anovulation, menopause, menopausal symptoms, hypothyroidism), pre-menstrual syndrome, premature labor, infertility, for example infertility due to repeated miscarriage, irregular menstrual cycles, abnormal uterine bleeding, osteoporosis, autoimmmune disease, multiple sclerosis, prostate enlargement, prostate cancer, and hypothyroidism. For example, by enhancing progesterone signaling, the PDE1 inhibitors may be used to encourage egg implantation through effects on the lining of uterus, and to help maintain pregnancy in women who are prone to miscarriage due to immune response to pregnancy or low progesterone function. The novel PDE1 inhibitors, e.g., as described herein, may also be useful to enhance the effectiveness of hormone replacement therapy, e.g., administered in combination with estrogen/estradiol/estriol and/or progesterone/progestins in postmenopausal women, and estrogen-induced endometrial hyperplasia and carcinoma. The methods of the invention are also useful for animal breeding, for example to induce sexual receptivity and/or estrus in a nonhuman female mammal to be bred.

[0056] In this aspect, PDE1 Inhibitors may be used in the foregoing methods of treatment or prophylaxis as a sole therapeutic agent, but may also be used in combination or for co-administration with other active agents, for example in conjunction with hormone replacement therapy. Thus, the invention further comprises a method of treating disorders that may be ameliorated by enhancement of progesterone signaling comprising administering simultaneously, sequentially, or contemporaneously therapeutically effective amounts of

(i) a PDE1 Inhibitor, e.g., a compound according to any of Formula V or 1.1-1.14, and

(ii) a hormone, e.g., selected from estrogen and estrogen analogues (e.g., estradiol, estriol, estradiol esters) and progesterone and progesterone analogues (e.g., progestins)

in free or pharmaceutically acceptable salt or prodrug form, to a human or animal patient in need thereof.

[0057] The invention also provides a method for enhancing or potentiating dopamine D1 intracellular signaling activity in a cell or tissue comprising contacting said cell or tissue with an amount of a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt or prodrug form, sufficient to inhibit PDE1 activity.

[0058] The invention also provides a method for treating a PDE1-related disorder, a dopamine D1 receptor intracellular signaling pathway disorder, or disorders that may be alleviated by the enhancement of the progesterone signaling pathway in a patient in need thereof comprising administering to the patient an effective amount of a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt or prodrug form, that inhibits PDE1, wherein PDE1 activity modulates phosphorylation of DARPP-32 and/or the GluR1 AMPA receptor.

[0059] In another aspect, the invention also provides a method for the treatment for glaucoma or elevated intraocular pressure comprising topical administration of a therapeutically effective amount of a PDE1 Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt form, in an ophthalmically compatible carrier to the eye of a patient in need thereof. However, treatment may alternatively include a systemic therapy. Systemic therapy includes treatment that can directly reach the bloodstream, or oral methods of administration, for example.

[0060] The invention further provides a pharmaceutical composition for topical ophthalmic use comprising a PDE1 inhibitor; for example an ophthalmic solution, suspension, cream or ointment comprising a PDE1 Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or ophthalmologically

acceptable salt form, in combination or association with an ophthalmologically acceptable diluent or carrier.

[0061] Optionally, the PDE1 inhibitor (e.g., any of Formula V or 1.1-1.14) may be administered sequentially or simultaneously with a second drug useful for treatment of glaucoma or elevated intraocular pressure. Where two active agents are administered, the therapeutically effective amount of each agent may be below the amount needed for activity as monotherapy. Accordingly, a subthreshold amount (i.e., an amount below the level necessary for efficacy as monotherapy) may be considered therapeutically effective and may also be referred alternatively as an effective amount. Indeed, an advantage of administering different agents with different mechanisms of action and different side effect profiles may be to reduce the dosage and side effects of either or both agents, as well as to enhance or potentiate their activity as monotherapy.

[0062] The invention thus provides the method of treatment of a condition selected from glaucoma and elevated intraocular pressure comprising administering to a patient in need thereof an effective amount, e.g., a subthreshold amount, of an agent known to lower intraocular pressure concomitantly, simultaneously or sequentially with an effective amount, e.g., a subthreshold amount, of a PDE1 Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt form, such that amount of the agent known to lower intraocular pressure and the amount of the PDE1 inhibitor in combination are effective to treat the condition.

[0063] In one aspect, one or both of the agents are administered topically to the eye. Thus the invention provides a method of reducing the side effects of treatment of glaucoma or elevated intraocular pressure by administering a reduced dose of an agent known to lower intraocular pressure concomitantly, simultaneously or sequentially with an effective amount of a PDE1 inhibitor. However, methods other than topical administration, such as systemic therapeutic administration, may also be utilized.

[0064] The optional additional agent or agents for use in combination with a PDE1 inhibitor may, for example, be selected from the existing drugs comprise typically of instillation of a prostaglandin, pilocarpine, epinephrine, or topical beta-blocker treatment,

e.g. with timolol, as well as systemically administered inhibitors of carbonic anhydrase, e.g. acetazolamide. Cholinesterase inhibitors such as physostigmine and echothiopate may also be employed and have an effect similar to that of pilocarpine. Drugs currently used to treat glaucoma thus include, e.g.,

1. Prostaglandin analogs such as latanoprost (Xalatan), bimatoprost (Lumigan) and travoprost (Travatan), which increase uveoscleral outflow of aqueous humor. Bimatoprost also increases trabecular outflow.
2. Topical beta-adrenergic receptor antagonists such as timolol, levobunolol (Betagan), and betaxolol, which decrease aqueous humor production by the ciliary body.
3. Alpha$_2$-adrenergic agonists such as brimonidine (Alphagan), which work by a dual mechanism, decreasing aqueous production and increasing uveo-scleral outflow.
4. Less-selective sympathomimetics like epinephrine and dipivefrin (Propine) increase outflow of aqueous humor through trabecular meshwork and possibly through uveoscleral outflow pathway, probably by a beta$_2$-agonist action.
5. Miotic agents (para-sympathomimetics) like pilocarpine work by contraction of the ciliary muscle, tightening the trabecular meshwork and allowing increased outflow of the aqueous humour.
6. Carbonic anhydrase inhibitors like dorzolamide (Trusopt), brinzolamide (Azopt), acetazolamide (Diamox) lower secretion of aqueous humor by inhibiting carbonic anhydrase in the ciliary body.
7. Physostigmine is also used to treat glaucoma and delayed gastric emptying.

[0065] For example, the invention provides pharmaceutical compositions comprising a PDE1 Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt form, and an agent selected from (i) the prostanoids, unoprostone, latanoprost, travoprost, or bimatoprost; (ii) an alpha adrenergic agonist such as brimonidine, apraclonidine, or dipivefrin and (iii) a muscarinic agonist, such as pilocarpine, in combination or association with a pharmaceutically acceptable diluent or carrier. For example, the invention provides ophthalmic formulations comprising a PDE-1 Inhibitor of the

Invention, e.g., a compound according to any of Formula V or 1.1-1.14, together with bimatoprost, abrimonidine, brimonidine, timolol, or combinations thereof, in free or ophthamalogically acceptable salt form, in combination or association with an ophthamologically acceptable diluent or carrier. In addition to selecting a combination, however, a person of ordinary skill in the art can select an appropriate selective receptor subtype agonist or antagonist. For example, for alpha adrenergic agonist, one can select an agonist selective for an alpha 1 adrenergic receptor, or an agonist selective for an $alpha_2$ adrenergic receptor such as brimonidine, for example. For a beta-adrenergic receptor antagonist, one can select an antagonist selective for either β_1, or β_2, or β_3, depending on the appropriate therapeutic application. One can also select a muscarinic agonist selective for a particular receptor subtype such as M_1-M_5.

[0066] The PDE1 inhibitor may be administered in the form of an ophthalmic composition, which includes an ophthalmic solution, cream or ointment. The ophthalmic composition may additionally include an intraocular-pressure lowering agent.

[0067] In yet another example, the PDE1 Inhibitors disclosed may be combined with a subthreshold amount of an intraocular pressure-lowering agent which may be a bimatoprost ophthalmic solution, a brimonidine tartrate ophthalmic solution, or brimonidine tartrate/timolol maleate ophthalmic solution.

[0068] In addition to the above-mentioned methods, it has also been surprisingly discovered that PDE1 inhibitors (e.g., any of Formula V or 1.1-1.14) are useful to treat psychosis, for example, any conditions characterized by psychotic symptoms such as hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking, e.g., schizophrenia, schizoaffective disorder, schizophreniform disorder, psychotic disorder, delusional disorder, and mania, such as in acute manic episodes and bipolar disorder. Without intending to be bound by any theory, it is believed that typical and atypical antipsychotic drugs such as clozapine primarily have their antagonistic activity at the dopamine D2 receptor. PDE1 inhibitors, however,

primarily act to enhance signaling at the dopamine D1 receptor. By enhancing D1 receptor signaling, PDE1 inhibitors can increase NMDA receptor function in various brain regions, for example in nucleus accumbens neurons and in the prefrontal cortex. This enhancement of function may be seen for example in NMDA receptors containing the NR2B subunit, and may occur e.g., via activation of the Src and protein kinase A family of kinases.

[0069] Therefore, the invention provides a new method for the treatment of psychosis, e.g., schizophrenia, schizoaffective disorder, schizophreniform disorder, psychotic disorder, delusional disorder, and mania, such as in acute manic episodes and bipolar disorder, comprising administering a therapeutically effective amount of a phosphodiesterase-1 (PDE1) Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt form, to a patient in need thereof.

[0070] PDE 1 Inhibitors may be used in the foregoing methods of treatment prophylaxis as a sole therapeutic agent, but may also be used in combination or for co-administration with other active agents. Thus, the invention further comprises a method of treating psychosis, e.g., schizophrenia, schizoaffective disorder, schizophreniform disorder, psychotic disorder, delusional disorder, or mania, comprising administering simultaneously, sequentially, or contemporaneously therapeutically effective amounts of:

(i) a PDE1 Inhibitor of the invention, in free or pharmaceutically acceptable salt form; and

(ii) an antipsychotic, e.g.,

Typical antipsychotics, e.g.,

Butyrophenones, e.g. Haloperidol (Haldol, Serenace), Droperidol (Droleptan);

Phenothiazines, e.g., Chlorpromazine (Thorazine, Largactil), Fluphenazine (Prolixin), Perphenazine (Trilafon), Prochlorperazine (Compazine), Thioridazine (Mellaril, Melleril), Trifluoperazine (Stelazine), Mesoridazine, Periciazine, Promazine,

Triflupromazine (Vesprin), Levomepromazine (Nozinan), Promethazine (Phenergan), Pimozide (Orap);
Thioxanthenes, e.g., Chlorprothixene, Flupenthixol (Depixol, Fluanxol), Thiothixene (Navane), Zuclopenthixol (Clopixol, Acuphase);
Atypical antipsychotics, e.g.,
Clozapine (Clozaril), Olanzapine (Zyprexa), Risperidone (Risperdal), Quetiapine (Seroquel), Ziprasidone (Geodon), Amisulpride (Solian), Paliperidone (Invega), Aripiprazole (Abilify), Bifeprunox; norclozapine,

in free or pharmaceutically acceptable salt form, to a patient in need thereof.

[0071] In a particular embodiment, the Compounds of the Invention are particularly useful for the treatment or prophylaxis of schizophrenia.

[0072] Compounds of the Invention, in free or pharmaceutically acceptable salt form, are particularly useful for the treatment of Parkinson's disease, schizophrenia, narcolepsy, glaucoma and female sexual dysfunction.

[0073] In still another aspect, the invention provides a method of lengthening or enhancing growth of the eyelashes by administering an effective amount of a prostaglandin analogue, e.g., bimatoprost, concomitantly, simultaneously or sequentially with an effective amount of a PDE1 inhibitor of the Invention, in free or pharmaceutically acceptable salt form, to the eye of a patient in need thereof.

[0074] In yet another aspect, the invention provides a method for the treatment or prophylaxis of traumatic brain injury comprising administering a therapeutically effective amount of a PDE1 Inhibitor of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, in free or pharmaceutically acceptable salt form, to a patient in need thereof. Traumatic brain injury (TBI) encompasses primary injury as well as secondary injury, including both focal and diffuse brain injuries. Secondary injuries are multiple, parallel, interacting and interdependent cascades of biological reactions arising from discrete subcellular processes (e.g., toxicity due to reactive oxygen species,

overstimulation of glutamate receptors, excessive influx of calcium and inflammatory upregulation) which are caused or exacerbated by the inflammatory response and progress after the initial (primary) injury.

[0075] The present invention also provides

(i) a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, as hereinbefore described, in free or pharmaceutically acceptable salt form for example for use in any method or in the treatment of any disease or condition as hereinbefore set forth,

(ii) the use of a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, as hereinbefore described, in free or pharmaceutically acceptable salt form, (in the manufacture of a medicament) for treating any disease or condition as hereinbefore set forth,

(iii) a pharmaceutical composition comprising a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, as hereinbefore described, in free or pharmaceutically acceptable salt form, in combination or association with a pharmaceutically acceptable diluent or carrier, and

(iv) a pharmaceutical composition comprising a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, as hereinbefore described, in free or pharmaceutically acceptable salt form, in combination or association with a pharmaceutically acceptable diluent or carrier for use in the treatment of any disease or condition as hereinbefore set forth.

[0076] Therefore, the invention provides use of a Compound of the Invention, e.g., a compound according to any of Formula V or 1.1-1.14, as hereinbefore described, in free or pharmaceutically acceptable salt form, or a Compound of the Invention in a pharmaceutical composition form (in the manufacture of a medicament) for the treatment or prophylactic treatment of any one or more of the following diseases: Parkinson's

disease, restless leg, tremors, dyskinesias, Huntington's disease, Alzheimer's disease, and/or drug-induced movement disorders; depression, attention deficit disorder, attention deficit hyperactivity disorder, bipolar illness, anxiety, sleep disorder, narcolepsy, cognitive impairment, e.g., cognitive impairment of schizophrenia, dementia, Tourette's syndrome, autism, fragile X syndrome, psychostimulant withdrawal, and/or drug addiction; cerebrovascular disease, stroke, congestive heart disease, hypertension, pulmonary hypertension, e.g., pulmonary arterial hypertension, and/or sexual dysfunction; asthma, chronic obstructive pulmonary disease, and/or allergic rhinitis, as well as autoimmune and inflammatory diseases; and/or female sexual dysfunction, exercise amenorrhoea, anovulation, menopause, menopausal symptoms, hypothyroidism, pre-menstrual syndrome, premature labor, infertility, irregular menstrual cycles, abnormal uterine bleeding, osteoporosis, multiple sclerosis, prostate enlargement, prostate cancer, hypothyroidism, and/or estrogen-induced endometrial hyperplasia and/or carcinoma; and/or any disease or condition characterized by low levels of cAMP and/or cGMP (or inhibition of cAMP and/or cGMP signaling pathways) in cells expressing PDE1, and/or by reduced dopamine D1 receptor signaling activity; and/or any disease or condition that may be ameliorated by the enhancement of progesterone signaling.

[0077] The invention also provides use of a Compound of the Invention, in free or pharmaceutically acceptable salt form, (the manufacture of a medicament) for the treatment or prophylactic treatment of any one or more of:

a) glaucoma, elevated intraocular pressure,

b) psychosis, for example, any conditions characterized by psychotic symptoms such as hallucinations, paranoid or bizarre delusions, or disorganized speech and thinking, e.g., schizophrenia, schizoaffective disorder, schizophreniform disorder, psychotic disorder, delusional disorder, and mania, such as in acute manic episodes and bipolar disorder,

c) traumatic brain injury, and/or

d) central and peripheral degenerative disorders particularly those with inflammatory components.

[0078] The phrase "Compounds of the Invention" or "PDE 1 inhibitors of the Invention" encompasses any and all of the compounds disclosed herewith, e.g., a Compound of Formula V or 1.1-1.14.

[0079] 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.

[0080] For methods of treatment, the word "therapeutically effective amount" as used herein refers to an amount of a drug (e.g., a PDE1 inhibitor) sufficient to treat or ameliorate the pathological effects a CNS or PNS disease, disorder, or injury. For example, a therapeutically effective amount of a PDE1 inhibitor may be an amount sufficient to, e.g., increase intracellular levels of cAMP or cGMP, decrease intracellular levels of calcium, and/or increase neuroregeneration. Where relevant, a therapeutically effective amount may also be the amount of a PDE1 inhibitor necessary to slow or prevent the development of CNS or PNS disease or disorder.

[0081] The term "patient" or "subject" refers to human or non-human (i.e., animal) patient. In a particular embodiment, the invention encompasses both human and nonhuman patients. In another embodiment, the invention encompasses nonhuman patients. In other embodiment, the term encompasses human patients.

[0082] The term "control subject" as used herein, refers to any human or nonhuman organism that does not have and/or is not suspected of having a CNS or PNS disorder, syndrome, disease, condition and/or symptom. The term "reference standard" as used herein, refers to the prior measurement and obtaining of results in a control subject or population of control subjects. In another aspect, the term "reference standard" refers to the prior measurement and obtaining of results in a patient prior to his or her development of a CNS or PNS disorder, syndrome, disease, condition and/or symptom.

[0083] The term "biological sample" as used herein, may include any sample comprising biological material obtained from, e.g., an organism, body fluid, waste

product, cell or part of a cell thereof, cell line, biopsy, tissue culture or other source containing a intracellular calcium, cAMP, or cGMP levels.

[0084] A "neurogenic agent" is defined as a chemical agent or reagent that can promote, stimulate, or otherwise increase the amount or degree or nature of neurogenesis *in vivo* or *ex vivo* or *in vitro,* relative to the amount, degree, or nature of neurogenesis in the absence of the agent or reagent.

[0085] A "CNS injury" as used herein may include, e.g., damage to retinal ganglion cells, a traumatic brain injury, a stroke-related injury, a cerebral aneurism- related injury, a spinal cord injury or trauma, including monoplegia, diplegia, paraplegia, hemiplegia and quadriplegia, a neuroproliferative disorder, or neuropathic pain syndrome. A "PNS injury" as used herein may include, e.g., damage to the spinal or cranial nerves, wherein that damage may include a lesion or some acute or chronic trauma.

[0086] Compounds of the Invention, (e.g., any of Formula V or 1.1-1.14) 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 with or for co-administration with other active agents.

[0087] 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, 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 of a

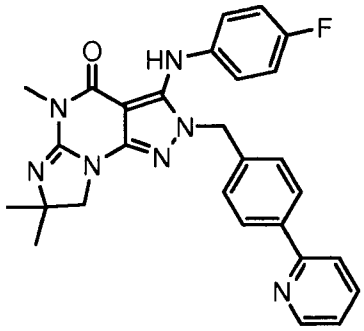
Compound of the Invention, together with a pharmaceutically acceptable diluent or carrier therefor.

[0088] 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.

EXAMPLES

Example 1

7,8-Dihydro-2-(4-(pyridine-2-yl)benzyl)-3-(4-fluorophenylamino)- 5,7,7-trimethyl-[2*H*]-imidazo-[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one



(a) 7-(4-Methoxybenzyl)-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione

[0089] A suspension of 7-(4-methoxybenzyl)-5-methyl-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (8.43 g, 29.4 mmol), 2-(4-(chloromethyl)phenyl)-pyridine (6.0 g, 29.4 mmol) and K_2CO_3 (4.07 g, 29.4 mmol) in DMF (100 mL) is stirred at room temperature overnight. Solvent is removed under reduced pressure. The obtained residue is treated with water (150 mL) and hexanes (25 mL). The mixture is stirred at room temperature for an hour, and then filtered. The filter cake is washed with water three times (3 × 50 mL), and then dried under vacuum to give 13 g of crude product

(yield: 97%), which is used in the next step without further purification. MS (ESI) m/z 454.2 $[M+H]^+$.

(b) 5-Methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione

[0090] TFA (50 mL) is added into a suspension of 7-(4-Methoxybenzyl)-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (13 g, 28.7 mmol) in methylene chloride (80 mL) to give a tan solution, and then TFMSA (4 mL) is added. The reaction mixture is stirred at room temperature overnight. Solvents are removed under reduced pressure. The obtained residue is treated with water (150 mL), cooled to 0 °C, and then adjusted to pH 8-9 with 28% ammonium hydroxide (approx. 35 mL). After filtration, the obtained solids are washed with water three times (3 × 50 mL), and then dried under vacuum to give 12.8g of crude product (crude yield: 134%), which is used in the next step without further purification. MS (ESI) m/z 334.1 $[M+H]^+$.

(c) 6-Chloro-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidin-4(5H)-one

[0091] 5-Methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dione (8.5 g, 25.5 mmol) is suspended in $POCl_3$ (300 mL), and then slowly heated to reflux. After the mixture is refluxed for 30h, $POCl_3$ is removed under reduced pressure. The obtained residue is treated with water (300 mL), cooled to 0 °C, and then adjusted to pH 8-9 with 28% ammonium hydroxide (approx. 30 mL). After filtration, the obtained solids are washed with water five times (5 × 50 mL), and then dried under vacuum to give 8.6 g of crude product (crude yield: 96%), which is used in the next step without further purification. MS (ESI) m/z 352.1 $[M+H]^+$.

(d) 6-(1-Hydroxy-2-methylpropan-2-ylamino)-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidin-4(5H)-one

[0092] A mixture of 6-Chloro-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (4.0 g, 11 mmol), 2-amino-2-methylpropan-1-ol (6.5 mL, 71

mmol) and DIPEA (3.4 mL, 20 mmol) in DMA (20 mL) is heated at 130 °C for an hour. Solvent is removed under reduced pressure. The obtained residue is treated with water (200 mL). After filtration, the filter cake is washed with water twice (2 × 50 mL), and then dried under vacuum to give 3.7 g of crude product (crude yield: 80%), which is used in the next step without further purification. MS (ESI) m/z 405.2 $[M+H]^+$.

