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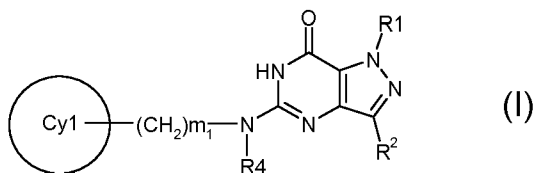
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(54) Title: NOVEL COMPOUNDS USEFUL FOR THE TREATMENT OF DEGENERATIVE & INFLAMMATORY DISEASES



(57) Abstract: The present invention relates to compounds that are inhibitors of PDE1A, a phosphodiesterase that is involved in the modulation of the degradation of cartilage, joint degeneration and diseases involving such degradation and/or inflammation, and particularly, to a compound according to formula (I), or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof. The invention extends to compositions, including pharmaceutical compositions, and corresponding uses thereof, and methods of prophylaxis and treatment involving the same.

**NOVEL COMPOUNDS USEFUL FOR THE TREATMENT OF DEGENERATIVE &
INFLAMMATORY DISEASES**

BACKGROUND OF THE INVENTION

Field of Invention

[0001] The present invention relates to compounds that are inhibitors of PDE1A, a phosphodiesterase that is involved in the modulation of the degradation of cartilage, joint degeneration and diseases involving such degradation and/or inflammation.

[0002] Cartilage is an avascular tissue of which chondrocytes are the main cellular component. The chondrocytes in normal articular cartilage occupy approximately 5% of the tissue volume, while the extra-cellular matrix makes up the remaining 95% of the tissue. The chondrocytes secrete the components of the matrix, mainly proteoglycans and collagens, which in turn supply the chondrocytes with an environment suitable for their survival under mechanical stress. In cartilage, collagen type II, together with the protein collagen type IX, is arranged in solid fibril-like structures, which provide cartilage with great mechanical strength. The proteoglycans can absorb water and are responsible for the resilient and shock absorbing properties of the cartilage.

[0003] One of the functional roles of cartilage in the joint is to allow bones to articulate on each other smoothly. Loss of articular cartilage, therefore, causes the bones to rub against each other leading to pain and loss of mobility. The degradation of cartilage can have various causes. In inflammatory arthritis, as in rheumatoid arthritis for example, cartilage degradation is caused by the secretion of proteases (e.g. collagenases) by inflamed tissues (the inflamed synovium for example). Cartilage degradation can also be the result of an injury of the cartilage, due to an accident or surgery, or exaggerated loading or 'wear and tear'. The ability of cartilage tissue to regenerate after such insults is limited. Chondrocytes in injured cartilage often display reduced cartilage synthesizing (anabolic) activity and/or increased cartilage degrading (catabolic) activity.

[0004] The degeneration of cartilage is the hallmark of various diseases, among which rheumatoid arthritis and osteoarthritis are the most prominent.

[0005] Rheumatoid arthritis (RA) is a chronic joint degenerative disease, characterized by inflammation and destruction of the joint structures. When the disease is unchecked, it leads to substantial disability and pain due to loss of joint functionality and even premature death. The aim of an RA therapy, therefore, is not to slow down the disease but to attain remission in order to stop the joint destruction. Besides the severity of the disease outcome, the high prevalence of RA (~ 0.8% of adults are affected worldwide) means a high socio-economic impact. (For reviews on RA, we refer to Smolen and Steiner (2003); Lee and Weinblatt (2001); Choy and Panayi (2001); O'Dell (2004) and Firestein (2003)).

[0006] Osteoarthritis (also referred to as OA, or wear-and-tear arthritis) is the most common form of arthritis and is characterized by loss of articular cartilage, often associated with hypertrophy of the bone and pain. The disease mainly affects hands and weight-bearing joints such as knees, hips and spines. This process thins the cartilage. When the surface area has disappeared due to the thinning, a grade I

osteoarthritis is reached; when the tangential surface area has disappeared, grade II osteoarthritis is reached. There are further levels of degeneration and destruction, which affect the deep and the calcified cartilage layers that border with the subchondral bone. For an extensive review on Osteoarthritis, refer to Wieland *et al.*, 2005.

[0007] The clinical manifestations of the development of the osteoarthritis condition include: increased volume of the joint, pain, crepitation and functional disability that, lead to pain and reduced mobility of the joints. When disease further develops, pain at rest emerges. If the condition persists without correction and/or therapy, the joint is destroyed leading to disability. Replacement surgery with total prosthesis is then required.

[0008] Therapeutic methods for the correction of the articular cartilage lesions that appear during the osteoarthritic disease have been developed, but so far none of them have been able to mediate the regeneration of articular cartilage *in situ* and *in vivo*.

[0009] Osteoarthritis is difficult to treat. At present, no cure is available and treatment focuses on relieving pain and preventing the affected joint from becoming deformed. Common treatments include the use of non-steroidal anti-inflammatory drugs (NSAID's). Although the dietary supplements as chondroitin and glucosamine sulphate have been advocated as safe and effective options for the treatment of osteoarthritis, a recent clinical trial revealed that both treatments did not reduce pain associated to osteoarthritis. (Clegg *et al.*, 2006). Taken together, no disease modifying osteoarthritic drugs are available.