(e) 7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[0093] Thionyl chloride (756 μL, 10.4 mmol) is added dropwise to a solution of crude 6-(1-hydroxy-2-methylpropan-2-ylamino)-5-methyl-2-(4-(pyridin-2-yl)benzyl)-2H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (4.2 g, 10.4 mmol) in DMF (84 mL). The reaction mixture is stirred at room temperature for 20 min. Water (5 mL) is added to quench the reaction. Solvents are removed under reduced pressure. The obtained residue is treated with methylene chloride, and then washed with 5% $NaHCO_3$ aqueous solution three times. The organic phase is evaporated to dryness to give 6.1g of crude product (crude yield: 152%), which is used in the next step without further purification. MS (ESI) m/z 387.2 $[M+H]^+$.

(f) 7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[0094] 1.0M LiHMDS (55.4 mL, 55.4 mmol) in THF is added dropwise to a solution of crude 7,8-dihydro-2-(4-(pyridin2-yl)benzyl)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (4.6 g, 11.9 mmol) and hexachloroethane (2.58 g, 10.9 mmol) in methylene chloride (130 mL) at 0 °C. The reaction mixture is stirred at 0 °C for 30 min, and then quenched with water (100 mL) and methylene chloride (150 mL). The organic phase is washed with water three times (3 × 70 mL), and then evaporated to dryness. The obtained crude product is purified on a neutral aluminum

oxide column to give 1.5 g of pure product (HPLC purity: 96%; yield: 30%). MS (ESI) m/z 421.1 $[M+H]^+$.

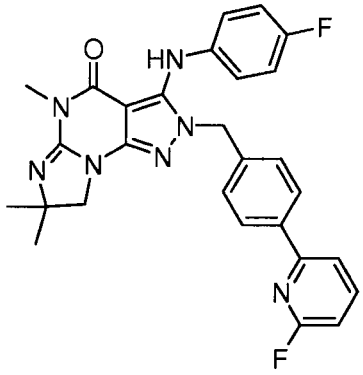
(g) 7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-3-(4-fluorophenylamino)- 5,7,7-trimethyl-[2*H*]-imidazo-[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one

[0095] 7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (550 mg, 1.31 mmol), 4-fluorobenzenamine (125 µL, 1.31 mmol) and potassium carbonate (361 mg, 2.61 mmol) in *tert*-amyl alcohol (3 mL) are degassed with argon and then Xantphos (15 mg, 0.026 mmol) and $Pd_2(dba)_3$ (12 mg, 0.013 mmol) are added. The suspension is degassed again, and then slowly heated to 110 °C. The reaction mixture is stirred at 110 °C under argon overnight. Another batch of $Pd_2(dba)_3$ (12 mg) and Xantphos (15 mg) is added. The reaction is heated at 110 °C for additional 24 h for complete conversion. After routine workup, the crude product is purified by silica-gel column chromatography to give 352 mg of final product as a beige solid (HPLC purity: 97.4%; yield: 54%). ^1H NMR (500 MHz, Chloroform-d) δ 8.68 (dt, J = 4.7, 1.3 Hz, 1H), 7.88 (d, J = 8.3 Hz, 2H), 7.74 (td, J = 7.7, 1.8 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.23 (ddd, J = 7.4, 4.8, 1.2 Hz, 1H), 7.06 (d, J = 8.3 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.94 – 6.87 (m, 2H), 6.79 (s, 1H), 4.90 (s, 2H), 3.71 (s, 2H), 3.35 (s, 3H), 1.40 (s, 6H). MS (ESI) m/z 496.2 $[M+H]^+$.

[0096] The compound of Example 1 shows good selectivity for PDE1 and inhibts PDE activity at an IC_{50} value of equal to or less than 5nM.

Example 2

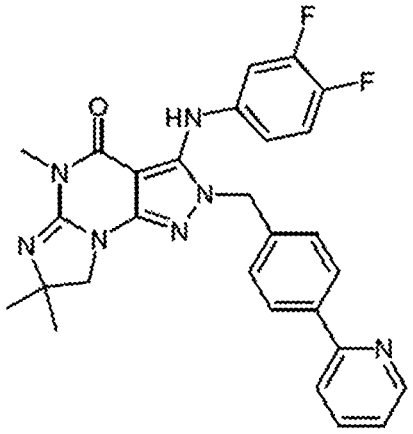
7,8-Dihydro-2-(4-(6-Fluoropyridin-2-yl)benzyl)-3-(4-fluorophenylamino)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one



[0097] The synthesis method is analogous to example 1 wherein 2-(4-(chloromethyl)phenyl)-6-fluoropyridine is added in step (a) instead of 2-(4-(chloromethyl)phenyl)-pyridine. Final product is obtained as a off-white solid (HPLC purity: 99%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.89 (d, J = 8.4 Hz, 2H), 7.83 (q, J = 8.0 Hz, 1H), 7.58 (dd, J = 7.5, 2.3 Hz, 1H), 7.05 (d, J = 8.3 Hz, 2H), 7.00 – 6.84 (m, 6H), 4.91 (s, 2H), 3.76 (s, 2H), 3.39 (s, 3H), 1.47 (s, 6H). MS (ESI) m/z 514.3 $[M+H]^+$

Example 3

7,8-Dihydro-2-(4-(pyridine-2-yl)benzyl)-3-(3,4-difluorophenylamino)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one



(a) 2-(4-Bromobenzyl)-7,8-dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[0098] The title compound is synthesized using the procedure analogous to the one described from step (a) to step (e) of Example 1 wherein 1-bromo-4-(bromomethyl)benzene was added in step (a) instead of 2-(4-(chloromethyl)phenyl)-pyridine. MS (ESI) m/z 388.1 $[M+H]^+$.

(b) 2-(4-Phenoxybenzyl)-7,8-dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[0099] 2-(4-Bromobenzyl)-7,8-dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (118 g, 304 mmol) is added to a suspension of phenol (57 g, 606 mmol) and cesium carbonate (200 g, 614 mmol) in NMP (900 mL), followed by 2,2,6,6-tetramethylheptane-3,5-dione (7 mL, 33.5 mmol) and CuCl (15 g, 152 mmol). The reaction mixture is heated at 120 °C under nitrogen atmosphere for 10 h. After the completion of the reaction, the mixture is diluted with water (4 L), and then extracted with ethyl acetate. The combined organic phase is evaporated to dryness. The obtained crude product is purified by silica gel column chromatography to give 103 g of product (yield: 84%). MS (ESI) m/z 402.2 $[M+H]^+$.

(c) 7,8-Dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[00100] TFA (600 mL) is added to a suspension of 2-(4-phenoxybenzyl)-7,8-dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (103 g, 257 mmol) in methylene chloride (210 mL) to give a tan solution, and then TFMSA (168 mL) is added. The reaction mixture is stirred at room temperature until the starting material disappears. The reaction mixture is poured into cold water (3 L). After filtration, the filter cake is washed with water twice, and then basified with ammonium hydroxide aqueous solution, followed by adding ethyl acetate with stirring. The precipitated solids are filtered, washed successively with water three times, ethyl acetate twice and methanol

once, and then dried under vacuum to give 45 g of product (yield: 80%). MS (ESI) m/z 220.2 $[M+H]^+$.

(d) 7,8-Dihydro-2-(4-(pyridin-2-yl)benzyl)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[00101] A suspension of 7,8-dihydro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (1.5 g, 6.84 mmol), 2-(4-(bromomethyl)phenyl)pyridine (1.7 g, 6.84 mmol) and K_2CO_3 (2.83 g, 20.5 mmol) in DMF (60 mL) is stirred at room temperature for 2-3 days. Solvent is removed under reduced pressure. The obtained residue is treated with water (100 mL), sonicated and then filtered. The filter cake is dried under vacuum to give 2.19 g of crude product (yield: 83%), which is used in the next step without further purification. MS (ESI) m/z 387.1 $[M+H]^+$.

(e) 7,8-Dihydro-2-(4-(pyridine-2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

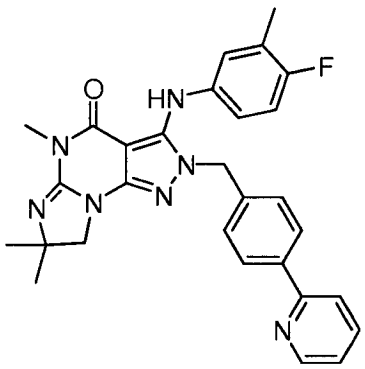
[00102] 1.0M LiHMDS (3.0 mL, 3.0 mmol) in THF is added dropwise to a solution of crude 7,8-dihydro-2-(4-(pyridin2-yl)benzyl)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (1.16 g, 3.0 mmol) and hexachloroethane (2.13 g, 9.0 mmol) in methylene chloride (30 mL). The reaction mixture is stirred at room temperature for 90 minutes, and is then quenched with cold water (200 mL). The mixture is extracted with methylene chloride three times (50 mL × 3), and the combined organic phase was washed with brine (30 mL), and then evaporated to dryness under reduced pressure. The obtained residue is purified on a neutral alumina oxide column to give 960 mg of pure product as an off-white solid (HPLC purity: 96.8%; yield: 76%). MS (ESI) m/z 421.2 $[M+H]^+$.

(f) 7,8-Dihydro-2-(4-(pyridine-2-yl)benzyl)-3-(3,4-difluorophenylamino)- 5,7,7-trimethyl-[2*H*]-imidazo-[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one

[00103] 7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (230 mg, 0.546 mmol), 3,4-difluorobenzenamine (106 mg, 0.821 mmol) and potassium carbonate (300 mg, 2.17 mmol) in *tert*-amyl alcohol (2.8 mL) are degassed with argon, and then Xantphos (26 mg, 0.045 mmol) and $Pd_2(dba)_3$ (20 mg, 0.022 mmol) are added. The suspension is degassed again, and then heated to 110 °C. The reaction mixture is stirred at 110 °C under argon overnight. After routine workup, the crude product is purified on a basic alumina oxide column to give 194 mg of final product as a beige solid (HPLC purity: 99%; yield: 69%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.69 (d, J = 4.5 Hz, 1H), 7.88 (d, J = 8.2 Hz, 2H), 7.76 (td, J = 7.8, 1.6 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.26 – 7.17 (m, 2H), 7.15 (d, J = 8.2 Hz, 2H), 7.03 (m, 1H), 6.69 (m, 1H), 6.60 (m, 1H), 5.05 (s, 2H), 3.79 (s, 2H), 3.29 (s, 3H), 1.47 (s, 6H). MS (ESI) m/z 514.2 $[M+H]^+$.

Example 4

7,8-Dihydro-2-(4-(pyridin2-yl)benzyl)-3-(4-fluoro-3-methylphenylamino)-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

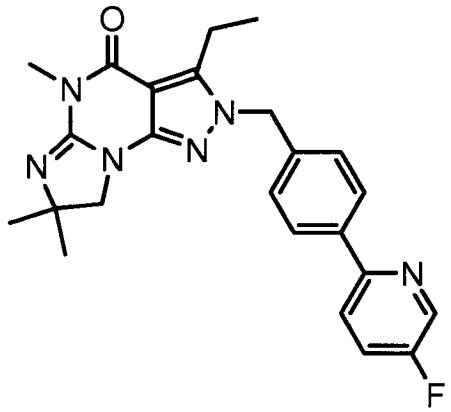


[00104] The synthesis method is analogous to example 3 wherein 4-fluoro-3-methylbenzenamine was added in step (f) instead of 3,4-difluorobenzenamine. Final product is obtained as an off-white solid (HPLC purity: 97%). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.70 (ddd, J = 4.8, 1.9, 1.0 Hz, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.77 (td, J = 7.7, 1.9 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.26 (m, 1H), 7.06 (d, J = 8.3 Hz, 2H), 6.97

– 6.86 (m, 2H), 6.81 – 6.69 (m, 2H), 4.91 (s, 2H), 3.81 (s, 2H), 3.40 (s, 3H), 2.13 (d, J = 1.4 Hz, 3H), 1.49 (s, 6H). MS (ESI) m/z 510.2 $[M+H]^+$

Example 5

7,8-Dihydro-2-(4-(5-fluoropyridin2-yl)benzyl)-3-ethyl-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one



(a) 7,8-Dihydro-2-(4-(5-fluoropyridin2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

[00105] The title compound is prepared using the procedure analogous to the one described in steps (a) to (f) of Example 1 wherein 2-(4-(chloromethyl)phenyl)-5-fluoropyridine was added in step (a) instead of 2-(4-(chloromethyl)phenyl)-pyridine. MS (ESI) m/z 439.2 $[M+H]^+$.

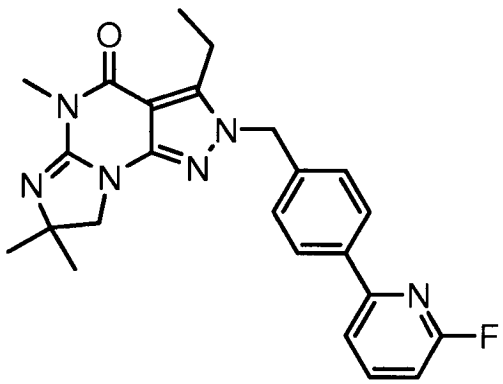
(b) 7,8-Dihydro-2-(4-(5-fluoropyridin2-yl)benzyl)-3-ethyl-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-on

[00106] Ethylmagnesium bromide (3.0 M in ether, 3 mL) is added dropwise to a reaction vial containing $ZnCl_2$ (1.2 g, 8.8 mmol) at 0 °C under argon. The mixture is stirred at room temperature for 20 min, and is then cooled to – 78 °C. 9-Methoxy-9-borabicyclo[3.3.1]nonane (1.0 M in hexanes, 8 mL) is added dropwise. After the completion of the addition, the mixture is stirred at room temperature for 40 min. 7,8-Dihydro-2-(4-(5-fluoropyridin2-yl)benzyl)-3-chloro-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one (352 mg, 0.8 mmol) in anhydrous DMF (15 mL)

is slowly added to the mixture, followed by 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (S-Phos, 38 mg) and palladium acetate (13 mg). The reaction vial is sealed and stirred at room temperature for 30 min, and is then heated at 100 °C for 4 days. The mixture is diluted with water (150 mL), and then extracted with dichloromethane (60 mL × 3). The combined organic phase is evaporated to dryness under reduced pressure. The residue is purified by a with a semi-preparative HPLC system equipped with a reversed-phase C18 column using a gradient of 0 – 26% acetonitrile in water containing 0.1% formic acid over 16 min to give 177 mg of product as a pale yellow solid (HPLC purity: 99.5%; yield: 51%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.53 (d, *J* = 2.9 Hz, 1H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.69 (dd, *J* = 8.8, 4.2 Hz, 1H), 7.47 (td, *J* = 8.4, 2.9 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 2H), 5.29 (s, 2H), 3.73 (s, 2H), 3.41 (s, 3H), 2.95 (q, *J* = 7.6 Hz, 2H), 1.42 (s, 6H), 1.18 (t, *J* = 7.5 Hz, 3H). MS (ESI) m/z 433.3 $[M+H]^+$.

Example 6

7,8-Dihydro-2-(4-(6-fluoropyridin2-yl)benzyl)-3-ethyl-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one



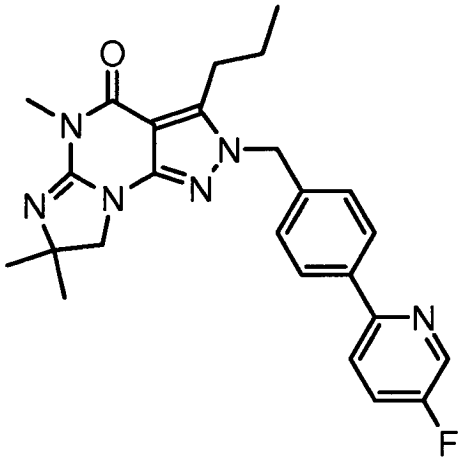
[00107] The title compound is prepared using the procedure analogous to the one described in Example 5 wherein 2-(4-(chloromethyl)phenyl)-6-fluoropyridine was added in step (a) instead of 2-(4-(chloromethyl)phenyl)-5-fluoropyridine. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.84 (m, 1H), 7.59 (dd, *J* = 7.5, 2.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 3H), 6.87 (dd, *J* = 8.1, 3.0 Hz, 1H), 5.28 (s, 2H), 3.71 (s, 2H), 3.38 (s,

3H), 2.94 (q, J = 7.5 Hz, 2H), 1.40 (s, 6H), 1.17 (t, J = 7.5 Hz, 3H). MS (ESI) m/z 433.2 $[M+H]^+$.

[00108] The compound of Example 5 shows good selectivity for PDE1 and inhibts PDE activity at an IC_{50} value of equal to or less than 30nM.

Example 7

7,8-Dihydro-2-(4-(5-fluoropyridin2-yl)benzyl)-3-propyl-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one

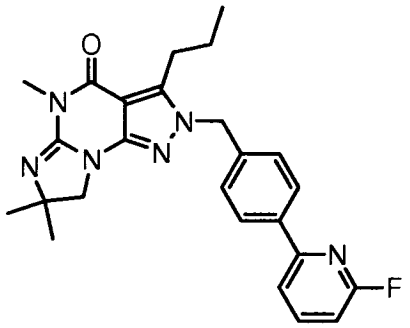


[00109] The title compound is prepared using the procedure analogous to the one described in Example 5 wherein propylmagnesium bromide was added in step (b) instead of ethylmagnesium bromide. MS (ESI) m/z 447.2 $[M+H]^+$.

[00110] The compound of Example 7 shows good selectivity for PDE1 and inhibts PDE activity at an IC_{50} value of equal to or less than 15nM.

Example 8

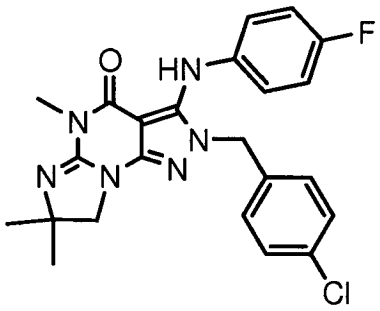
7,8-Dihydro-2-(4-(6-fluoropyridin2-yl)benzyl)-3-propyl-5,7,7-trimethyl-[2H]-imidazo-[1,2-a]pyrazolo[4,3-e]pyrimidin-4(5H)-one



[00111] The title compound is prepared using the procedure analogous to the one described in Example 5 wherein propylmagnesium bromide was added in step (b) instead of ethylmagnesium bromide, and 2-(4-(chloromethyl)phenyl)-6-fluoropyridine was added in step (a) instead of 2-(4-(chloromethyl)phenyl)-5-fluoropyridine. MS (ESI) m/z 447.2 $[M+H]^+$.

Example 9

7,8-Dihydro-2-(4-chlorobenzyl)-3-(4-fluorophenylamino)- 5,7,7-trimethyl-[2*H*]-imidazo-[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one



[00112] The title compound is prepared using the procedure analogous to the one described in Example 1 wherein 1-chloro-4-(chloromethyl)benzene was added in step (a) instead of 2-(4-(chloromethyl)phenyl)-pyridine. MS (ESI) m/z 453.2 $[M+H]^+$

[00113] The compound of Example 9 shows good selectivity for PDE1 and inhibts PDE activity at an IC_{50} value of equal to or less than 5nM.

Example 10: Measurement of PDEIB inhibition in vitro using IMAP Phosphodiesterase Assay Kit

[00114] 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.

[00115] 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-5 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.

[00116] In the phosphodiesterase assay, cGMP-fluorescein, which cannot be bound to IMAP, and therefore retains little fluorescence polarization, is converted to GMPfluorescein, which, when bound to IMAP, yields a large increase in fluorescence polarization (mp). Inhibition of phosphodiesterase, therefore, is detected as a decrease in mp.

Enzyme assay

[00117] 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).

[00118] 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 μmol 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), 10 mM Tris-HCl pH 7.2, 10 mM $MgCl_2$, 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 μL 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.

[00119] The FL-GMP conversion reaction is initiated by combining 4 parts enzyme and inhibitor mix with 1 part substrate solution (0.225 μL) 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 (mp).

[00120] A decrease in GMP concentration, measured as decreased mp, is indicative of inhibition of PDE activity. IC_{50} 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 IC_{50} values to be estimated using nonlinear regression software (XLFit; IDBS, Cambridge, MA)

[00121] Various compounds of Examples 1-9 demonstrate good selectivity for PDE1, and can inhibit PDE1 at IC_{50} values equal to or less than 50nM in the present assay.

EXAMPLE 11

[00122] A selective PDE1 inhibitor of the present invention demonstrates microsomal stability in human microsomal stability assays. The aforementioned selective PDE1

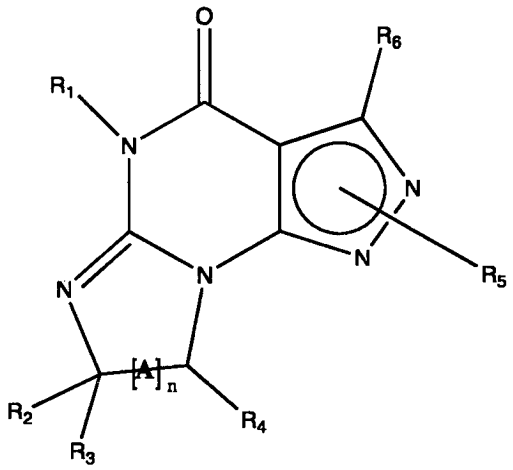
inhibitor demonstrates a K value less than 0.01, and demonstrates a half-life of T1/2 of about 100-1800 minutes.

EXAMPLE 12

[00123] A selective PDE1 inhibitor of the present invention demonstrates the ability to cross the blood-brain barrier. Following an injection of 10mg/kg in a suitable mouse model, the aforementioned selective PDE1 inhibitor is detectable at about 3 μM less than about 0.5 hours following the injection.

What is claimed:

1. A compound of Formula V



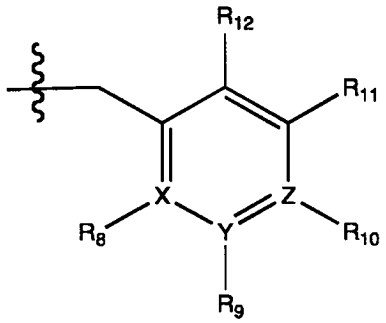
Formula V

wherein

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

(ii) R_4 is H 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);

(iii) 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

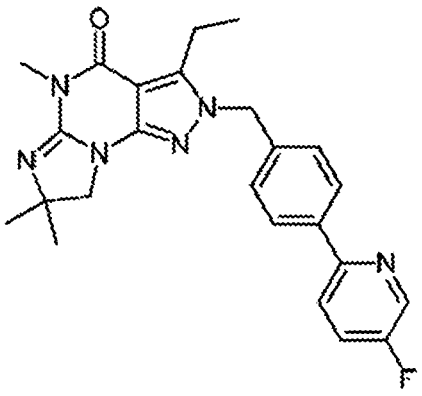
wherein X, Y and Z are C, and R_8, R_9, R_{11} and R_{12} are H, and R_{10} is halogen, or heteroaryl optionally substituted with halogen, alkyl, haloalkyl, hydroxy or carboxy (e.g., pyridyl or 2-halopyridyl, (for example, pyrid-2-yl, 5-fluoropyrid-2-yl or 6-fluoropyrid-2-yl)); and

(iv) R_6 is H, C_{1-4}alkyl, arylamino optionally substituted with C_{1-4}alkyl or halogen (e.g., phenylamino or 4-fluorophenylamino); and

(v) n=0;

in free or pharmaceutically acceptable salt form.

2. A compound according to claim 1, wherein wherein R_1 is methyl.
3. A compound according to any of claims 1-2, wherein R_2 and R_3 are C_{1-4} alkyl.
4. A compound according to any of claims 1-3, wherein R_2 and R_3 are both methyl.
5. A compound according to any of claims 1-4, wherein R_{10} is heteroaryl optionally substituted with halogen.
6. A compound according to any of claims 1-5, wherein R_{10} is pyrid-2-yl.
7. A compound according to any of claims 1-5, wherein R_{10} is 5-fluoro-pyrid-2-yl.
8. A compound according to any of claims 1-5, wherein R_{10} is 6-fluoro-pyrid-2-yl.
9. A compound according to any of claims 1-8, wherein R_6 is C_{1-4}alkyl.
10. A compound according to any of claims 1-9, wherein R_6 is ethyl.
11. A compound according to any of claims 1-9, wherein R_6 is propyl.
12. A compound according to any of claims 1-8, wherein R_6 is arylamino optionally substituted with C_{1-4}alkyl or halogen.
13. A compound according to any of claims 1-8 or 12, wherein R_6 is 4-fluorophenylamino.
14. A compound according to any one of claims 1-13, wherein the compound is selected from:



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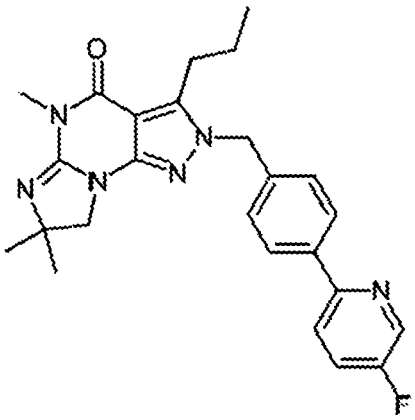

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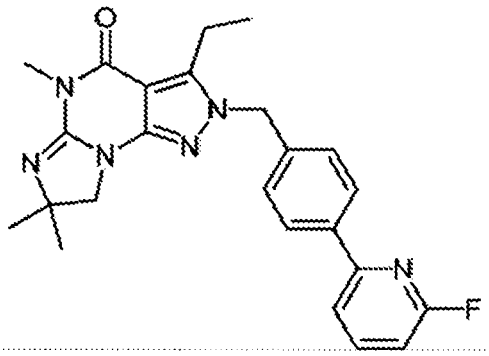

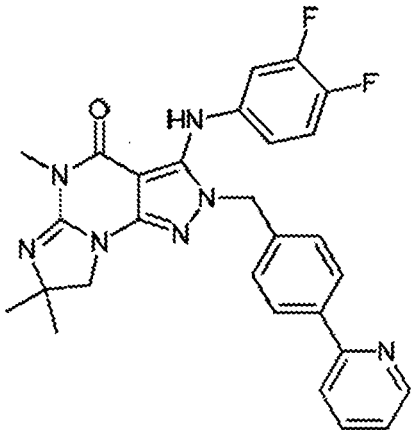

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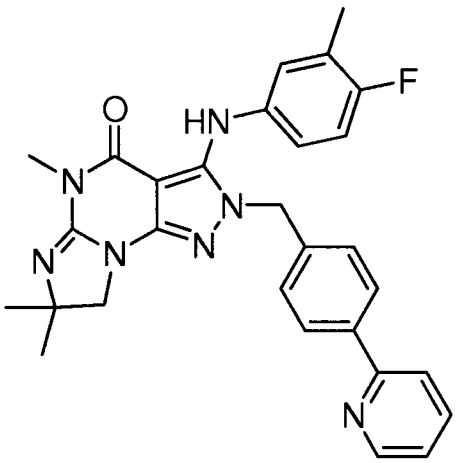
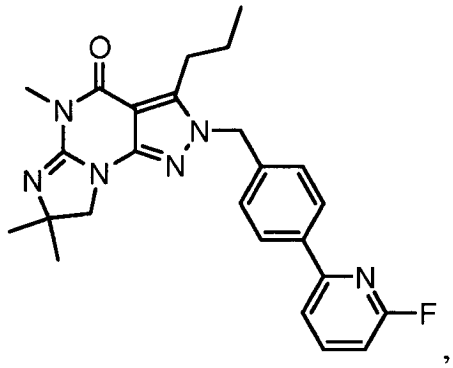


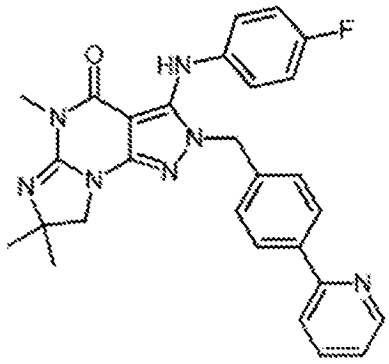
, or

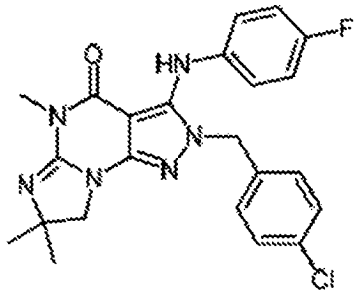


,

in free or pharmaceutically acceptable salt form.

15. A compound according to any one of claims 1-13, wherein the compound is selected from:





, or

in free or pharmaceutically acceptable salt form.

16. A pharmaceutical composition comprising a compound according to any one of claims 1-15 in admixture with at least one pharmaceutically acceptable carrier or excipient.

17. A method for the prophylaxis and/or treatment of a CNS disease, disorder, and/or injury, wherein the method comprises the administration of an effective amount of a PDE1 inhibitor to a subject, wherein the administration of the PDE1 inhibitor modulates the subject's level of intracellular cAMP, wherein the PDE1 inhibitor is a compound according to any one of claim 1-15 or a pharmaceutical composition according to claim 16.

18. A method according to claim 17, wherein the CNS disease, disorder, or injury is a spinal cord injury.

19. The method according to claim 17, wherein the CNS disease, disorder, or injury relates to motor neuron trauma.

20. The method according to any of claim 17-19, wherein the CNS disease, disorder, or injury is selected from the group consisting of: neurological traumas and injuries, surgery related trauma and/or injury, retinal injury and trauma, injury related to epilepsy, spinal cord injury, brain injury, brain surgery, trauma related brain injury, trauma related to spinal cord injury, brain injury related to cancer treatment, spinal cord injury related to cancer treatment, brain injury related to infection, brain injury related to inflammation, spinal cord injury related to infection, spinal cord injury related to inflammation, brain injury related to environmental toxins, and spinal cord injury related to environmental toxins.

21. The method according to any of claims 17-20, wherein the CNS disease, disorder, or injury is a neurodegenerative disorder.

22. The method according to claim 21, wherein the neurodegenerative disease, disorder, or injury is selected from the group consisting of: Alzheimer's disease, Multiple Sclerosis, Glaucoma, Frontotemporal dementia, Dementia with Lewy bodies, Corticobasal degeneration, Progressive supranuclear palsy, Prion disorders, Huntington's disease, Multiple system atrophy, Parkinson's disease, Amyotrophic lateral sclerosis, Hereditary spastic paraparesis, Spinocerebellar atrophies, Friedreich's ataxia, Amyloidoses, Metabolic (diabetes) related disorders, Toxin related disorders, chronic CNS inflammation, and Charcot Marie Tooth disease.

23. A method of treatment or prophylaxis of a PNS disease, disorder, or injury, wherein the method comprises administration of an effective amount of a PDE1 inhibitor to a subject in order to increase the subject's intracellular levels of cAMP, wherein the PDE1 inhibitor is a compound according to any one of claim 1-15 or a pharmaceutical composition according to claim 16.

24. A method according to any one of claims 17-23, wherein the PDE1 inhibitor is administered to a patient that is shown to have elevated intracellular calcium

levels compared to a control subject (e.g., reference standard).

25. A method of prophylaxis of the development of a CNS disease or disorder in a subject that is at risk for developing a CNS disease or disorder, wherein the method comprises:

1.) Obtaining a CNS sample from the subject;

2.) Measuring the levels of intracellular calcium from the sample;

3.) Comparing the levels of intracellular calcium in the biological sample to a reference standard;

4.) Determining whether a patient is at risk for developing a CNS disease or disorder based upon the level of intracellular calcium compared to the reference standard;

5.) Administering a PDE1 inhibitor to a subject based upon the subject's levels of intracellular calcium put them at risk for the development of a CNS disease or disorder (e.g., administration of a PDE1 inhibitor to a subject because they have elevated intracellular calcium levels compared to the reference standard), wherein the PDE1 inhibitor is a compound according to any one of claim 1-15 or a pharmaceutical composition according to claim 16.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/50814

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/519 (2015.01)
CPC - C07D 487/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/519 ; A61P 25/28, 25/16, 25/18, (2015.01)
CPC - C07D 487/14

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); ProQuest; Ebsco; Google; Google Scholar; SureChem; PubMed; PubChem; 7,8-dihydro-imidazo[1,2-alpha]pyrazolo[4,3-e]pyrimidin-4-one; Phosphodiesterase 1; PDE1 inhibitors; Central nervous system; CNS diseases; disorders; injury; Parkinson's disease; Depression; schizophrenia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/133261 A2 (INTRA-CELLULAR THERAPIES, INC) 14 December 2006; abstract; paragraph [0008].	1-2 and 3/1-2
A	US 8,697,710 B2 (INTRA-CELLULAR THERAPIES, INC) 15 April 2014; abstract; column 3, line 1 to column 5, line 35.	1-2 and 3/1-2
A	US 2013/0239234 A1 (GREENGARD P et al.) 12 September 2013; abstract; paragraphs [0308]-[0311].	1-2 and 3/1-2

☐ 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
13 November 2015 (13.11.2015)	18 DEC 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas 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/US15/50814

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.: 4-25
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.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2017.08.29

(21)申请号 201580062061.3
(22)申请日 2015.09.17
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2017.05.16
(86)PCT国际申请的申请数据
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(87)PCT国际申请的公布数据
WO2016/044667 EN 2016.03.24
(71)申请人 细胞内治疗公司
地址 美国纽约州
(72)发明人 P·李 H·郑 J·赵
L·文诺格勒
(74)专利代理机构 北京市中咨律师事务所 11247
代理人 杨春刚 黄革生
(51)Int.Cl.
A61K 31/519(2006.01)

权利要求书4页 说明书31页

(54)发明名称

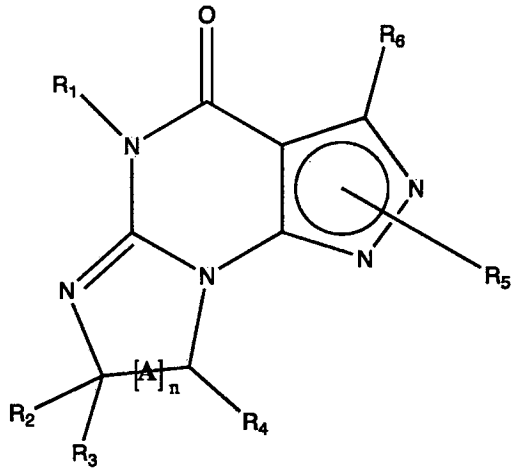
化合物和方法

(57)摘要

本申请的主题广泛涉及治疗和/或预防CNS疾病、病症和/或损伤的化合物和方法。在一方面，本申请的主题涉及作为神经保护剂和/或神经再生剂的磷酸二酯酶1(PDE1)抑制剂。在另一方面，本申请的主题涉及具有发生CNS疾病或病症的风险的个体。

CN 107106563 A

1.游离或药学上可接受的盐形式的式V化合物



式V

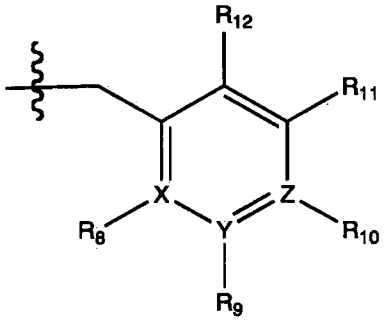
其中

(i)R_1是C_{1-4}烷基(例如甲基)；

(ii)R_4是H，且R_2和R_3独立地是H或C_{1-4}烷基

(例如R_2和R_3都是甲基，或者R_2是H，而R_3是异丙基)；

(iii)R_5连接至式V的吡唑并部分的一个氮上，并且是式A的基团



式A

其中，X、Y和Z是C，并且R_8、R_9、R_{11}和R_{12}是H，且R_{10}是卤素或者任选被卤素、烷基、卤代烷基、羟基或羧基取代的杂芳基(例如吡啶基或2-卤代吡啶基(例如吡啶-2-基、5-氟吡啶-2-基或6-氟吡啶-2-基))；且

(iv)R_6是H、C_{1-4}烷基、任选被C_{1-4}烷基或卤素取代的芳基氨基(例如苯基氨基或4-氟苯基氨基)；且

(v)n＝0。

2.根据权利要求1所述的化合物，其中R_1是甲基。

3.根据权利要求1-2中任何一项所述的化合物，其中R_2和R_3是C_{1-4}烷基。

4.根据权利要求1-3中任何一项所述的化合物，其中R_2和R_3都是甲基。

5.根据权利要求1-4中任何一项所述的化合物，其中R_{10}是任选被卤素取代的杂芳基。

6.根据权利要求1-5中任何一项所述的化合物，其中R_{10}是吡啶-2-基。

7.根据权利要求1-5中任何一项所述的化合物，其中R_{10}是5-氟-吡啶-2-基。

8.根据权利要求1-5中任何一项所述的化合物，其中R_{10}是6-氟-吡啶-2-基。

9.根据权利要求1-8中任何一项所述的化合物，其中R_6是C_{1-4}烷基。

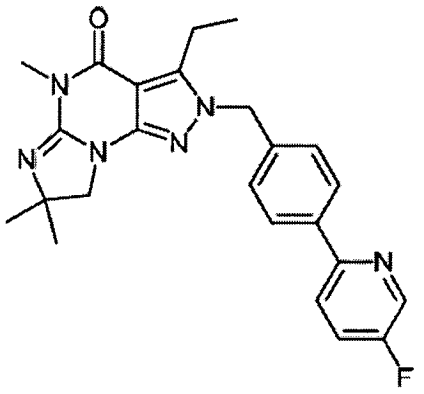
10.根据权利要求1-9中任何一项所述的化合物，其中R_6是乙基。

11.根据权利要求1-9中任何一项所述的化合物，其中R_6是丙基。

12.根据权利要求1-8中任何一项所述的化合物，其中R_6是任选被C_{1-4}烷基或卤素取代的芳基氨基。

13.根据权利要求1-8或12中任何一项所述的化合物，其中R_6是4-氟苯基氨基。

14.根据权利要求1-13中任何一项所述的化合物，其中所述化合物选自游离或药学上可接受的盐形式的：



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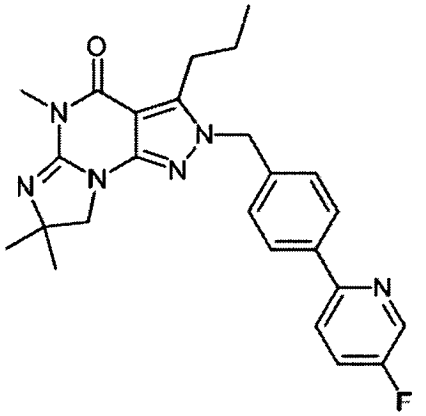

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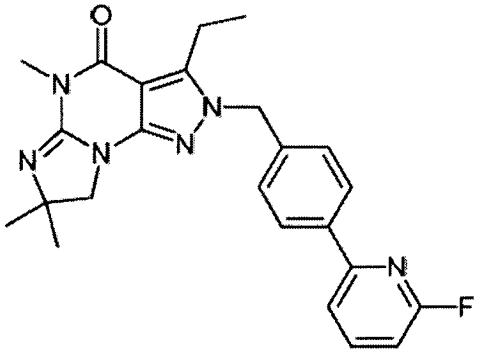

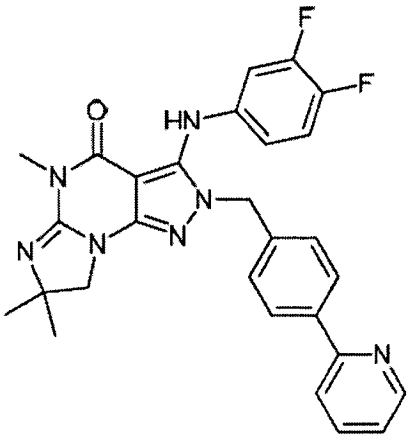

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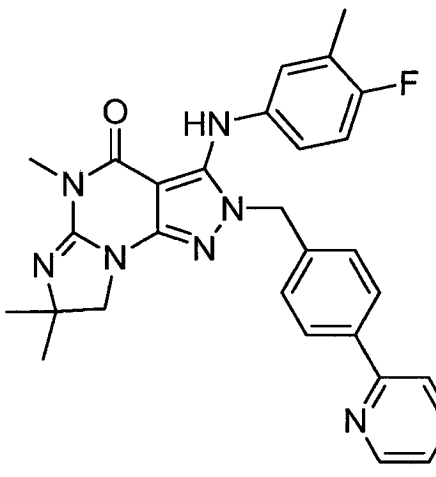
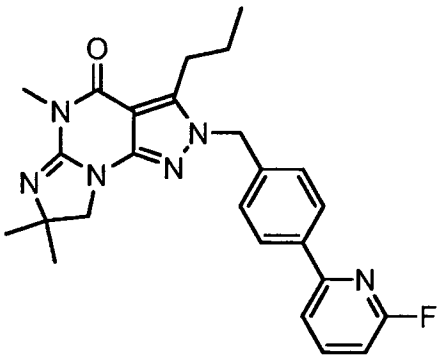
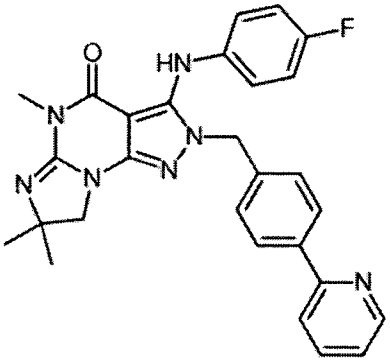


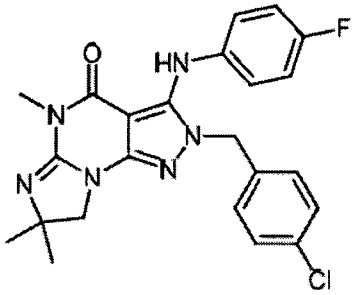
或



15.根据权利要求1-13中任何一项所述的化合物，其中所述化合物选自游离或药学上可接受的盐形式的：



或



16.药物组合物，其含有根据权利要求1-15中任何一项所述的化合物，以及至少一种药学上可接受的载体或赋形剂。

17.用于预防和/或治疗CNS疾病、病症和/或损伤的方法，其中该方法包括向个体施用有效量的PDE1抑制剂，其中PDE1抑制剂的施用调节个体的细胞内cAMP水平，其中PDE1抑制剂是根据权利要求1-15中任何一项所述的化合物或者根据权利要求16所述的药物组合物。

18.根据权利要求17所述的方法，其中CNS疾病、病症和/或损伤是脊髓损伤。

19.根据权利要求17所述的方法，其中CNS疾病、病症和/或损伤与运动神经元创伤相关。

20.根据权利要求17-19中任何一项所述的方法，其中CNS疾病、病症和/或损伤选自：神经创伤和损伤、手术相关的创伤和/或损伤、视网膜损伤和创伤、与癫痫相关的损伤、脊髓损伤、脑损伤、脑部手术、创伤相关的脑损伤、创伤相关的脊髓损伤、与癌症治疗相关的脑损伤、与癌症治疗相关的脊髓损伤、与感染相关的脑损伤、与炎症相关的脑损伤、与感染相关的脊髓损伤、与炎症相关的脊髓损伤、与环境毒素相关的脑损伤和与环境毒素相关的脊髓损伤。

21.根据权利要求17-20中任何一项所述的方法，其中CNS疾病、病症和/或损伤是神经退行性病症。

22.根据权利要求21所述的方法，其中神经退行性疾病、病症或损伤选自：阿尔茨海默氏病、多发性硬化、青光眼、额颞叶型痴呆、路易体痴呆、皮质基底变性、进行性核上麻痹、朊病毒病症、亨廷顿氏病、多系统萎缩、帕金森氏病、肌萎缩侧索硬化、遗传性痉挛性轻截瘫、脊髓小脑萎缩、弗莱德里希共济失调、淀粉样变性、代谢(糖尿病)相关的病症、毒素相关的