[0010] In severe cases, joint replacement may be necessary. This is especially true for hips and knees. If a joint is extremely painful and cannot be replaced, it may be fused. This procedure stops the pain, but results in the permanent loss of joint function, making walking and bending difficult.

[0011] Another possible treatment is the transplantation of cultured autologous chondrocytes. Here chondral cellular material is taken from the patient, sent to a laboratory where it is expanded. The material is then implanted in the damaged tissues to cover the tissue's defects.

[0012] Another treatment includes the intra-articular instillation of Hylan G-F 20 (Synvisc, Hyalgan, Artz etc.), a substance that improves temporarily the rheology of the synovial fluid, producing an almost immediate sensation of free movement and a marked reduction of pain.

[0013] Other reported methods include application of tendinous, periosteal, fascial, muscular or perichondral grafts; implantation of fibrin or cultured chondrocytes; implantation of synthetic matrices, such as collagen, carbon fiber; administration of electromagnetic fields. All of these have reported minimal and incomplete effects, resulting in a poor quality tissue that can neither support the weighted load nor allow the restoration of an articular function with normal movement.

[0014] Stimulation of the anabolic processes, blocking catabolic processes, or a combination of these two, may result in stabilization of the cartilage, and perhaps even reversion of the damage, and therefore prevent further progression of the disease. Various triggers may stimulate anabolic stimulation of chondrocytes. Insulin-like growth factor-I (IGF-I) is the predominant anabolic growth factor in synovial fluid and stimulates the synthesis of both proteoglycans and collagen. It has also been shown that

members of the bone morphogenetic protein (BMP) family, notably BMP2, BMP4, BMP6, and BMP7, and members of the human transforming growth factor- β (TGF- β) family can induce chondrocyte anabolic stimulation (Chubinskaya and Kuettner, 2003). A compound has recently been identified that induces anabolic stimulation of chondrocytes (US 6,500,854; EP 1.391211). However, most of these compounds show severe side effects and, consequently, there is a strong need for compounds that stimulate chondrocyte differentiation without these side effects.

[0015] Adenosine 3', 5'-cyclic monophosphate (cyclic AMP or cAMP) and guanosine 3', 5'-cyclic monophosphate (cyclic GMP or cGMP) are key second messenger molecules in cells which are synthesized by guanylyl and adenylyl cyclases. These molecules, by playing a role as 'relay' on signal transduction pathways, are key in controlling normal and pathological cell responses. Cyclic nucleotide phosphodiesterases (PDE's) are enzymes that hydrolyse cyclic nucleotides and thereby control the cellular levels of these second messenger molecules. Because of their key role in cellular signaling, PDE's are considered new therapeutic targets. Inhibition of PDE4 and PDE5 are accepted approaches for the treatment of asthma/chronic obstructive pulmonary disease and erectile dysfunction, respectively. As such, pharmaceutical industry has recently deployed a lot of efforts to develop PDE4 inhibitors (e.g. Cilomilast) and PDE5 inhibitors (e.g. sildenafil), some of which are marketed.

[0016] The diversity of the PDE family of enzymes (11 gene families (PDE1 – PDE11) encoding more than 20 different PDE genes) allows a refined control over a variety of cellular processes. For an extensive review on PDE's, we refer to Lugnier, 2006. PDE's classically contain a catalytic domain, which is well conserved among different PDEs. In addition, PDE's contain regulatory domains. The activity of enzymes of the PDE1 subfamily, for example, is regulated by Ca^{2+} and calmodulin as well as by phosphorylation. As such, the PDE1 enzymes are involved in the complex interaction between the Ca^{2+} and cyclic nucleotide second messenger systems. Another feature of PDE1 enzymes is their dual substrate specificity as they have the capacity to hydrolyse both cAMP and cGMP (Zhang *et al.*, 2004).

[0017] The generation of transgenic animals represents the best tool for the understanding of the specific physiological role of individual PDEs. In the PDE1 subfamily, a PDE1B knockout mouse has been generated and characterized. PDE1B(-/-) mice showed exaggerated hyperactivity after acute D-methamphetamine administration. PDE1B(-/-) and PDE1B(+/-) mice demonstrated spatial-learning deficits. These results indicate that enhancement of cyclic nucleotide signaling by inactivation of PDE1B-mediated cyclic nucleotide hydrolysis plays a significant role in the central nervous system, especially on the dopaminergic function (Reed *et al.*, 2002). Less is known about the physiological role of the other members of the PDE1 superfamily. A role for PDE1 enzymes (PDE1C in particular) in vascular tone (e.g.; in pulmonary hypertension) has been suggested (Murray *et al.*, 2006). For an extensive review on PDE1 enzymes, we refer to Kakkar *et al.*, 1999 and Goraya and Cooper, 2005.