病症、慢性CNS炎症和夏科-马里-图斯病。

23.治疗或预防PNS疾病、病症或损伤的方法，其中所述方法包括向个体施用有效量的PDE1抑制剂，以便升高个体的细胞内cAMP水平，其中PDE1抑制剂是根据权利要求1-15中任何一项所述的化合物或者根据权利要求16所述的药物组合物。

24.根据权利要求17-23中任何一项所述的方法，其中将PDE1抑制剂施用至患者，与对照个体(例如参考标准)相比，所述患者显示具有升高的细胞内钙水平。

25.在具有发生CNS疾病或病症风险的个体中预防CNS疾病或病症发生的方法，其中所述方法包括：

1.)从个体获得CNS样品；

2.)测量样品的细胞内钙水平；

3.)将生物样品中的细胞内钙水平与参考标准进行比较；

4.)与参考标准相比，基于细胞内钙水平，确定患者是否具有发生CNS疾病或病症的风险；

5.)基于个体的使他们具有发生CNS疾病或病症的风险的细胞内钙水平，向个体施用PDE1抑制剂(例如向个体施用PDE1抑制剂，因为与参考标准相比，其具有升高的细胞内钙水平)，其中PDE1抑制剂是根据权利要求1-15中任何一项所述的化合物或者根据权利要求16所述的药物组合物。

化合物和方法

[0001] 相关申请的交叉引用

[0002] 本国际申请要求2014年9月17日提交的早期提交的美国临时申请US 62/051,735和2014年9月18日提交的US 62/052,283的优先权，将其以全部引入文中作为参考。

发明领域

[0003] 本领域广泛涉及治疗和/或预防中枢神经系统(CNS)疾病、病症和/或损伤的化合物和方法。一方面，本领域涉及作为神经保护剂和/或神经再生剂的磷酸二酯酶1(PDE1)抑制剂。另一方面，本领域涉及在具有患CNS疾病或病症的风险的个体中，预防CNS疾病或病症的发生。

[0004] 发明背景

[0005] 环核苷酸磷酸二酯酶(PDEs)通过将这些环核苷酸水解为其各自的5'-单磷酸(5'AMP和5'GMP)下调细胞内cAMP和cGMP信号转导。已鉴定了11个家族的磷酸二酯酶，但是仅第I家族的PDEs，即经Ca^{2+}-钙调蛋白活化的Ca^{2+}-钙调蛋白-依赖性磷酸二酯酶(CaM-PDE)，已经被证明介导钙和环核苷酸(例如cAMP和cGMP)信号通路。三种已知的CaM-PDE基因，即PDE1A、PDE1B和PDE1C，均在中枢神经系统组织中有表达。PDE1A在脑的各处均有表达，在海马区的CA1至CA3层以及小脑中的表达水平较高，在纹状体中的表达水平低。PDE1A还在肺和心脏中表达。PDE1B主要在纹状体、齿状回、嗅束和小脑中表达，并且其表达与具有高水平多巴胺能神经分布的脑区域有关。虽然PDE1B主要在中枢神经系统中表达，但是其可以在心脏中被检测到，存在于嗜中性粒细胞中，并且已被证明参与该细胞的炎症反应。PDE1C在嗅上皮、小脑颗粒细胞、纹状体、心脏和血管平滑肌中表达。

[0006] CaM-PDE在介导脑细胞内、特别是称为基底核或纹状体的脑区域内的脑细胞的信号转导方面起关键作用。例如，NMDA-型谷氨酸受体激活和/或多巴胺D2受体激活导致细胞内钙浓度增加，从而导致效应子如钙调蛋白依赖性激酶II(CaMKII)和钙调磷酸酶的激活以及CaM-PDE的激活，导致cAMP和cGMP降低。另一方面，多巴胺D1受体激活导致腺苷酸环化酶的激活，从而致使cAMP增加。该环核苷酸又激活蛋白激酶A(PKA；cAMP-依赖性蛋白激酶)。cGMP的产生已知通过各种刺激例如细胞内高钙水平诱导的一氧化氮产生发生在与认知功能相关的组织，并已知随后活化蛋白激酶G(PKG；cGMP-依赖性蛋白激酶)。PKG和PKA磷酸化下游信号传导通路元件如DARPP-32(多巴胺和cAMP-调节的磷蛋白)和cAMP应答元件结合蛋白(CREB)。磷酸化的DARPP-32接着抑制蛋白磷酸-1(PP-1)的活性，由此增加底物蛋白质例如孕酮受体(PR)的磷酸化状态，致使诱导生理反应。在精神分裂症中D1受体信号传导被破坏，引起所述疾病中的认知损害。已在动物试验中完全确定cAMP和cGMP在认知功能方面中的作用。啮齿动物中的研究已表明通过多巴胺D1或孕酮受体的活化，诱导cAMP和cGMP合成，促进与各种生理反应相关的孕酮信号传导，包括与一些啮齿动物交配感受性相关的脊柱前凸应答。参见Mani等人，Science(2000)287:1053，将其内容引入文中作为参考。

[0007] 因此，CaM-PDE可影响基底核(纹状体)中的多巴胺调节的和其它的细胞内信号传导通路，包括但不限于一氧化氮、去甲肾上腺素能、神经降压素、CCK、VIP、血清素、谷氨酸

(例如NMDA受体、AMPA受体)、GABA、乙酰胆碱、腺苷(例如A2A受体)、大麻素受体、利尿钠肽(例如ANP、BNP、CNP)、DARPP-32和内啡肽细胞内信号通路。

[0008] 磷酸二酯酶(PDE)活性、特别是磷酸二酯酶1(PDE1)活性在脑组织中作为自主活动和学习及记忆的调节器起作用。PDE1是调节细胞内信号通路的治疗靶点,优选在神经系统中,包括但不限于多巴胺D1受体、多巴胺D2受体、一氧化氮、去甲肾上腺素能、神经降压素、CCK、VIP、血清素、谷氨酸(例如NMDA受体、AMPA受体)、GABA、乙酰胆碱、腺苷(例如A2A受体)、大麻素受体、利尿钠肽(例如ANP、BNP、CNP)、内啡肽细胞内信号通路和孕酮信号通路。例如,PDE1B的抑制通过保护cGMP和cAMP免受降解应当加强多巴胺D1激动剂的作用,并且通过抑制PDE1活性(这是D2受体介导的细胞内钙增加的结果)应当类似地抑制多巴胺D2受体信号通路。细胞内钙水平的慢性增加与多种病症中的细胞死亡有关,特别是与神经变性疾病如阿尔茨海默氏病、帕金森病和亨廷顿病以及导致中风和心肌梗塞的循环系统病症中的细胞死亡有关。因此,PDE1抑制剂可潜在地用于特征在于多巴胺D1受体信号传导活性降低的疾病,例如帕金森病、不宁腿综合征、抑郁、发作性睡病和认知缺损如与精神分裂症相关的认知缺损。PDE1抑制剂还可用于可通过增加孕酮信号传导而缓和的疾病例如雌性性功能障碍。

[0009] 此外,神经发生是动物和人脑内的生命过程,据此,在有机体的整个生命期内,新的神经细胞不断产生。新形成的细胞能分化成神经系统的功能细胞,并整合于脑中现存的神经回路中。神经发生已知在成年期持续于哺乳动物脑部的两个区域:侧脑室的室下区(SVZ)和海马的齿状回。在所述区域,多能神经祖细胞(NPCs)继续分裂,并产生新的功能神经元和胶质细胞。已表明各种因素可刺激成人海马神经发生例如肾上腺切除术、自觉锻炼、富裕环境、海马依赖的学习和抗抑郁剂。其他因素例如肾上腺激素、应激、年龄和药物滥用负面影响神经发生。

[0010] 尽管神经发生的重要性不能被夸大,然而脊髓损伤后,轴突再生的失败仍然是医学和神经科学面临的最大挑战之一。不同于周围神经系统的有髓鞘的轴突,中枢神经系统的有髓鞘的轴突切断后不再生。然而,重要进展一直是识别围绕CNS轴突的髓鞘中的抑制蛋白。一些生物活性分子看起来抑制轴突生长,致使CNS神经元再生失败。髓磷脂含有一些已显示其抑制轴突生长过程的蛋白质。NogoA,即网状内皮素(reticulon)家族的一员,是确定为轴突生长抑制剂的第一种蛋白质。它由少突细胞和一些神经元表达,并可发现于细胞内和细胞表面(特别是在轴突的髓鞘上)。可有助于抑制轴突再生的其他蛋白质包括髓磷脂相关的糖蛋白(MAG)、少突细胞-髓磷脂糖蛋白(OMgp)和蛋白聚糖versican。

[0011] 因此,看起来CNS环境限制损伤后的轴突再生。事实上,已将CNS髓磷脂确定为促使再生失败的主要因素。存在证据表明髓鞘中存在的CNS蛋白质抑制轴突生长和再生。

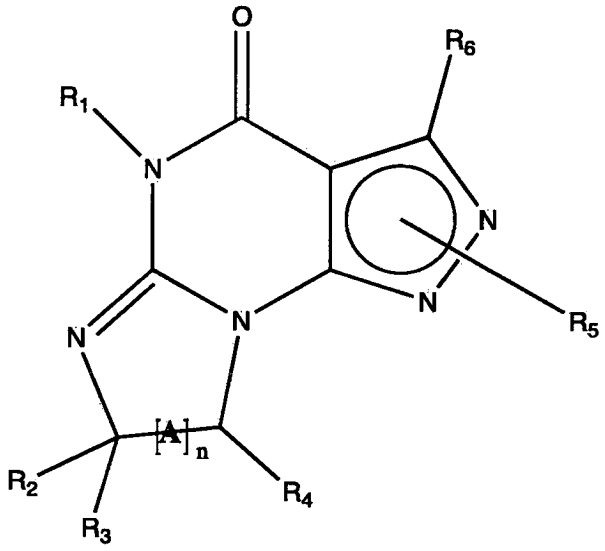
[0012] 对于克服轴突再生的抑制,已提出多种策略。一直有效的一种策略是提高细胞内cAMP水平。所述可用几种方法实现,例如:条件性外周损伤、cAMP类似物的施用、用神经营养因子引发或者用磷酸二酯酶抑制剂咯利普兰(PDE4抑制剂)治疗。cAMP的作用可以是转录依赖的,并且cAMP-介导的CREB活化可引起基因例如精氨酸酶I和白细胞介素-6的上调和表达。认为这些基因的产物促进轴突再生,其增加其他cAMP-调节的基因可产生其他物质的可能性,所述物质将有利于脊髓损伤的治疗。然而,对于增加IL-6的表达,这一作用机制的一个显著缺点可能是IL-6是一个潜在有害的促炎细胞因子,意味着,可能的是高水平的IL-6

实际上能恶化脊髓损伤后发生的炎症，然后，这可引起细胞死亡的增加。实际上，支持该问题的因素是已观察到IL-6转基因小鼠具有广泛的星形胶质细胞增生、神经变性和血脑屏障的破坏。

[0013] 发明简述

[0014] 本发明提供游离、盐或前药形式的式V化合物，包括其对映体、非对映异构体和外消旋物：

[0015]



式 V

[0016] 其中

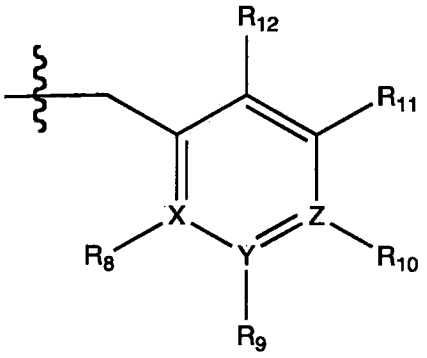
[0017] (i) R_1是C_{1-4}烷基(例如甲基)；

[0018] (ii) R_4是H，且R_2和R_3独立地是H或C_{1-4}烷基

[0019] (例如R_2和R_3都是甲基，或者R_2是H，而R_3是异丙基)；

[0020] (iii) R_5连接至式V的吡唑并部分的一个氮上，并且是式A的基团

[0021]



式 A

[0022] 其中，X、Y和Z是C，并且R_8、R_9、R_{11}和R_{12}是H，以及R_{10}是卤素(例如氯)或者任选被卤素、烷基、卤代烷基、羟基或羧基取代的杂芳基(例如吡啶基或2-卤代吡啶基，(例如吡啶-2-基、5-氟吡啶-2-基或6-氟吡啶-2-基))；且

[0023] (iv) R_6是H、C_{1-4}烷基(例如甲基、乙基或丙基)、任选被C_{1-4}烷基或卤素取代的芳基氨基(例如苯基氨基或4-氟苯基氨基)或者硫C_{1-4}烷基(例如硫乙基(thioethyl))；且

[0024] (v) n＝0。

[0025] 另一方面，本发明考虑PDE1抑制剂(例如式V)是根据下面任何一式所述的式V化合物：

[0026] 1.1式V化合物，其中R_1是甲基；

[0027] 1.2式V或1.1化合物，其中R_2和R_3是C_{1-4}烷基；

[0028] 1.3式V或1.1-1.2中任何一式的化合物，其中R_2和R_3都是甲基；

[0029] 1.4式V或1.1-1.3中任何一式的化合物，其中R_{10}是任选被卤素取代的杂芳基；

[0030] 1.5式V或1.1-1.4中任何一式的化合物，其中R_{10}是吡啶-2-基；

[0031] 1.6式V或1.1-1.4中任何一式的化合物，其中R_{10}是5-氟-吡啶-2-基；

[0032] 1.7式V或1.1-1.4中任何一式的化合物，其中R_{10}是6-氟-吡啶-2-基；

[0033] 1.8式V或1.1-1.7中任何一式的化合物，其中R_6是C_{1-4}烷基；

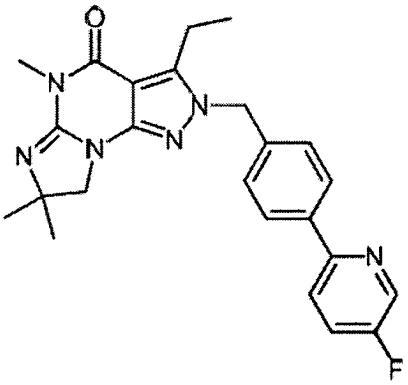
[0034] 1.9式V或1.1-1.8中任何一式的化合物，其中R_6是乙基；

[0035] 1.10式V或1.1-1.8中任何一式的化合物，其中R_6是丙基；

[0036] 1.11式V或1.1-1.7中任何一式的化合物，其中R_6是任选被C_{1-4}烷基或卤素取代的芳基氨基；

[0037] 1.12式V或1.1-1.7中任何一式的化合物，其中R_6是4-氟苯基氨基；

[0038] 1.13上述任何一式，其中所述化合物选自游离、盐或前药形式的下列化合物，包括其对映体、非对映异构体和外消旋物：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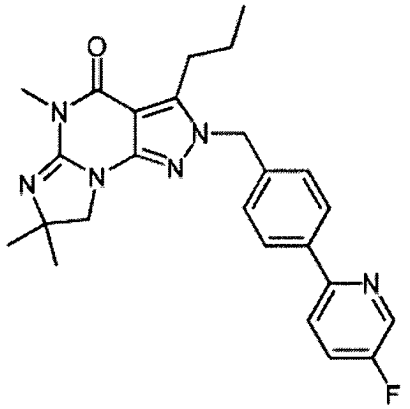
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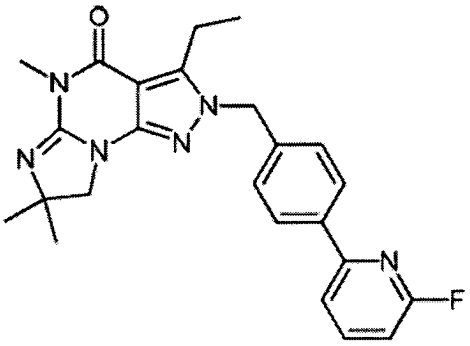
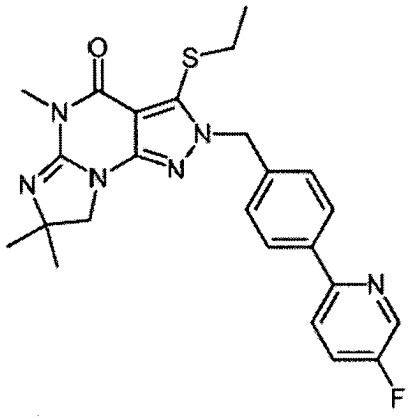
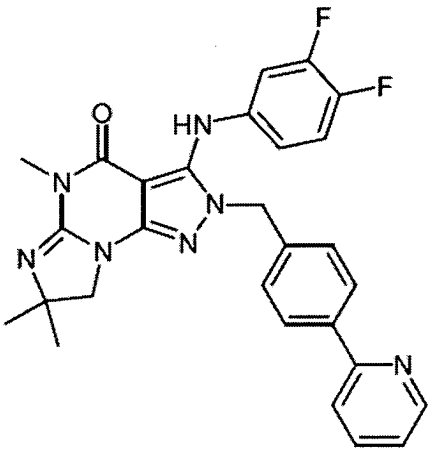
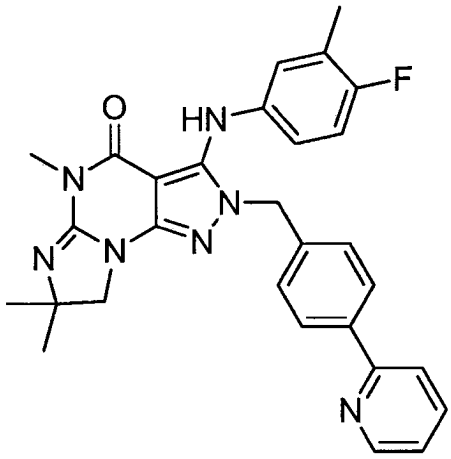
[0039]


或
，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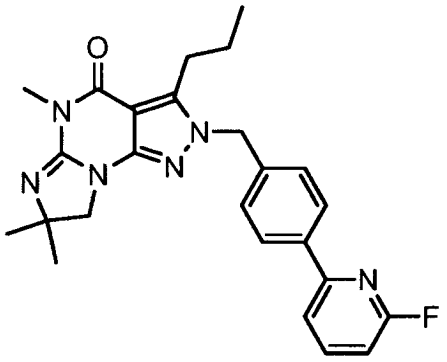
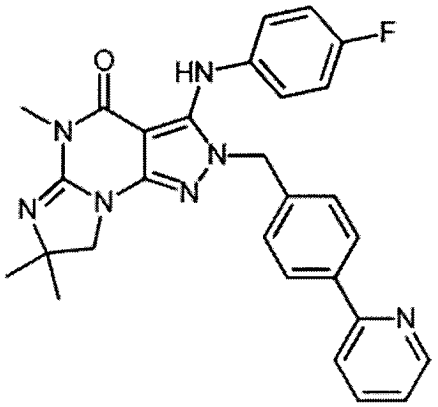
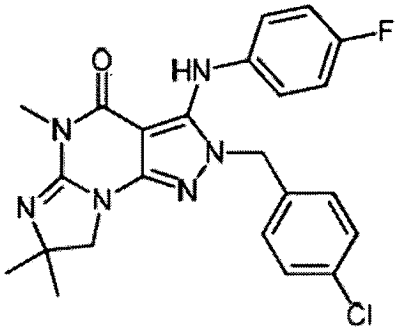
[0040] 

或

[0041] 1.14上述任何一式，其中所述化合物选自游离、盐或前药形式的下列化合物，包括其对映体、非对映异构体和外消旋物：

[0042] 

或



[0043] 一方面，前述任何一式(例如式V或1.1-1.14)的选择性PDE1抑制剂是抑制磷酸二酯酶-介导的(例如PDE1-介导的，尤其是PDE1A或PDE1C-介导的)cGMP水解的化合物，例如在固定化金属亲和颗粒试剂PDE测试中，游离或盐形式的优选化合物具有低于1M、优选低于500nM，并更优选低于50nM的IC_{50}。

[0044] 本发明的优点之一是PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)可作为神经保护剂和/或神经再生剂。对于CNS损伤(例如脊髓损伤)、疾病或病症，即使在轴突再生抑制剂存在下，文中公开的化合物和方法可以用于辅助或提高轴突生长和轴突再生。

[0045] 不受任何具体理论的束缚，认为：本发明的至少一个优点是PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)的施用可增加细胞内cAMP水平和启动基因转录，所述基因是在CNS疾病、病症或损伤情况下，克服轴突再生抑制和促进轴突生长和/或轴突再生所必需的。例如，增加的细胞内cAMP，例如由PDE1抑制引起的，将使cAMP-依赖蛋白例如蛋白激酶C(PKC)的活性增加。

[0046] 此外，认为：PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)的施用可升高cAMP和cGMP的细胞内水平。未受理论束缚，所述cAMP和cGMP的升高可抗衡与缓慢升高的细胞内钙水平相关的潜在有害效应。已观察到：细胞内钙水平升高可能与各种退行性疾病的发展相关。例如，一个可能解释是细胞内钙水平的升高(例如细胞内钙水平的慢性升高)引起PDE1活化，然后，PDE1刺激cAMP水解。然后，降低的cAMP浓度灭活cAMP-依赖蛋白例如蛋白激酶C(PKC)。

[0047] 然而，不受任何理论束缚，认为：施用PDE1抑制剂(例如式V或1.1-1.14中任何一式

的化合物)的另一个潜在益处是细胞内cGMP的增加。所述细胞内cGMP的增加可引起PKG活性的增加,预防细胞内钙水平的进一步增加。因此,不受任何理论束缚,PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)的施用可具有双重益处,例如在轴突再生(和/或神经保护)中起有益作用,并且同时降低可能与细胞内钙水平升高相关的有害效应。

[0048] 在一实施方案中,本发明包括治疗或预防CNS疾病、病症或损伤(例如脊髓损伤,例如脊髓性肌萎缩,例如运动神经元损伤)的组合物和方法,其中所述方法包括施用有效量的PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物),以调控cAMP和/或cGMP的细胞内水平。在一实施方案中,该细胞内cAMP的增加是神经保护的和/或有助于神经发生的增加或刺激(例如PDE1抑制剂增加轴突生长和/或轴突再生)。

[0049] 还在另一实施方案中,本发明包括治疗或预防周围神经系统(PNS)损伤的组合物和方法,其中所述方法包括施用PDE1抑制剂,以增加cAMP和/或cGMP的细胞内水平,其直接或间接增加神经再生和/或保护免于进一步神经损伤。

[0050] 另一实施方案中,本发明包括在具有形成CNS疾病或病症风险的个体中,预防CNS疾病或病症的组合物和方法,其中所述方法包括:

[0051] 1.)从个体中获得CNS样品;

[0052] 2.)测量样品中细胞内的钙水平;

[0053] 3.)将所述生物样品中的细胞内钙水平与参照标准进行比较;

[0054] 4.)与参考标准相比,基于细胞内钙水平,确定患者是否处于形成CNS疾病或病症的风险中;

[0055] 5.)基于个体的细胞内钙水平,将PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)施用至个体(例如将PDE1抑制剂施用至个体,因为与参考标准相比,其具有升高的细胞内钙水平)。

[0056] 上下文若无特别说明或不是可以清楚地看出的话,如本文所用的下述术语具有如下含义:

[0057] (a)如本文所用的"烷基"是饱和或不饱和的烃基团,优选是饱和的,优选有1至6个碳原子,其可以是直链或支链的,并且可以任选例如被卤素(例如氯或氟)、羟基或羧基单-、二-或三-取代。

[0058] (b)如本文所用的"环烷基"是饱和或不饱和的非芳香性的烃基团,优选是饱和的,优选包含3至9个碳原子,其中至少一些原子形成非芳性的单环或二环或者桥环结构,并且其可以任选被取代,例如被卤素(例如氯或氟)、羟基或羧基取代。其中,环烷基任选包含1个或多个选自N和O和/或S的原子,所述环烷基还可以是杂环烷基。

[0059] (c)除非另外说明,"杂环烷基"是饱和或不饱和的非芳香性的烃基团,优选饱和的,优选包含3-9个碳原子,至少其中一些原子形成非芳香性的单-或双环或者桥环结构,其中至少一个碳原子被N、O或S取代,所述杂环烷基可以是任选被取代的,例如被卤素(例如氯或氟)、羟基或羧基取代。

[0060] (d)如本文所用的"芳基"是单环或二环芳族烃,优选是苯基,其是任选被取代的,例如被烷基(例如甲基)、卤素(例如氯或氟)、卤代烷基(例如三氟甲基)、羟基、羧基或另外的芳基或杂芳基(例如联苯基或吡啶基苯基)取代。

[0061] (e)如本文所用的"杂芳基"是其中构成芳环的原子中的一个或多个是硫或氮而非

碳的芳族基团，例如吡啶基或噻二唑基，其可以任选被取代，例如被烷基、卤素、卤代烷基、羟基或羧基取代。

[0062] (f)预期的是当取代基以“ene”结尾(“即为亚基”)时，例如亚烷基、亚苯基或芳基亚烷基，所述取代基旨在桥接或者连接两个其他取代基。因此，亚甲基预期是-CH_2-，并且亚苯基预期是-C_6H_4-，并且芳基亚烷基预期是例如-C_6H_4-CH_2-或-CH_2-C_6H_4-。

[0063] 在本说明书中，除非另外指示，诸如“本发明化合物”的表述将理解为包括任意形式的化合物，例如游离或酸加成盐形式，或者当化合物含有酸性取代基时，还包括碱加成盐形式。本发明化合物意欲用作药物，因而优选药学上可接受的盐。不适于药物用途的盐可能是有用的，例如对于本发明化合物或其药学上可接受的盐的分离或纯化而言，因而这类盐也被包括在内。

[0064] 本发明化合物(包含文中公开的任何化合物)可以以游离或盐形式、例如酸加成盐形式存在。在本说明书中，除非另外指明，诸如“本发明化合物”的表述将理解为包括任意形式的化合物，例如游离或酸加成盐形式，或者当化合物含有酸性取代基时，碱加成盐形式。本发明化合物意欲用作药物，因而优选药学上可接受的盐。不适于药物用途的盐可能是有用的，例如对于本发明游离化合物或其药学上可接受的盐的分离或纯化而言，因而这类盐也被包括在内。

[0065] 本发明化合物在某些情况下以前药形式存在。前药是在体内转化成本发明化合物的化合物。例如，当本发明化合物含有羟基或羧基取代基时，这些取代基可以形成生理学上可水解的和可接受的酯。如本文所用的“生理学上可水解的和可接受的酯”指在生理条件下可以水解产生酸(在本发明化合物具有羟基取代基的情况下)或醇(在本发明化合物具有羧基取代基的情况下)的本发明化合物的酯，它们自身在欲施用剂量下是生理学上可耐受的。因此，当本发明化合物包含羟基基团例如化合物-OH时，所述化合物的酰基酯前药即化合物-O-C(O)-C_{1-4}烷基可在体内水解，一方面形成生理学上可水解的醇(化合物-OH)，且另一方面形成羧酸(例如HOC(O)-C_{1-4}烷基)。或者，当本发明化合物含有羧酸例如化合物-C(O)OH时，所述化合物的酸酯前药即化合物-C(O)O-C_{1-4}烷基可水解，形成化合物-C(O)OH和醇HO-C_{1-4}烷基。正如将被理解的那样，该术语因而囊括常规的药用前药形式。

[0066] 另一方面，本发明还提供药物组合物，其含有游离或药学上可接受的盐形式的本发明化合物以及药学上可接受的载体。

[0067] 制备本发明化合物的方法

[0068] 本发明化合物及它们的药学上可接受的盐可以采用本文所描述和所举例的方法、与之类似的方法和化学领域已知的方法来制备。这类方法包括但不限于下文所述的那些。如果不能商业获得，那么用于这些方法的原料可以通过从化学领域所选择的方法采用类似于或相似于已知化合物的合成的技术来制备。

[0069] 各种原料和/或本发明化合物可利用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、US 2014/0194396、PCT/US14/30412中所述的方法制备，并将各参考文献整体引入本文作为参考。

[0070] 本发明化合物包括其对映异构体、非对映异构体和外消旋物，以及其多晶型物、水

合物、溶剂合物和复合物。本发明范围内的一些个体化合物可以含有双键。在本发明中双键的陈述包括双键的E和Z异构体两者。另外，本发明范围内的一些化合物可以含有一个或多个不对称中心。本发明包括任一种光学纯的立体异构体以及立体异构体的任意组合的应用。

[0071] 还预期的是本发明化合物包括其稳定和不稳定的同位素。也就是说，本发明化合物包括用所述原子的任何稳定或不稳定同位素变体代替或富集结构中的任一原子或多于一个原子。同位素是含有不同中子数的相同元素的原子。同位素变体是除其天然最丰富同位素之外，任何元素的任何同位素。与相同元素天然最丰富的核素相比，同位素变体将包含1个或多个额外的或者1个或多个更少的中子。同位素可以是稳定的(非放射性的)或者不稳定的(放射性的)。例如，碳的天然最丰富的核素是^{12}C，并且1个已知的碳的稳定同位素是^{13}C。元素的同位素通常共享相同的电子和化学特性。预期的是包含所述同位素的化合物的活性将保持，并且所述化合物还将具有用于测量非同位素类似物的药代动力学的实用性。例如，本发明化合物的1个或多个原子位置的氢原子可以被氘取代(或富集氘)。已知的稳定同位素的示例包括但不限于氘(^{2}H)、^{13}C、^{15}N和^{18}O。已知的不稳定同位素的示例包括^{3}H、^{123}I、^{131}I、^{125}I、^{11}C、^{18}F。不稳定同位素可用于放射-成像和/或本发明化合物的药代动力学研究。本发明化合物的1个或多个原子位置可被任何已知的同位素变体替换或者富集任何已知的同位素变体。化学品和试剂的天然来源通常不是同位素纯的，以至于通过传统化学方法制备的本发明化合物一般将含有同位素丰度的正常、天然变化。例如元素碳的自然丰度大约由98.93%^{12}C和1.07%^{13}C构成。因此，通过传统化学手段制备的本发明化合物在结构的各碳原子上，通常将由大约98.93%^{12}C和1.07%^{13}C构成。富集指化学结构中较少的同位素的多于天然丰度的存在。因此，例如可就1个或多个碳原子位置的^{13}C的存在富集本发明化合物。如文中所用，“替换”指大于大约95%的同位素变体的富集。

[0072] 熔点未经校正，且(dec)表示分解。温度以摄氏度(℃)给出；除非另有说明，否则操作在室温或环境温度、即18至25℃下进行。色谱法意指快速硅胶色谱法；薄层色谱法(TLC)在硅胶板上进行。NMR数据以主要特征质子的δ值提供，以相对于作为内标的四甲基甲硅烷(TMS)的百万分率(ppm)给出。使用信号形状的常规缩写。偶合常数(J)以Hz给出。对于质谱法(MS)，对其中同位素分裂导致多重质谱峰的分子，报道最低质量的主要离子。溶剂混合物组成以体积百分比或体积比给出。当NMR光谱是复杂的时，仅报道特征信号。

[0073] 术语和缩略语：

[0074] BOC＝叔-丁氧羰基

[0075] BOP＝苯并三唑-1-基-氧基-三(二甲基氨基)鏻六氟磷酸盐

[0076] BuLi＝正丁基锂

[0077] $Bu^{t}OH$＝叔丁醇

[0078] CAN＝硝酸铈(IV)铵

[0079] DBU＝1,8-二氮杂二环[5.4.0]十一碳-7-烯，

[0080] DIPEA＝二异丙基乙胺

[0081] DMF＝N,N-二甲基甲酰胺

[0082] DMSO＝二甲基亚砜

[0083] Et_2O＝乙醚

[0084] EtOAc＝乙酸乙酯

[0085] equiv.＝当量

[0086] h＝小时

[0087] HPLC＝高效液相色谱法

[0088] LDA＝二异丙基氨基锂

[0089] MeOH＝甲醇

[0090] NBS＝N-溴代琥珀酰亚胺

[0091] NCS＝N-氯代琥珀酰亚胺

[0092] $NaHCO_3$＝碳酸氢钠

[0093] NH_4OH＝氢氧化铵

[0094] $Pd_2(dba)_3$＝三[二亚苄基丙酮]二钯(0)

[0095] PMB＝对甲氧基苄基

[0096] $POCl_3$＝氧氯化磷

[0097] $SOCl_2$＝亚硫酰氯

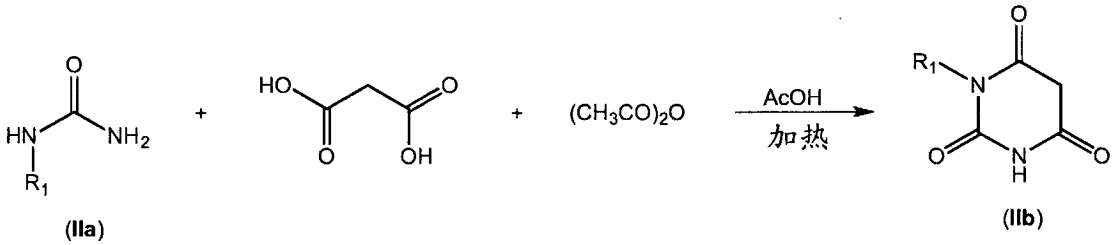
[0098] TFA＝三氟乙酸

[0099] TFMSA＝三氟甲磺酸

[0100] THF＝四氢呋喃

[0101] 下文举例说明了本发明中可用的合成方法。基团R的定义如上文对式I或1.1-1.14中任何一式所述，另有指示除外。

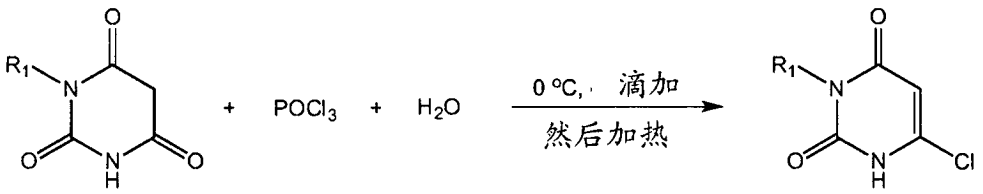
[0102] 式IIb的中间体化合物可以如下制备：使式IIa化合物与丙二酸和乙酸酐在乙酸中反应，任选加热(例如至大约90℃，保持大约3小时)：

[0103] 

(IIa) (IIb)

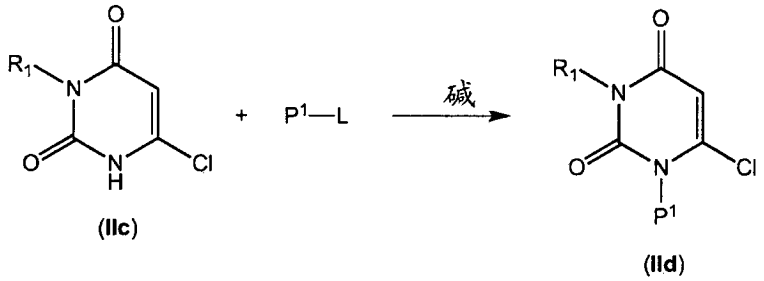
[0104] 其中R_1是C_{1-4}烷基例如甲基。

[0105] 式IIc中间体可如下制备：使式IIb化合物与氯化化合物例如$POCl_3$反应，有时带有少量水和/或加热(例如，加热至大约80℃，保持约4小时)：

[0106] 

(IIb) (IIc)

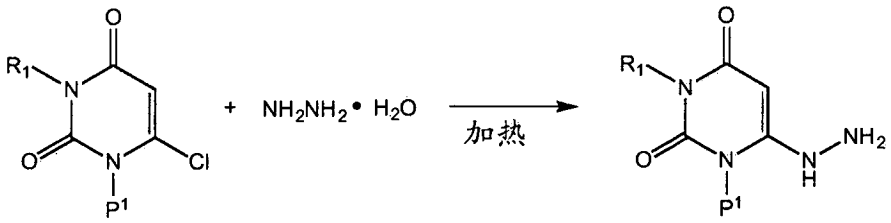
[0107] 式IId中间体可以通过使式IIc化合物与例如试剂P^1-X和碱如碳酸钾、碳酸氢钠、碳酸铯、氢氧化钠、三乙胺、二异丙基乙胺等在溶剂如DMF中于室温或者在加热下反应来制备：

[0108] 

(IIc) (IId)

[0109] 其中P^1是保护基[例如PMB或BOC]；且L是离去基，例如卤素、甲磺酸酯或甲苯磺酸酯。优选地，P^1是PMB，且碱是碳酸钾。

[0110] 式IIe中间体可以如下制备：使式IId化合物与肼或肼水合物在溶剂如甲醇中反应，优选在加热下(例如回流约4小时)：

[0111] 

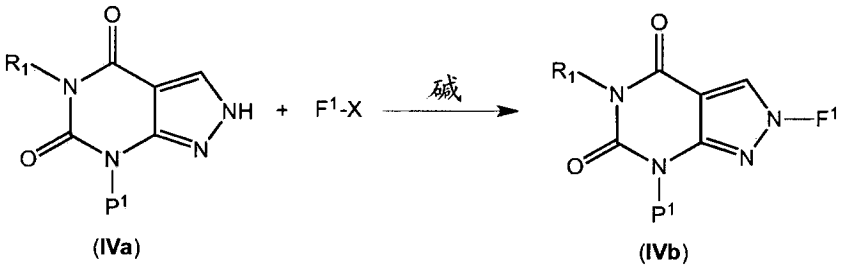
(IId) (IIe)

[0112] 式IVa中间体可以通过式IIe化合物与$POCl_3$和DMF反应而制备：

[0113] 

(IIe) (IVa)

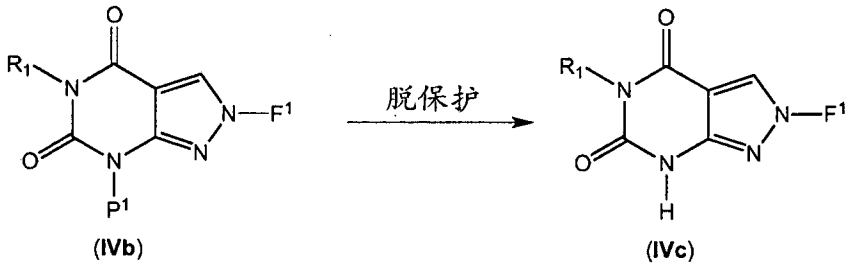
[0114] 式IVb中间体可以通过式IVa化合物与试剂式F^1-X和碱例如碳酸钾在溶剂例如DMF中于室温反应而制备：

[0115] 

(IVa) (IVb)

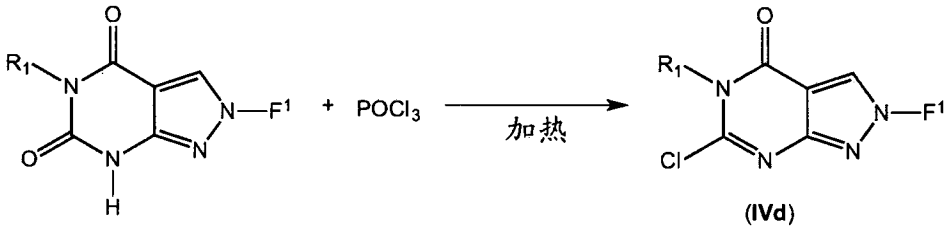
[0116] 其中F^1是保护基(例如取代的苄基如4-溴苄基)，并且X是卤素(例如Br)。

[0117] 式IVc中间体可以通过利用适当方法除去保护基P^1，由式IVb化合物而制备。例如，如果P^1是PMB基团，那么可在环境温度或升高的温度下，用TFA/TFMSA将其除去，而如果P1是BOC，那么可利用酸例如TFA或盐酸，将其除去：

[0118] 

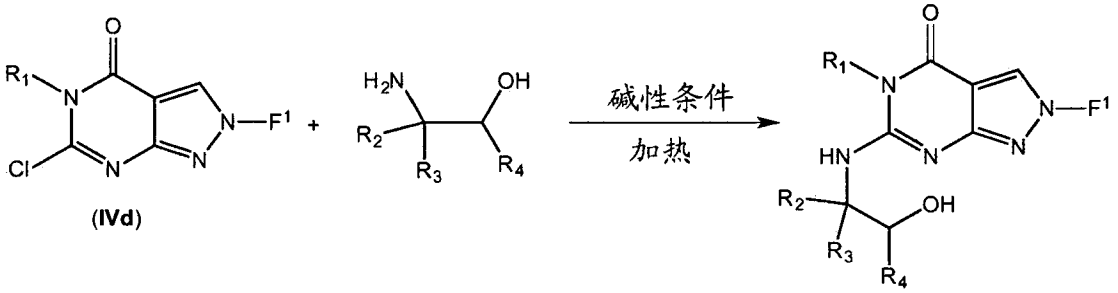
(IVb) (IVc)

[0119] 式IVd中间体可通过式IVc化合物与氯化化合物例如$POCl_3$，任选在加热下(例如回流2天或更长时间或者于密封瓶中在150-200℃下微波辐射5-10分钟)反应而制备：

[0120] 

(IVc) (IVd)

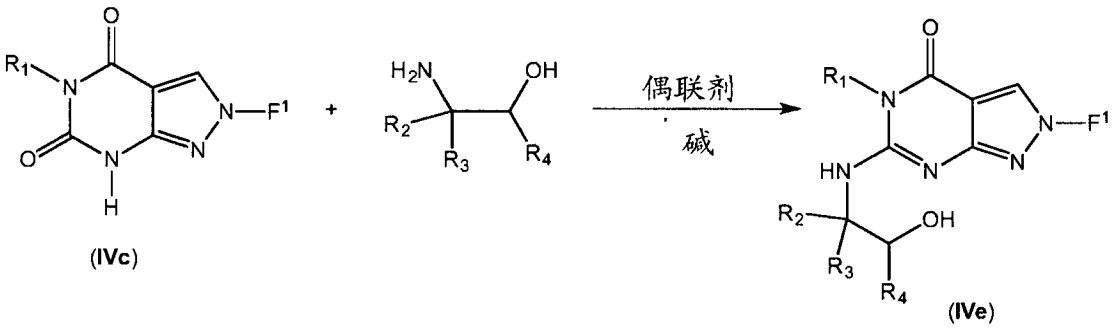
[0121] 式IVe中间体可以如下制备：在碱性条件下，使式IVd化合物与氨基醇在溶剂例如DMF中，任选加热下反应：

[0122] 

(IVd) (IVe)

[0123] 其中R_1、R_2、R_3和R_4同前面对式V或1.1-1.14中任何一式所定义的。

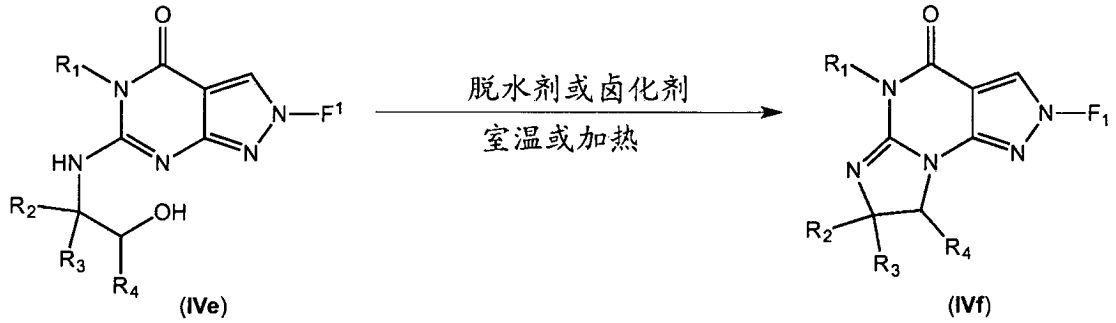
[0124] 或者，中间体IVe可通过在碱例如DBU存在下，使氨基醇和偶联剂例如BOP反应，由式IVc化合物直接制备：

[0125] 

(IVc) (IVe)

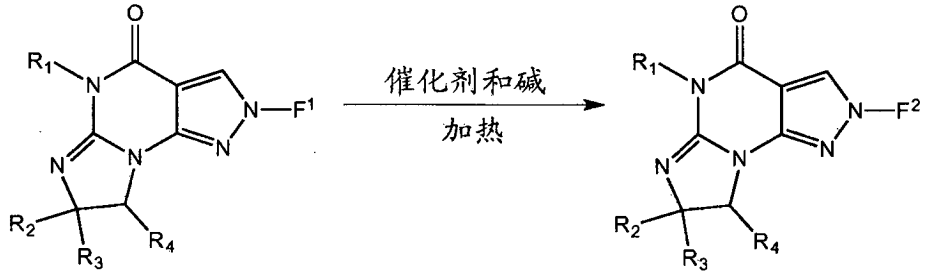
[0126] 其中，R_1、R_2、R_3和R_4同上文对式V或1.1-1.14中任何一式所定义的。

[0127] 式IVf中间体可如下制备：式IVe化合物与脱水剂/卤化剂例如$SOCl_2$在溶剂例如二氯甲烷中、于室温或35℃加热下反应：

[0128] 

(IVe) (IVf)

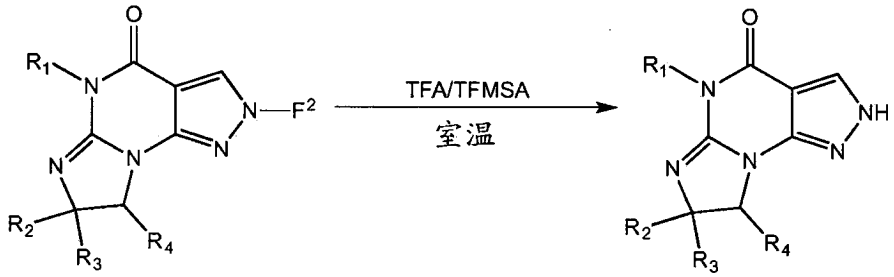
[0129] 式IVg中间体可以如下制备:式IVf化合物与催化剂例如铜盐和2,2,6,6-四甲基庚烷-3,5-二酮和碱例如碳酸铯在溶剂例如NMP中、于加热下反应:

[0130] 

(IVf) (IVg)

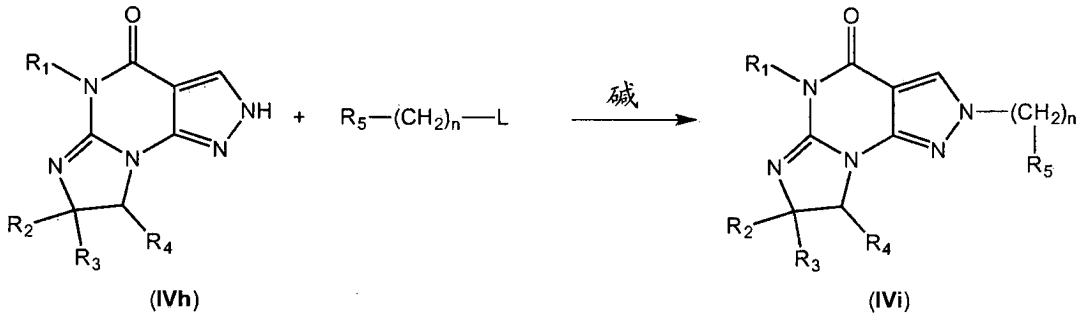
[0131] 其中,F^2是二芳基醚。

[0132] 式IVh中间体可通过式IVg化合物与酸性系统例如TFA和TFMSA在溶剂例如二氯甲烷中、于室温下反应而制备:

[0133] 

(IVg) (IVh)

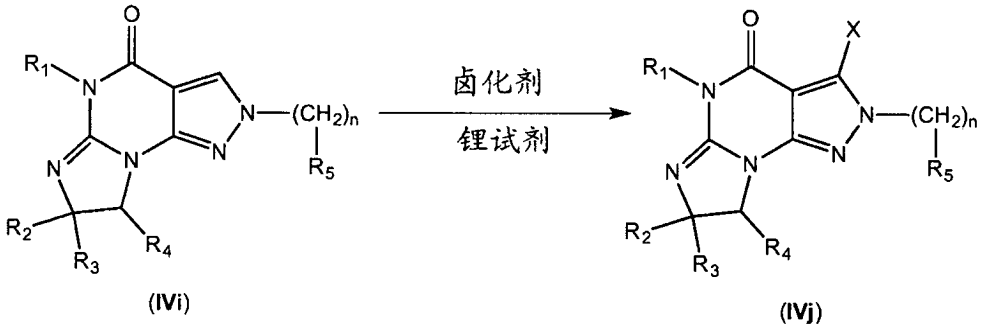
[0134] 式IVi中间体可通过在碱例如碳酸钾存在下,使式IVh化合物与试剂式$R_5-(CH_2)_n-L$在溶剂例如DMF中、于室温下反应而制备:

[0135] 

(IVh) (IVi)

[0136] 其中n是0,且R_5是式A基团,如同上文对式V或1.1-1.14中任何一式所定义的,并且L是离去基团例如卤素(例如Br)。

[0137] 式IVj中间体(其中X是卤素(例如Cl))可通过式IVi化合物与卤化剂(例如NCS或NBS)和碱例如LiHMDS在溶剂例如THF中、于低温下反应而制备:

[0138] 

(IVi) (IVj)

[0139] 然后，可通过本领域技术人员已知的方法，由式IVj化合物制备本发明化合物。例如，通过用芳基胺或烷基硫醇置换卤素X。

[0140] 本发明化合物的使用方法

[0141] 本发明还提供方法I，其中方法I包括中枢神经系统疾病、病症和损伤的预防和/或治疗，其中所述方法包括施用有效量的PDE1抑制剂(例如式V或1.1-1.14的任何化合物)，以调节细胞内cAMP水平。

[0142] 例如，方法I还包括：

[0143] 1.1.方法I，其中施用PDE1抑制剂增强轴突生长或再生，和/或减缓或逆转神经退行性病患中所述细胞的损失。

[0144] 1.2.前面以及下列方法-I中的任何一项，其中CNS疾病、病症或损伤指直接或间接影响CNS正常功能的损害。

[0145] 1.3.前面以及下列方法-I中的任何一项，其中CNS疾病、病症或损伤可以是结构、物理或机械损害，并可以由物理冲击例如神经纤维的破碎、挤压或拉伸引起。

[0146] 1.4.前面以及下列方法-I中的任何一项，其中CNS疾病、病症或损伤是脊髓损伤。

[0147] 1.5.方法1.4，其中PDE1抑制剂延缓或阻止脊髓损伤的恶化。

[0148] 1.6.前面以及下列方法-I中的任何一项，其中PDE1抑制剂延缓或阻止轴突纤维降解。

[0149] 1.7.前面以及下列方法-I中的任何一项，其中CNS疾病、病症或损伤与运动神经元创伤相关。

[0150] 1.8.前面以及下列方法-I中的任何一项，其中所述疾病、病症或损伤选自：神经创伤和损伤、手术相关的创伤和/或损伤、视网膜损伤和创伤、与癫痫相关的损伤、脊髓损伤、脑损伤、脑部手术、创伤相关的脑损伤、创伤相关的脊髓损伤、与癌症治疗相关的脑损伤、与癌症治疗相关的脊髓损伤、与感染相关的脑损伤、与炎症相关的脑损伤、与感染相关的脊髓损伤、与炎症相关的脊髓损伤、与环境毒素相关的脑损伤和与环境毒素相关的脊髓损伤。

[0151] 1.9.前面以及下列方法-I中的任何一项，其中所述CNS疾病、病症或损伤包括疾病(例如帕金森氏病)、化学物质失调或生理功能障碍例如缺氧症(例如中风)、动脉瘤或再灌注损伤破坏或降解的神经元或神经纤维。

[0152] 1.10.前面以及下列方法-I中的任何一项，其中所述CNS疾病、病症或损伤是神经退行性病症。

[0153] 1.11.方法1.10，其中退行性疾病、病症或损伤选自：阿尔茨海默氏病、多发性硬化、脊髓性肌萎缩、青光眼、额颞叶型痴呆、路易体痴呆、皮质基底变性、进行性核上麻痹、朊病毒病症、亨廷顿氏病、多系统萎缩、帕金森氏病、肌萎缩侧索硬化、遗传性痉挛性轻截瘫、

脊髓小脑萎缩、弗莱德里希(Friedreich's)共济失调、淀粉样变性、代谢(糖尿病)相关的病症、毒素相关的病症、慢性CNS炎症、夏科-马里-图斯(Charcot Marie Tooth)病、糖尿病神经病变、癌症化疗(例如长春花生物碱和多柔比星)引起的损伤、与中风相关的脑损伤、与中风相关的缺血、并且神经系统病症包括但不限于与神经退行相关的各种周围神经和神经性病症,包括但不限于三叉神经痛、舌咽神经痛、贝尔(Bell)麻痹、重症肌无力、肌营养不良、肌萎缩侧索硬化、进行性肌萎缩、进行性延髓遗传性肌萎缩、疝气型、破裂型或脱出型椎间盘综合征、颈椎病、神经丛障碍、胸廓出口破坏综合征、周围神经病例如由诸如铅、丙烯酰胺类、γ-二酮类、二硫化碳、氨苯砜、蜱引起的那些、卟啉症和吉兰-巴雷(Gullain-Barre)综合征。

[0154] 1.12.前面以及下列方法-I中的任何一项,其中CNS疾病、病症或损伤是CNS损害、发作或由发作(例如癫痫性发作)引起的损伤、放射性损伤、化疗引起的损伤和/或中风或其他缺血性损伤。

[0155] 1.13.前面以及下列方法-I中的任何一项,其中将PDE1抑制剂的施用用于补充、置换和/或增补神经元和/或胶质细胞。

[0156] 1.14.前面以及下列方法-I中的任何一项,其中将PDE1抑制剂施用至需要其的个体或患者。

[0157] 1.15.前面以及下列方法-I中的任何一项,其中PDE1抑制剂升高细胞内cAMP的水平或表达。

[0158] 1.16.前面以及下列方法-I中的任何一项,其中PDE1抑制剂降低细胞内cAMP的水平或表达。

[0159] 1.17.前面以及下列方法-I中的任何一项,其中PDE1调节PKA或PKG的活性。

[0160] 1.18.前面以及下列方法-I中的任何一项,其中PDE1抑制剂增加PKA或PKG的活性。

[0161] 1.19.前面以及下列方法-I中的任何一项,其中PDE1抑制剂的施用增加cAMP和cGMP水平。

[0162] 1.20.前面以及下列方法-I中的任何一项,其中PDE1抑制剂的施用升高细胞内cAMP的水平,并且其中所述细胞内cAMP水平的升高具有神经保护和/或神经再生作用。

[0163] 1.21.前面以及下列方法-I中的任何一项,包含向患者施用有效量的PDE1抑制剂,所述患者患细胞内钙水平升高(例如慢性升高)相关的疾病或病症,并且其中PDE1抑制剂防止所述钙水平的进一步升高。

[0164] 1.22.前面以及下列方法-I中的任何一项,其中将PDE1抑制剂单独施用或者与其它活性物质组合施用。

[0165] 1.23.前面以及下列方法-I中的任何一项,其中所述疾病、病症和损伤是与运动神经元相关的,并且其中运动神经元疾病、病症或损伤是多发性硬化。

[0166] 1.24.前面以及下列方法-II中的任何一项,其中将PDE1抑制剂与其他活性物质组合施用,以治疗多发性硬化。

[0167] 1.25.2.11的方法,其中所述活性物质选自干扰素、醋酸格拉默(Glatiramer acetate)、那他珠单抗(Natalizumab)、**Gilenya®**(芬戈莫德)、**Fampyra®**、免疫抑制剂和肾上腺皮质激素。

[0168] 在另一实施方案中,本发明提供方法II,其中方法II包括治疗或预防周围神经系

统(PNS)疾病、病症或损伤的组合物和方法，其中所述方法包括施用有效量的PDE1抑制剂(例如式V或1.1-1.14中的任何化合物)，以提高细胞内的cAMP水平。

[0169] 例如，方法II还包括：

[0170] 2.1.方法II，其中PNS疾病、病症或损伤指直接或间接影响CNS正常功能的损害。

[0171] 2.2.前面以及下列方法-II中的任何一项，其中将PDE1抑制剂施用至需要其的个体或患者。

[0172] 2.3.前面以及下列方法-II中的任何一项，其中PDE1抑制剂升高细胞内cAMP的水平或表达。

[0173] 2.4.前面以及下列方法-II中的任何一项，其中PDE1抑制剂(例如直接或间接)调节PKA和/或PKG活性。

[0174] 2.5.前面以及下列方法-II中的任何一项，其中PDE1抑制剂(例如直接或间接)升高PKA和/或PKG活性。

[0175] 2.6.前面以及下列方法-II中的任何一项，其中PDE1抑制剂的施用升高cAMP和/或cGMP水平。

[0176] 2.7.前面以及下列方法-II中的任何一项，其中PDE1抑制剂的施用升高细胞内cAMP水平，并且其中所述细胞内cAMP水平的升高保护神经纤维，再生神经纤维或者促进神经纤维生长(例如轴突再生)。

[0177] 2.8.前面以及下列方法-II中的任何一项，其包含向患者施用有效量的PDE1抑制剂，所述患者患细胞内钙水平升高(例如慢性升高)相关的疾病或病症。

[0178] 2.9.前面以及下列方法-II中的任何一项，其中将PDE1抑制剂单独施用或者与其它活性物质组合施用。

[0179] 2.10. 2.9的方法，其中所述活性物质选自IGF(例如IGF-1)或类固醇。

[0180] 2.11.前面以及下列方法-II中的任何一项，其中PNS疾病、病症或损伤选自神经病(例如周围神经病、自主神经病变和单神经病)、坐骨神经痛、腕管综合征、多神经病、糖尿病神经病变、带状疱疹后神经痛和胸廓出口综合征。

[0181] 在另一实施方案中，本发明提供方法III，其中方法III包括在患所述疾病或病症风险的个体中预防CNS疾病或病症的组合物和方法，其中所述方法包括：

[0182] 1.)从个体获得样品；

[0183] 2.)测量样品的细胞内钙水平；

[0184] 3.)将生物样品中的细胞内钙水平与参考标准进行比较；

[0185] 4.)与参考标准相比，基于细胞内钙水平，确定患者是否具有患CNS疾病或病症的风险；

[0186] 5.)基于个体的细胞内钙水平，向个体施用PDE1抑制剂(例如式V或1.1-1.14中任何一式的化合物)(例如向个体施用PDE1抑制剂，因为与参考标准相比，其具有升高的细胞内钙水平)。

[0187] 例如，方法III还包括：

[0188] 3.1.方法III，其中样品是生物样品。

[0189] 3.2.前面以及下面方法-III中的任何一项，其中利用化学荧光探针，测量患者细胞内的钙水平。

[0190] 3.3.前面以及下面方法-III中的任何一项，其中与对照(例如参考标准)相比，患者细胞内的钙水平是升高的。

[0191] 3.4.前面以及下面方法-III中的任何一项，其中将PDE1抑制剂施用至患者，与对照(例如参考标准)相比，所述患者显示具有升高的细胞内钙水平。

[0192] 3.5.前面以及下面方法-III中的任何一项，其中PDE1抑制剂的施用延缓或防止CNS和/或PNS疾病或病症的形成，其中CNS疾病或病症是与升高(例如慢性升高)的细胞内钙水平相关的。

[0193] 3.6.前面以及下面方法-III中的任何一项，其中PDE1抑制剂的施用降低个体将患CNS和/或PNS疾病或病症的可能性，其中CNS和/或PNS疾病或病症是与升高(例如慢性升高)的细胞内钙水平相关的(例如方法I等和方法II等中所列疾病、病症或损伤中的任何一种)。

[0194] 3.7.前面以及下面方法-III中的任何一项，其中所述方法任选地包含测量患者的cAMP或cGMP的细胞内水平。

[0195] 3.8.前面以及下面方法-III中的任何一项，其中将PDE1抑制剂单独施用或者与其它活性物质组合施用。

[0196] 3.9.前面以及下面方法-III中的任何一项，其中施用PDE1抑制剂，因为与对照个体相比，患者具有低水平的cAMP和/或cGMP。

[0197] 本发明化合物可用于治疗特征在于cAMP和cGMP介导的通路被破坏或损害的疾病，例如作为归因于环核苷酸合成诱导剂如多巴胺和一氧化氮(NO)抑制或水平降低的PDE1表达增加或者cAMP和cGMP表达降低的结果。通过防止cAMP和cGMP被PDE1降解并由此增加cAMP和cGMP的细胞内水平，本发明化合物增强环核苷酸合成诱导剂的活性。

[0198] 在另一实施方案中，本发明还提供了治疗方法，该方法包括施用有效量的PDE1抑制剂(例如式V或1.1-1.14中的任何化合物)以治疗下述病患中任意一种或多种：

[0199] (i)神经变性疾病，包括帕金森病、不宁腿、震颤、运动障碍、亨廷顿病、阿尔茨海默病和药物引起的运动障碍；

[0200] (ii)精神障碍，包括抑郁、注意力缺陷障碍、注意缺陷多动症、双相性疾病(bipolar illness)、焦虑、睡眠障碍例如发作性睡病、认知损害例如精神分裂症的认知损害、痴呆、图雷特氏综合征、孤独症、脆性X综合征、精神兴奋剂戒断和药物成瘾；

[0201] (iii)循环和心血管病症，包括脑血管疾病、中风、充血性心脏病、高血压、肺性高血压例如肺动脉高压和性功能障碍，包括国际申请号PCT/US2014/16741中所述的心脏血管疾病和相关病症，将其内容引入文中作为参考；

[0202] (iv)呼吸系统和炎性病症，包括哮喘、慢性阻塞性肺疾病和变应性鼻炎，以及自身免疫性和炎性疾病；

[0203] (v)通过增强孕酮信号可缓解的疾病例如雌性性功能障碍；

[0204] (vi)疾病或病症例如精神病、青光眼或升高的眼内压；

[0205] (vii)创伤性脑损伤；

[0206] (viii)特征在于在表达PDE1的细胞中的低水平cAMP和/或cGMP(或者cAMP和/或cGMP信号通路抑制)的任意疾病或病患；和/或

[0207] (ix)以降低的多巴胺D1受体信号活性为特征的疾病或病患，

[0208] 包括向需要其的人或动物患者施用有效量的本发明化合物，例如游离或药学上可

接受的盐或前药形式的根据(例如式V或1.1-1.14中的任何化合物)的化合物。

[0209] 一方面,本发明提供治疗或预防发作性睡病的方法。在本实施方案中,可将PDE1抑制剂(例如式V或1.1-1.14中的任何化合物)用作唯一治疗物质,但是还可以与其他活性物质组合使用或者与其他活性物质同时施用。因此,本发明还包括治疗发作性睡病的方法,其包括同时、依次或同时向需要其的人或动物患者施用治疗有效量的游离或药学上可接受的盐或前药形式的

[0210] (i)PDE1抑制剂,例如根据(例如式V或1.1-1.14中的任何化合物)的化合物,和

[0211] (ii)促进觉醒或调节睡眠的化合物,例如选自(a)中枢神经系统兴奋剂-苯丙胺类和苯丙胺样化合物,例如哌醋甲酯、右旋安非他命、甲基苯丙胺和匹莫林;(b)莫达非尼,(c)抗抑郁剂例如三环抗抑郁剂(包括丙咪嗪、地昔帕明、氯米帕明和普罗替林)和选择性血清再摄取抑制剂(包括氟西汀和舍曲林);和/或(d) γ-羟基丁酸(GHB)。

[0212] 另一方面,本发明还提供治疗或预防可通过增加孕酮信号传导而缓和的病患的方法,该方法包括向需要其的人或动物患者施用有效量的游离或药学上可接受的盐或前药形式的本发明化合物例如根据式V或1.1-1.14中任何一式的化合物。通过增加孕酮信号传导可缓和的疾病或病患包括但不限于雌性性功能障碍,继发闭经(例如运动性闭经、停止排卵、绝经、更年期症状、甲状腺功能减退),月经前综合征,早产分娩,不育例如反复流产引起的不育,不规则月经周期,异常子宫出血,骨质疏松症,自身免疫疾病,多发性硬化,前列腺肥大,前列腺癌和甲状腺功能减退。例如,通过升高孕酮信号传导,PDE1抑制剂可通过对子宫内层的作用而用于促进卵着床,并用于对由于妊娠免疫应答或低孕酮功能而易于流产的女性中帮助维持妊娠。例如文中所述的新PDE1抑制剂还可用于增强激素替代疗法在绝经后妇女、雌激素-诱导的子宫内膜增生症和癌症中的效果,例如与雌激素/雌二醇/雌三醇和/或孕酮/孕激素类联合施用。本发明方法还可用于动物繁殖,例如在要被繁殖的非人雌性哺乳动物中诱导性感受性和/或动情期。

[0213] 在这方面,PDE1抑制剂可作为唯一治疗物质,用于上述治疗或预防方法中,但是还可以与其他活性物质联合使用或者同时施用,例如与激素替代疗法联合。因此,本发明还包含治疗可通过增强孕酮信号传导而缓和的病症的方法,该方法包括同时、依次或同期地(contemporaneously)向需要其的人或动物患者施用治疗有效量的游离或药学上可接受的盐或前药形式的

[0214] (i)PDE1抑制剂,例如根据式V或1.1-1.14中任何一式的化合物,和

[0215] (ii)激素,例如选自雌激素和雌激素类似物(例如雌二醇、雌三醇、雌二醇酯类)和孕酮与孕酮类似物(例如孕激素(progestin))。

[0216] 本发明还提供增加或增强细胞或组织中多巴胺D1细胞内信号传导活性的方法,其包括使所述细胞或组织与足够抑制PDE1活性的量的游离或药学上可接受的盐或前药形式的本发明化合物例如根据式V或1.1-1.14中任何一式的化合物接触。

[0217] 本发明还提供在需要其的患者中治疗PDE1-相关病症、多巴胺D1受体细胞内信号通路障碍或者可通过增加孕酮信号通路而缓和的病症的方法,该方法包括向患者施用抑制PDE1的有效量的游离或药学上可接受的盐或前药形式的本发明化合物,例如根据式V或1.1-1.14中任何一式的化合物,其中PDE1活性调节DARPP-32和/或GluR1AMPA受体的磷酸化。

[0218] 另一方面，本发明还提供用于治疗青光眼或眼压升高的方法，该方法包括将眼科相容载体中的治疗有效量的游离或药学上可接受的盐形式的本发明PDE1抑制剂局部施用至需要其的患者的眼睛，本发明化合物例如是根据式V或1.1-1.14中任何一式的化合物。然而，替代地，治疗可以包括全身治疗。全身治疗包括例如能直接到达血流的治疗或者口服施用方法。

[0219] 本发明还提供用于局部眼科应用的包含PDE1抑制剂的药物组合物；例如包含与眼科可接受的稀释剂或载体组合或者联合的游离或眼科可接受的盐形式的本发明的PDE1抑制剂例如根据式V或1.1-1.14中任何一式的化合物的眼科溶液、混悬液、乳剂或软膏剂。

[0220] 任选地，PDE1抑制剂(例如式V或1.1-1.14中任何一式)可以与用于治疗青光眼或眼压升高的第二种药物依次或同时施用。当施用两种活性物质时，各物质的治疗有效量可以是低于作为单一疗法的活性所需的量。因此，可以认为亚阈值量(即低于单一疗法的效果所需水平的量)是治疗有效的，并还可以替代地称为有效量。实际上，施用具有不同作用机制和不同副作用模式的不同物质的优点可能是降低单种或两种物质的剂量和副作用，以及增加或增强其单一疗法的活性。

[0221] 因此，本发明提供治疗选自青光眼和眼压升高病患的方法，该方法包括向需要其的患者同期、同时或依次施用有效量例如亚阈值量的降低眼压的已知物质以及有效量例如亚阈值量的游离或药学上可接受的盐形式的本发明PDE1抑制剂，例如根据式V或1.1-1.14中任何一式的化合物，以便降低眼压的已知物质的量和PDE1抑制剂的量组合在一起对治疗所述病患是有效的。

[0222] 一方面，将一种或两种物质局部施用至眼睛。因此，本发明提供降低治疗青光眼或眼压升高的副作用的方法，这通过将降低剂量的降低眼内压的已知物质与有效量的PDE1抑制剂同期、同时或依次施用来实现。然而，还可以使用除局部施用之外的方法，例如全身治疗施用。

[0223] 用于与PDE1抑制剂联合使用的任选的其它物质可以例如选自现存药物，通常包括前列腺素、匹鲁卡品、肾上腺素的滴注剂或者局部β-阻断剂治疗例如用噻吗洛尔，以及全身施用的碳酸酐酶抑制剂，例如乙酰唑胺。还可以使用胆碱酯酶抑制剂例如毒扁豆碱和乙膦硫胆碱(echothiopate)，并且其具有类似于匹鲁卡品的作用。因此，目前用于治疗青光眼的药物包括，例如

[0224] 1.前列腺素类似物例如拉坦前列素(适利达(Xalatan))，比马前列素(卢美根(Lumigan))和曲伏前列素(Travatan)，其增加眼房水的眼色素层巩膜流出。比马前列素还增加小梁流出。

[0225] 2.局部β-肾上腺素能受体拮抗剂例如噻吗洛尔，左布诺洛尔(贝他根(Betagan))和倍他洛尔，其降低睫状体的房水产生。

[0226] 3.α_2-肾上腺素能激动剂例如溴莫尼定(阿法根(Alphagan))，其通过双重机制起作用，降低房水产生和增加眼色素层巩膜流出。

[0227] 4.更低选择性的拟交感神经药如肾上腺素和地匹福林(普罗品(Propine))增加房水经小梁网的流出，以及可能经眼色素层巩膜流出途径的流出，可能通过β_2-激动剂作用。

[0228] 5.缩瞳剂(拟副交感神经药)如匹鲁卡品通过收缩睫状肌而起作用，紧固小梁网并保证房水流出增加。

[0229] 6.碳酸酐酶抑制剂如多佐胺(舒静露(Trusopt))、布林唑胺(派立明(Azopt))、乙酰唑胺(Diamox)通过抑制睫状体的碳酸酐酶而降低房水分泌。

[0230] 7.毒扁豆碱也用于治疗青光眼和延缓胃排空。

[0231] 例如,本发明提供药物组合物,其含有与药学上可接受的稀释剂或载体组合或联合的游离或药学上可接受盐形式的本发明PDE1抑制剂,例如根据式V或1.1-1.14中任一式的化合物,以及选自下述的物质:(i)前列腺素类,乌诺前列酮,拉坦前列素,曲伏前列素或比马前列素;(ii)α肾上腺素能激动剂例如溴莫尼定,阿可乐定或地匹福林和(iii)毒蕈碱激动剂例如匹鲁卡品。例如,本发明提供眼科制剂,其含有游离或眼科可接受盐形式的本发明PDE-1抑制剂例如根据式V或1.1-1.14中任何一式的化合物以及比马前列素、abrimonidine、溴莫尼定、噻吗洛尔或其组合,它们与眼科可接受的稀释剂或载体组合或联合。然而,除了选择组合,本领域普通技术人员可选择适当选择性受体亚型激动剂或拮抗剂。例如,对于α肾上腺素能激动剂,人们可选择α1肾上腺素能受体选择性激动剂或者α2肾上腺素能受体选择性激动剂例如溴莫尼定。对于β-肾上腺素能受体拮抗剂,根据适当的治疗应用,人们可选择β_1或β_2或β_3选择性拮抗剂。人们还可选择具体受体亚型例如M_1-M_5的选择性毒蕈碱激动剂。

[0232] 可将PDE1抑制剂以眼科组合物的形式施用,其包括眼科溶液剂、乳剂或软膏剂。眼科组合物可另外包括降低眼内压的物质。

[0233] 还在另一实施例中,可将公开的PDE1抑制剂与亚阈值量的降低眼内压物质组合,所述物质可以是比马前列素眼科溶液剂、酒石酸溴莫尼定眼科溶液剂、或者酒石酸溴莫尼定/马来酸噻吗洛尔眼科溶液剂。

[0234] 除了上述方法,还已惊讶地发现PDE1抑制剂(例如式V或1.1-1.14中的任何一式)可用于治疗精神病例如以精神病症候例如幻觉、妄想狂或稀奇古怪的妄想或者言语和思想紊乱为特征的任何病患,例如精神分裂症、情感性分裂症、精神分裂症样精神障碍、精神障碍、妄想症和躁狂,例如急性躁狂性发作和双相情感障碍。预期不受任何理论束缚,认为典型和非典型抗精神病药例如氯氮平主要具有对多巴胺D2受体的拮抗活性。然而,PDE1抑制剂主要起增加多巴胺D1受体信号传导的作用。通过增强D1受体信号传导,PDE1抑制剂可增加各种脑区域例如在伏核神经元和前额叶皮质中的NMDA受体功能。所述功能的增强可见于例如含有NR2B亚单元的NMDA受体,并可例如通过Src和蛋白激酶A家族激酶的活化而发生。

[0235] 因此,本发明提供治疗精神病例如精神分裂症、情感性分裂症、精神分裂症样精神障碍、精神障碍、妄想症和躁狂的新方法,例如治疗急性躁狂性发作和双相情感障碍的新方法,其包括向需要其的患者施用治疗有效量的游离或药学上可接受盐的形式的本发明磷酸二酯酶-1(PDE1)抑制剂,例如根据式V或1.1-1.14中任何一式的化合物。

[0236] PDE1抑制剂可作为单一治疗物质而用于治疗预防的上述方法中,但是还可用于与其他活性物质组合或共同施用。因此,本发明还包括治疗精神病例如精神分裂症、情感性分裂症、精神分裂症样精神障碍、精神障碍、妄想症或躁狂的方法,包括向需要其的患者同时、依次或同期施用治疗有效量的:

[0237] (i)本发明的PDE1抑制剂,其为游离或药学上可接受盐的形式的;和

[0238] (ii)抗精神病药物,例如

[0239] 经典抗精神病药,例如

[0240] 苯丁酮类例如氟哌啶醇(Haldol、Serenace)、氟哌利多(Droleptan)；

[0241] 吩噻嗪类例如氯丙嗪(Thorazine、Largactil)、氟奋乃静(Prolixin)、奋乃静(Trilafon)、普鲁氯嗪(Compazine)、硫利达嗪(Mellaril、Melleril)、三氟拉嗪(Stelazine)、美索达嗪、哌氰嗪、普马嗪、三氟丙嗪(Vesprin)、左美丙嗪(Nozinan)、异丙嗪(Phenergan)、匹莫齐特(Orap)；

[0242] 噻吨类例如氯普噻吨、三氟噻吨(Depixol,Fluanxol)、氨砜噻吨(Navane)、珠氯噻醇(Clopixol,Acuphase)；

[0243] 非典型抗精神病药，例如，

[0244] 氯氮平(Clozaril)、奥氮平(再普乐)、利培酮(维思通)、喹硫平(思瑞康)、齐拉西酮(Geodon)、氨磺必利(Solian)、帕潘哌酮(Invega)、阿立哌唑(Abilify)、联苯芦诺(Bifeprunox)；去甲氯氮平，

[0245] 其为游离或药学上可接受盐的形式的。

[0246] 在一具体实施方案中，本发明化合物特别用于治疗或预防精神分裂症。

[0247] 游离或药学上可接受盐形式的本发明化合物特别可用于治疗帕金森氏病、精神分裂症、发作性睡病、青光眼和雌性性功能障碍。

[0248] 还在另一方面，本发明提供延长或增加睫毛生长的方法，该方法通过向需要其的患者的眼睛同期、同时或依次施用有效量的前列腺素类似物例如比马前列素与有效量的游离或药学上可接受盐形式的本发明PDE1抑制剂来实现。

[0249] 还在另一方面，本发明提供治疗或预防创伤性脑损伤的方法，其包括向需要其的患者施用治疗有效量的游离或药学上可接受盐形式的本发明PDE1抑制剂例如根据式V或1.1-1.14中任何一式的化合物。创伤性脑损伤(TBI)包括原发性损伤和继发性损伤，包括局部和弥漫性脑损伤。继发性损伤是源自离散亚细胞过程的多发、平行、相互影响和相互依赖的级联生物反应(例如活性氧、谷氨酸受体过刺激、钙的过度流入和炎症上调引起的毒性)，其通过起始(原发性)损伤后的炎症反应和进展引起或恶化。

[0250] 本发明还提供

[0251] (i)上文所述的游离或药学上可接受盐形式的本发明化合物，例如根据式V或1.1-1.14中任何一式的化合物，其用于上文所述的任何方法中或者任何疾病或病症的治疗中，

[0252] (ii)上文所述的游离或药学上可接受盐形式的本发明化合物例如根据式V或1.1-1.14中任何一式的化合物用于治疗上文所述的任何疾病或病症的用途(在制备用于治疗上文所述的任何疾病或病症的药物中的用途)，

[0253] (iii)药物组合物，其含有上文所述的游离或药学上可接受盐形式的本发明化合物例如根据式V或1.1-1.14中任何一式的化合物，该化合物与药学上可接受的稀释剂或载体组合或联合，和

[0254] (iv)药物组合物，其含有上文所述的游离或药学上可接受盐形式的本发明化合物例如根据式V或1.1-1.14中任何一式的化合物，该化合物与药学上可接受的稀释剂或载体组合或联合，

[0255] 所述药物组合物用于治疗上文所述任何疾病或病症。

[0256] 因此，本发明提供游离或药学上可接受盐形式的上文所述本发明化合物例如根据式V或1.1-1.14中任何一式的化合物或者药物组合物形式的本发明化合物用于治疗或预防

性治疗下述疾病任何一种或多种的用途，或在制备用于治疗或预防性治疗下述疾病任何一种或多种的药物中的用途：帕金森病、不宁腿综合征、震颤、运动失调、亨廷顿氏病、阿尔茨海默氏病、和/或药物引起的运动障碍；抑郁、注意缺陷障碍、注意力缺陷伴多动障碍、双相性疾病(bipolar illness)、焦虑、睡眠障碍、发作性睡病、认知损害例如精神分裂症的认知损害、痴呆、图雷特氏综合征、孤独症、脆性X综合征、精神兴奋剂戒断和/或药物成瘾；脑血管疾病、中风、充血性心脏病、高血压、肺性高血压例如肺动脉高压和/或性功能障碍；哮喘、慢性阻塞性肺疾病和/或变应性鼻炎，以及自身免疫性和炎性疾病；和/或雌性性功能障碍、运动性闭经、停止排卵、绝经、更年期症状、甲状腺功能减退、月经前综合征、早产分娩、不育、不规则月经周期、异常子宫出血、骨质疏松症、多发性硬化、前列腺肥大、前列腺癌和甲状腺功能减退和/或雌激素-诱导的子宫内膜增生症和/或癌症；特征在于在表达PDE1的细胞中的低水平cAMP和/或cGMP(或者cAMP和/或cGMP信号通路的抑制)的任意疾病或病患和/或以降低的多巴胺D1受体信号活性为特征的任意疾病或病患和/或可通过孕酮信号传导增强而缓和的任意疾病或病患。

[0257] 本发明还提供游离或药学上可接受盐形式的本发明化合物用于治疗或预防性治疗下述任何一种或多种疾病的用途或在制备治疗或预防性治疗下述任何一种或多种疾病的药物中的用途：

[0258] a)青光眼，眼压升高，

[0259] b)精神病，例如以精神病症候例如幻觉、妄想狂或稀奇古怪的妄想或者言语和思想紊乱为特征的任何病患，例如精神分裂症、情感性分裂症、精神分裂症样精神障碍、精神障碍、妄想症和躁狂，例如急性躁狂性发作和双相情感障碍，

[0260] c)创伤性脑损伤，和/或

[0261] d)中枢和周围退行性病症，特别是具有炎症性成分的那些。

[0262] 短语“本发明化合物”或“本发明的PDE1抑制剂”包括文中公开的任何和所有化合物例如式V或1.1-1.14化合物。

[0263] 用语“治疗”相应地将理解为囊括疾病症状的预防和治疗或改善以及疾病病因的治疗。

[0264] 对于治疗方法，文中所用的用语“治疗有效量”指足以治疗或缓和CNS或PNS疾病、病症或损伤的病理学效应的药物(PDE1抑制剂)的量。例如，治疗有效量的PDE1抑制剂可以是足以例如增加细胞内cAMP或cGMP水平、降低细胞内钙水平和/或增加神经再生的量。当相关时，治疗有效量还可以是延缓或防止CNS或PNS疾病或病症发展所需的PDE1抑制剂的量。

[0265] 术语“患者”或“个体”指人或非人(即动物)患者。在一具体实施方案中，本发明囊括人和非人患者。在另一实施方案中，本发明囊括非人患者。在另一实施方案中，该术语囊括人患者。

[0266] 文中所用的术语“对照个体”指任何人或非人有机体，其未患和/或未被怀疑患CNS或PNS病症、综合征、疾病、病患和/或症状。文中所用的术语“参考标准”指对照个体或对照个体群中，预先测量和获得结果。另一方面，术语“参考标准”指在其患CNS或PNS病症、综合征、疾病、病患和/或症状之前，患者的预先测量和获得结果。

[0267] 文中所用的术语“生物样品”可包括含有自例如有机体、体液、废弃物、细胞或其细胞部分、细胞株、生检、组织培养物或含细胞内钙、cAMP或cGMP水平的其他来源获得的生物

材料的任何样品。

[0268] 将“神经发生剂”定义为相对于无物质或试剂时神经发生的量或程度或性质，能促进、刺激或另外增加体内或离体或体外神经发生的量或程度或性质的化学物质或试剂。

[0269] 文中所用的“CNS损伤”可包括例如对视网膜神经节细胞的损害，创伤性脑损伤，中风-相关的损伤，大脑动脉瘤-相关的损伤，脊髓损伤或创伤，包括单瘫、双侧瘫痪、截瘫、偏瘫和四肢麻痹，神经增殖障碍或者神经性疼痛综合征。文中所用的“PNS损伤”可包括例如对脊髓神经或颅神经的损害，其中所述损害可包括伤害(lesion)或者一些急性或慢性创伤。

[0270] 上文所述的游离或药学上可接受盐形式的本发明化合物(例如式V或1.1-1.14中的任何一式)可用作单一治疗物质，但是还可以用于与其他活性物质组合使用或者用于与其他活性物质共同施用。

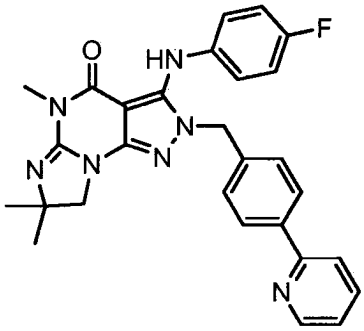
[0271] 实施本发明所采用的剂量当然将根据例如要治疗的具体疾病或病患、所用的具体的本发明化合物、施用方式以及所希望的疗法而改变。本发明化合物可以通过任意适宜的途径来施用，包括口服、胃肠道外、经皮或通过吸入施用，但是优选通过口服施用。通常而言，以约0.01至2.0mg/kg级别的剂量口服施用被指示可以获得令人满意的结果，例如对于治疗上文所述的疾病而言。因此，在较大型哺乳动物如人中，口服施用的指示日剂量将为约0.75至150mg，方便地一次施用或者以分开剂量每天施用2至4次施用或者以缓释形式施用。因而，口服施用的单位剂量形式例如可以含有约从0.2至75或150mg、例如约从0.2或2.0至50、75或100mg的本发明化合物以及用于其的药学上可接受的稀释剂或载体。

[0272] 包含本发明化合物的药物组合物可以采用盖仑领域已知的常规稀释剂或赋形剂和技术来制备。因而口服剂量形式可以包括片剂、胶囊剂、溶液剂和混悬剂等。

实施例

[0273] 实施例1

[0274] 7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-(4-氟苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0275] 

[0276] (a)7-(4-甲氧基苄基)-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4,6(5H,7H)-二酮

[0277] 将7-(4-甲氧基苄基)-5-甲基-2H-吡唑并[3,4-d]嘧啶-4,6(5H,7H)-二酮(8.43g，29.4mmol)、2-(4-(氯甲基)苯基)-吡啶(6.0g，29.4mmol)和K_2CO_3(4.07g，29.4mmol)的DMF(100mL)混悬液在室温下搅拌过夜。加压除去溶剂。将得到的残留物用水(150mL)和己烷(25mL)处理。将混合物在室温搅拌1小时，然后过滤。将滤饼用水冲洗3次(3×50mL)，然后真空干燥，得到13g粗产物(产率：97%)，不需进一步纯化，将其用于下一步骤。MS(ESI)m/z

454.2[M+H]$^+$。

[0278] (b)5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4,6(5H,7H)-二酮

[0279] 将TFA(50mL)加入到7-(4-甲氧基苄基)-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4,6(5H,7H)-二酮(13g,28.7mmol)的二氯甲烷(80mL)混悬液中,得到黄褐色溶液,然后加入TFMSA(4mL)。将反应混合物在室温搅拌过夜。减压除去溶剂。将得到的残留物用水(150mL)处理,冷却至0℃,然后用28%氢氧化铵(大约35mL)调至pH 8-9。过滤后,将得到的固体物用水冲洗3次(3×50mL),然后真空干燥,得到12.8g粗产物(粗产率:134%),未经进一步纯化,将其用于下一步骤中。MS(ESI)m/z334.1[M+H]$^+$。

[0280] (c)6-氯-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4(5H)-酮

[0281] 将5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4,6(5H,7H)-二酮(8.5g,25.5mmol)混悬于$POCl_3$(300mL)中,然后缓慢加热至回流。将混合物回流30h后,减压除去$POCl_3$。将得到的残留物用水(300mL)处理,冷却至0℃,然后用28%氢氧化铵(大约30mL)调至pH 8-9。过滤后,将得到的固体物用水冲洗5次(5×50mL),然后真空干燥,得到8.6g粗产物(粗产率:96%),未经进一步纯化,将其用于下一步骤中。MS(ESI)m/z 352.1[M+H]$^+$。

[0282] (d)6-(1-羟基-2-甲基丙-2-基氨基)-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4(5H)-酮

[0283] 将6-氯-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4(5H)-酮(4.0g,11mmol)、2-氨基-2-甲基丙烷-1-醇(6.5mL,71mmol)和DIPEA(3.4mL,20mmol)的DMA(20mL)混合物在130℃下加热1小时。减压除去溶剂。将得到的残留物用水(200mL)处理。过滤后,将滤饼用水冲洗2次(2×50mL),然后真空干燥,得到3.7g粗产物(粗产率:80%),未经进一步纯化,将其用于下一步骤中。MS(ESI)m/z 405.2[M+H]$^+$。

[0284] (e)7,8-二氢-2-(4-(吡啶-2-基)苄基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0285] 将亚硫酰氯(756μL,10.4mmol)滴加到粗6-(1-羟基-2-甲基丙-2-基氨基)-5-甲基-2-(4-(吡啶-2-基)苄基)-2H-吡唑并[3,4-d]嘧啶-4(5H)-酮(4.2g,10.4mmol)的DMF(84mL)溶液中。将反应混合物在室温搅拌20min。加入水(5mL),以淬灭反应。减压除去溶剂。将得到的残留物用二氯甲烷处理,然后用5%$NaHCO_3$水溶液冲洗3次。将有机相蒸发至干,得到6.1g粗产物(粗产率:152%),未经进一步纯化,将其用于下一步骤中。MS(ESI)m/z 387.2[M+H]$^+$。

[0286] (f)7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0287] 在0℃下,将1.0M LiHMDS(55.4mL,55.4mmol)的THF溶液滴加到粗7,8-二氢-2-(4-(吡啶2-基)苄基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(4.6g,11.9mmol)和六氯乙烷(2.58g,10.9mmol)的二氯甲烷(130mL)溶液中。将反应混合物在0℃搅拌30min,然后用水(100mL)和二氯甲烷(150mL)淬灭。将有机相用水冲洗3次(3×70mL),然后蒸发至干。将得到的粗产物经中性氧化铝柱纯化,得到1.5g粗产物(HPLC纯度:96%;产率:30%)。MS(ESI)m/z 421.1[M+H]$^+$。

[0288] (g)7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-(4-氟苯基氨基)-5,7,7-三甲基-[2H]-

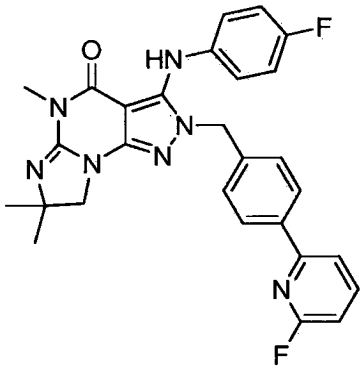
咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0289] 将7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(550mg,1.31mmol)、4-氟苯胺(125μL,1.31mmol)和碳酸钾(361mg,2.61mmol)的叔-戊醇(3mL)混合物用氩气脱气,然后加入Xantphos(15mg,0.026mmol)和$Pd_2(dba)_3$(12mg,0.013mmol)。将混悬液再脱气,然后缓慢加热至110℃。在氩气气氛下,将反应混合物于110℃下搅拌过夜。加入另一批$Pd_2(dba)_3$(12mg)和Xantphos(15mg)。将反应物在110℃下再加热24h,以完全转化。常规后处理后,将粗产物经硅胶柱层析纯化,得到352mg终产物,为米色固体物(HPLC纯度:97.4%;产率:54%)。^1H NMR(500MHz,氯仿-d)δ8.68(dt,J=4.7,1.3Hz,1H),7.88(d,J=8.3Hz,2H),7.74(td,J=7.7,1.8Hz,1H),7.68(d,J=8.0Hz,2H),7.23(ddd,J=7.4,4.8,1.2Hz,1H),7.06(d,J=8.3Hz,2H),7.00-6.93(m,2H),6.94-6.87(m,2H),6.79(s,1H),4.90(s,2H),3.71(s,2H),3.35(s,3H),1.40(s,6H).MS(ESI)m/z 496.2$[M+H]^+$.

[0290] 实施例1的化合物对PDE1显示良好选择性,并以等于或低于5nM的IC_{50}值抑制PDE活性。

[0291] 实施例2

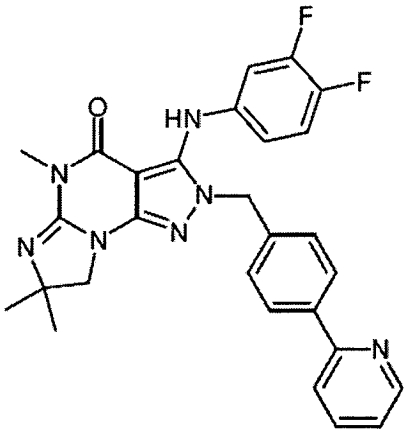
[0292] 7,8-二氢-2-(4-(6-氟吡啶-2-基)苄基)-3-(4-氟苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0293] 

[0294] 合成方法类似于实施例1,其中在步骤(a)中,加入2-(4-(氯甲基)苯基)-6-氟吡啶,代替2-(4-(氯甲基)苯基)-吡啶。得到终产物,作为灰白色固体物(HPLC纯度:99%)。^1H NMR(500MHz,氯仿-d)δ7.89(d,J=8.4Hz,2H),7.83(q,J=8.0Hz,1H),7.58(dd,J=7.5,2.3Hz,1H),7.05(d,J=8.3Hz,2H),7.00-6.84(m,6H),4.91(s,2H),3.76(s,2H),3.39(s,3H),1.47(s,6H).MS(ESI)m/z 514.3$[M+H]^+$

[0295] 实施例3

[0296] 7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-(3,4-二氟苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0297] 

[0298] (a)2-(4-溴苄基)-7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0299] 利用类似于实施例1的步骤(a)至步骤(e)中所述方法的方法,合成标题化合物,其中在步骤(a)中,加入1-溴-4-(溴甲基)苯,代替2-(4-(氯甲基)苯基)-吡啶。MS(ESI)m/z 388.1[M+H]$^+$.

[0300] (b)2-(4-苯氧基苄基)-7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0301] 将2-(4-溴苄基)-7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(118g,304mmol)加入到苯酚(57g,606mmol)和碳酸铯(200g,614mmol)的NMP(900mL)混悬液中,随后加入2,2,6,6-四甲基庚烷-3,5-二酮(7mL,33.5mmol)和CuCl(15g,152mmol)。将反应混合物在氮气气氛下于120℃下加热10h。完成反应后,将混合物用水(4L)稀释,然后用乙酸乙酯萃取。将合并的有机相蒸发至干。得到的粗产物经硅胶柱色谱纯化,得到103g产物(产率:84%)。MS(ESI)m/z 402.2[M+H]$^+$。

[0302] (c)7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0303] 将TFA(600mL)加入到2-(4-苯氧基苄基)-7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(103g,257mmol)的二氯甲烷(210mL)混悬液中,得到黄褐色溶液,然后加入TFMSA(168mL)。将反应混合物在室温下搅拌,直到原料消失。将反应混合物倒入冷水(3L)中。过滤后,将滤饼用水冲洗两次,然后用氢氧化铵水溶液碱化,随后在搅拌下,加入乙酸乙酯。将沉淀的固体物过滤,依次用水冲洗三次,用乙酸乙酯冲洗两次,以及用甲醇冲洗一次,然后真空干燥,得到45g产物(产率:80%)。MS(ESI)m/z 220.2[M+H]$^+$。

[0304] (d)7,8-二氢-2-(4-(吡啶-2-基)苄基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0305] 将7,8-二氢-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(1.5g,6.84mmol)、2-(4-(溴甲基)苯基)吡啶(1.7g,6.84mmol)和K_2CO_3(2.83g,20.5mmol)的DMF(60mL)混悬液在室温下搅拌2-3天。减压除去溶剂。得到的残留物用水(100mL)处理,超声处理,然后过滤。将滤饼真空干燥,得到2.19g粗产物(产率:83%),未经进一步纯化,将其用于下一步骤中。MS(ESI)m/z 387.1[M+H]$^+$。

[0306] (e)7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-

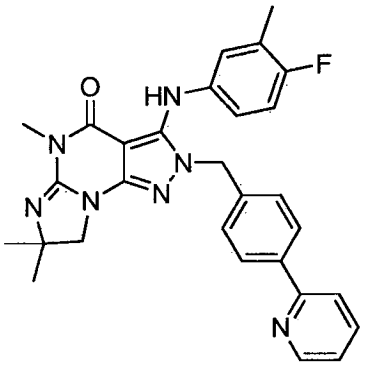
a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0307] 将1.0M LiHMDS(3.0mL,3.0mmol)的THF溶液滴加到粗7,8-二氢-2-(4-(吡啶-2-基)苄基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(1.16g,3.0mmol)和六氯乙烷(2.13g,9.0mmol)的二氯甲烷(30mL)溶液中。将反应混合物在室温搅拌90min,然后用冷水(200mL)淬灭。将混合物用二氯甲烷萃取3次(50mL×3),并将合并的有机相用盐水(30mL)冲洗,然后减压蒸发至干。将得到的残留物经中性氧化铝柱纯化,得到960mg纯产物,为灰白色固体物(HPLC纯度:96.8%;产率:76%)。MS(ESI)m/z 421.2$[M+H]^+$。

[0308] (f)7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-(3,4-二氟苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0309] 将7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(230mg,0.546mmol)、3,4-二氟苯胺(106mg,0.821mmol)和碳酸钾(300mg,2.17mmol)的叔-戊醇(2.8mL)混合物用氩气脱气,然后加入Xantphos(26mg,0.045mmol)和$Pd_2(dba)_3$(20mg,0.022mmol)。将混悬液再脱气,然后加热至110℃。将反应混合物在氩气气氛下于110℃搅拌过夜。常规后处理后,将粗产物在碱性氧化铝柱上纯化,得到194mg终产物,为米黄色固体物(HPLC纯度:99%;产率:69%)。^1H NMR(500MHz,氯仿-d)δ8.69(d,J=4.5Hz,1H),7.88(d,J=8.2Hz,2H),7.76(td,J=7.8,1.6Hz,1H),7.67(d,J=7.9Hz,1H),7.26-7.17(m,2H),7.15(d,J=8.2Hz,2H),7.03(m,1H),6.69(m,1H),6.60(m,1H),5.05(s,2H),3.79(s,2H),3.29(s,3H),1.47(s,6H).MS(ESI)m/z 514.2$[M+H]^+$.

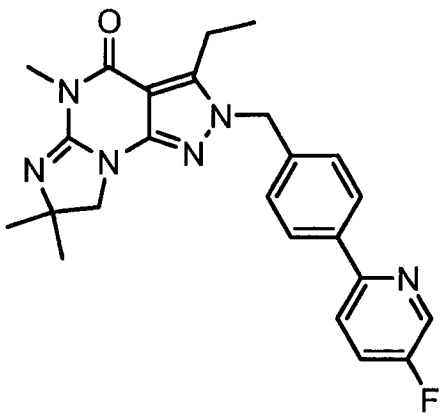
[0310] 实施例4 7,8-二氢-2-(4-(吡啶-2-基)苄基)-3-(4-氟-3-甲基苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0311] 

[0312] 合成方法类似于实施例3,其中在步骤(f)中,加入4-氟-3-甲基苯胺,代替3,4-二氟苯胺。得到终产物,为灰白色固体物(HPLC纯度:97%).^1H NMR(500MHz,氯仿-d)δ8.70(ddd,J=4.8,1.9,1.0Hz,1H),7.86(d,J=8.3Hz,2H),7.77(td,J=7.7,1.9Hz,1H),7.68(d,J=8.0Hz,1H),7.26(m,1H),7.06(d,J=8.3Hz,2H),6.97-6.86(m,2H),6.81-6.69(m,2H),4.91(s,2H),3.81(s,2H),3.40(s,3H),2.13(d,J=1.4Hz,3H),1.49(s,6H).MS(ESI)m/z 510.2$[M+H]^+$

[0313] 实施例5

[0314] 7,8-二氢-2-(4-(5-氟吡啶-2-基)苄基)-3-乙基-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0315] 

[0316] (a)7,8-二氢-2-(4-(5-氟吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

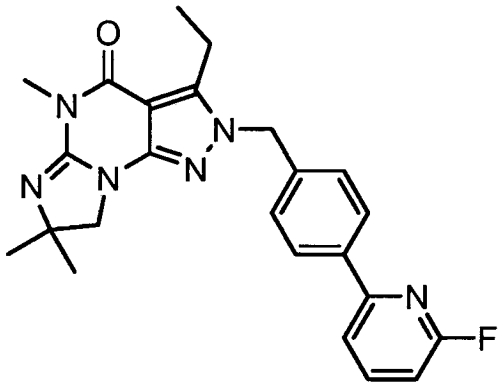
[0317] 利用类似于实施例1的步骤(a)～(f)中所述的方法,制备标题化合物,其中在步骤(a)中,加入2-(4-(氯甲基)苯基)-5-氟吡啶,代替2-(4-(氯甲基)苯基)-吡啶。MS(ESI)m/z 439.2[M+H]$^+$。

[0318] (b)7,8-二氢-2-(4-(5-氟吡啶-2-基)苄基)-3-乙基-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0319] 在0℃下,氩气气氛下,将溴化乙基镁(3.0M的乙醚溶液,3mL)滴加到含有$ZnCl_2$(1.2g,8.8mmol)的反应管中。将混合物在室温搅拌20min,然后冷却至-78℃。滴加9-甲氧基-9-硼双环[3.3.1]壬烷(1.0M的己烷溶液,8mL)。加入完成后,将混合物在室温下搅拌40min。向混合物中,缓慢加入7,8-二氢-2-(4-(5-氟吡啶-2-基)苄基)-3-氯-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮(352mg,0.8mmol)的无水DMF(15mL)溶液,随后加入2-二环己基膦基-2′,6′-二甲氧基联苯(S-Phos,38mg)和醋酸钯(13mg)。将反应管密封,并在室温下搅拌30min,然后在100℃下加热4天。将混合物用水(150mL)稀释,然后用二氯甲烷(60mL×3)萃取。将合并的有机相减压蒸发至干。将残留物经装配反相C18柱的半制备HPLC系统纯化,利用含有0.1%甲酸的0-26%梯度(历经16min)的乙腈水溶液,得到177mg产物,为浅黄色固体物(HPLC纯度:99.5%;产率:51%)。^{1}H NMR(500MHz,氯仿-d)δ8.53(d,J=2.9Hz,1H),7.92(d,J=8.3Hz,2H),7.69(dd,J=8.8,4.2Hz,1H),7.47(td,J=8.4,2.9Hz,1H),7.25(d,J=9.0Hz,2H),5.29(s,2H),3.73(s,2H),3.41(s,3H),2.95(q,J=7.6Hz,2H),1.42(s,6H),1.18(t,J=7.5Hz,3H).MS(ESI)m/z 433.3[M+H]$^+$.

[0320] 实施例6

[0321] 7,8-二氢-2-(4-(6-氟吡啶-2-基)苄基)-3-乙基-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

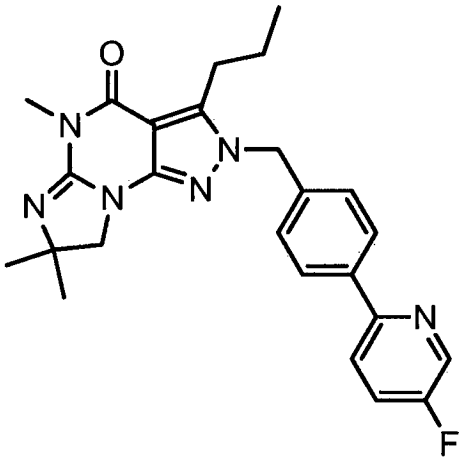
[0322] 

[0323] 利用类似于实施例5中所述的方法，制备标题化合物，其中在步骤(a)中，加入2-(4-(氯甲基)苯基)-6-氟吡啶，代替2-(4-(氯甲基)苯基)-5-氟吡啶。^{1}H NMR(400MHz,氯仿-d)δ7.98(d,J=8.4Hz,2H),7.84(m,1H),7.59(dd,J=7.5,2.4Hz,2H),7.25(d,J=8.4Hz,3H),6.87(dd,J=8.1,3.0Hz,1H),5.28(s,2H),3.71(s,2H),3.38(s,3H),2.94(q,J=7.5Hz,2H),1.40(s,6H),1.17(t,J=7.5Hz,3H).MS(ESI)m/z 433.2[M+H]$^+$.

[0324] 实施例5的化合物显示良好的PDE1选择性，并以等于或低于30nM的IC_{50}值，抑制PDE活性。

[0325] 实施例7

[0326] 7,8-二氢-2-(4-(5-氟吡啶-2-基)苄基)-3-丙基-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

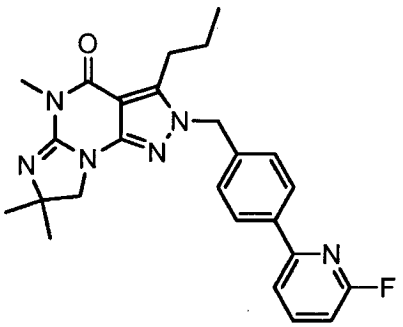
[0327] 

[0328] 利用类似于实施例5中所述的方法，制备标题化合物，其中在步骤(b)中，加入溴化丙基镁，代替溴化乙基镁。MS(ESI)m/z 447.2[M+H]$^+$。

[0329] 实施例7的化合物显示良好的PDE1选择性，并以等于或低于15nM的IC_{50}值，抑制PDE活性。

[0330] 实施例8

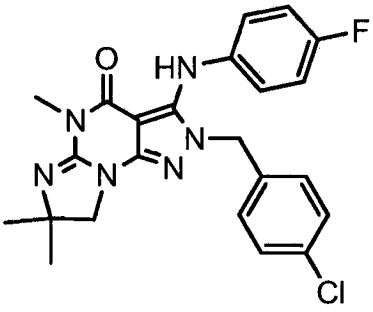
[0331] 7,8-二氢-2-(4-(6-氟吡啶-2-基)苄基)-3-丙基-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0332] 

[0333] 利用类似于实施例5中所述的方法，制备标题化合物，其中在步骤(b)中，加入溴化丙基镁，代替溴化乙基镁，并在步骤(a)中，加入2-(4-(氯甲基)苯基)-6-氟吡啶，代替2-(4-(氯甲基)苯基)-5-氟吡啶。MS(ESI)m/z 447.2[M+H]$^+$.

[0334] 实施例9

[0335] 7,8-二氢-2-(4-氯苄基)-3-(4-氟苯基氨基)-5,7,7-三甲基-[2H]-咪唑并-[1,2-a]吡唑并[4,3-e]嘧啶-4(5H)-酮

[0336] 

[0337] 利用类似于实施例1中所述的方法，制备标题化合物，其中在步骤(a)中，加入1-氯-4-(氯甲基)苯，代替2-(4-(氯甲基)苯基)-吡啶。MS(ESI)m/z 453.2[M+H]$^+$

[0338] 实施例9的化合物显示良好的PDE1选择性，并以等于或低于5nM的IC_{50}值，抑制PDE活性。

[0339] 实施例10：采用IMAP磷酸二酯酶分析试剂盒测定PDEIB体外抑制

[0340] 磷酸二酯酶IB(PDEIB)是将环鸟苷一磷酸(cGMP)转化为5'-鸟苷一磷酸(5'-GMP)的钙/钙调蛋白依赖性磷酸二酯酶。PDEIB还可以将修饰的cGMP底物、例如荧光分子cGMP-荧光素转化为相应的GMP-荧光素。由cGMP-荧光素生成GMP-荧光素可以采用例如IMAP(Molecular Devices,Sunnyvale,CA)固定化金属亲和颗粒试剂进行定量。

[0341] 简言之，IMAP试剂以高亲和力与见于GMP-荧光素中的而不是见于cGMP-荧光素中的游离5'-磷酸结合。所得的GMP-荧光素-IMAP复合物相对于cGMP-5荧光素而言是大的。在大的、缓慢滚动(tumbling)的复合物中建立的小的荧光团，可以与未结合的荧光团区分开，因为它们发荧光时所释放的光子保持与用于激发荧光的光子相同的极性。

[0342] 在磷酸二酯酶测定中，cGMP-荧光素(其不能与IMAP结合并因而几乎不保留荧光偏振)被转化为GMP-荧光素，其当与IMAP结合时使得荧光偏振(mp)产生大的增加。因此，磷酸二酯酶的抑制被作为mp的降低来检测。

[0343] 酶测定

[0344] 材料：除了IMAP试剂(反应缓冲液，结合缓冲液，FL-GMP和IMAP珠)外，所有化学物质可自Sigma-Aldrich(St.Louis,MO)获得，IMAP试剂可自Molecular Devices(Sunnyvale,

CA)获得。

[0345] 测定:可使用下面的磷酸二酯酶酶:3',5'-环核苷酸特异性牛脑磷酸二酯酶(Sigma,St.Louis,MO)(主要是PDEIB)和重组全长人PDE1 A和PDE1B(分别是r-hPDE1 A和r-hPDE1B),其可由本领域技术人员在例如HEK或SF9细胞中生产。将PDE1酶用50%甘油重构至2.5U/ml。于30℃、pH 7.5下,1单位的酶每分钟将1.0μmol 3',5'-cAMP水解为5'-AMP。将一份酶加入1999份反应缓冲液(30μM $CaCl_2$,10U/ml钙调蛋白(Sigma P2277)、10mM Tris-HCl pH 7.2、10mM $MgCl_2$、0.1%BSA、0.05%NaN_3)中,得到终浓度为1.25mU/ml。将99μl稀释的酶溶液加入平底96孔聚乙烯板的各孔中,向其中加入1μl溶于100%DMSO中的受试化合物。将化合物与酶混合并于室温预孵育10分钟。

[0346] 在384孔微量滴定板中,通过合并4份酶与抑制剂混合物和1份底物溶液(0.225μL)启动FL-GMP转化反应。将反应物于室温在暗处孵育15分钟。向384孔板的各孔中加入60μl结合试剂(IMAP珠在补充有1:1800消泡剂稀释液的结合缓冲液中的1:400稀释液)阻止反应。将板于室温孵育1小时,以使得IMAP结合进行完全,然后置于Envision多模式微量板读数器(PerkinElmer,Shelton,CT)中以测定荧光偏振(mp)。

[0347] 作为降低的mp测得的GMP浓度的降低指示PDE活性的抑制。IC_{50}值通过测定在0.0037nM至80,000nM范围内的8至16个浓度的化合物存在下的酶活性,然后将药物浓度对AmP作图来确定,其允许采用非线性回归分析软件(XLFit;IDBS,Cambrige,MA)来估计IC_{50}值。

[0348] 在本测定中,实施例1-9的各化合物显示良好的PDE1选择性,并能以等于或低于50nM的IC_{50}值,抑制PDE1。

[0349] 实施例11

[0350] 本发明的选择性PDE1抑制剂在人微粒体稳定性测定中,显示微粒体稳定性。上述选择性PDE1抑制剂显示低于0.01的K值,并显示大约100-1800分钟的半衰期T1/2。

[0351] 实施例12

[0352] 本发明的选择性PDE1抑制剂显示透过血脑屏障的能力。在适当小鼠模型中注射10mg/kg后,上述选择性PDE1抑制剂在注射后小于大约0.5小时,可以以大约3μM检测到。

Abstract

The subject matter generally relates to compounds and methods of treatment and/or prophylaxis of CNS diseases, disorders, and/or injuries. In one aspect, the subject matter relates to inhibitors of phosphodiesterase 1 (PDE1) as neuroprotective agents and/or neural regenerative agents. In a further aspect, the subject matter relates to individuals that are at risk for the development of CNS disease or disorder.