[0018] Several data point to a role of PDEs in chondrocyte biology. First, PDE4 and PDE1 were identified as major PDE activities in chondrocytes (Tenor *et al.*, 2002). The involvement of PDEs in cartilage catabolic events was further evidenced as follows. The IL1 cytokine is responsible for cartilage catabolism by reducing the expression of matrix components, by inducing the expression of collagenases and inducible nitric oxide synthase (iNOS), which mediates the production of nitric oxide (NO). This

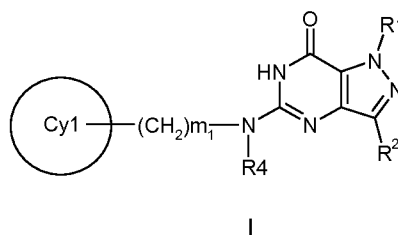
event appears dependent on PDE activity, as IBMX, PDE5 inhibitor and PDE4 inhibitor treatment of chondrocytes reduced the induction of iNOS expression by IL1 (Geng *et al.*, 1998, Tenor *et al.*, 2002). The ability of PDE inhibitors to reduce iNOS expression appeared dependent on autocrine PGE2 production by the chondrocytes. Taken together, these data suggest a role for PDEs in cartilage catabolic events.

[0019] The current therapies are not satisfactory and therefore there remains a need to identify further compounds that may be of use in the treatment of degenerative joint diseases, e.g. osteoarthritis, rheumatoid arthritis and osteoporosis. The present invention therefore provides compounds, methods for their manufacture and a pharmaceutical comprising a compound of the invention together with a suitable pharmaceutical carrier. The present invention also provides for the use of a compound of the invention in the preparation of a medicament for the treatment of degenerative joint diseases.

SUMMARY OF THE INVENTION

[0020] The present invention is based on the discovery that inhibitors of PDE1A are useful for the treatment of diseases involving cartilage degradation, joint degradation and/or inflammation, for example osteoarthritis. The present invention also provides methods for the production of these compounds, pharmaceutical compositions comprising these compounds and methods for treating diseases involving cartilage degradation, joint degradation and/or inflammation by administering a compound of the invention.

[0021] Accordingly, the present invention relates to compounds, according to formula (I):



wherein:

Cy1 is substituted or unsubstituted cycloalkyl or substituted or unsubstituted heterocycloalkyl;

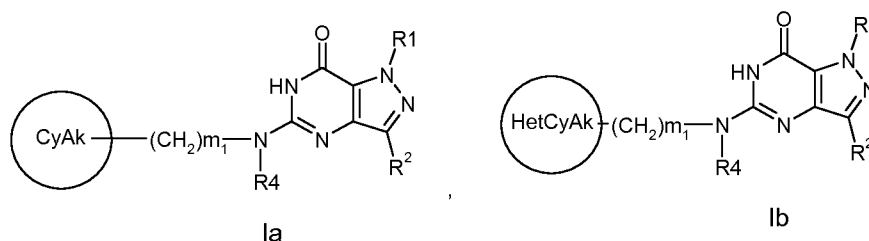
R¹ is H, substituted or unsubstituted alkyl;

R² is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

R⁴ is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl; and m₁ is 0, 1, 2, 3, or 4; provided that when Cy1 is heterocycloalkyl and the heterocycloalkyl group is joined to -(CH₂)_{m1}- via an N atom of said heterocycloalkyl group then m₁ is 2, 3 or 4;

or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof.

[0022] In one embodiment, with respect to compounds of formula I, the compound is according to formulae Ia or Ib:



wherein R^1 , R^2 , R^4 and m_1 are as described for formula I;

CyAk is substituted or unsubstituted cycloalkyl; and HetCyAk is substituted or unsubstituted heterocycloalkyl.

[0023] In one embodiment, with respect to compounds of formulae Ia-Ib, R^1 is alkyl. In another embodiment R^1 is Me.

[0024] In one embodiment, with respect to compounds of formulae Ia-Ib, R^2 is alkyl or cycloalkyl. In another embodiment R^2 is alkyl.

[0025] In one embodiment, with respect to compounds of formulae Ia-Ib, R^4 is alkyl.

[0026] In one embodiment, with respect to compounds of formulae Ia-Ib, R^4 is substituted alkyl and the substitution is selected from substituted or unsubstituted cycloalkyl and heterocycloalkyl.

[0027] Another aspect of this invention relates to the use of the present compound in a therapeutic method, a pharmaceutical composition, and the manufacture of such composition, useful for the treatment of a disease involving inflammation, and in particular, a disease characteristic of abnormal PDE1A activity. This invention also relates to processes for the preparation of the present compounds.

[0028] Other objects and advantages will become apparent to those skilled in the art from a consideration of the ensuing detailed description with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1. Shows the mechanism of the primary screening assay using the cAMP dynamic htrf kit from Cisbio.

DETAILED DESCRIPTION

Definitions

[0030] The following terms are intended to have the meanings presented therewith below and are useful in understanding the description and intended scope of the present invention.

[0031] When describing the invention, which may include compounds, pharmaceutical compositions containing such compounds and methods of using such compounds and compositions, the following terms, if present, have the following meanings unless otherwise indicated. It should also be understood that when described herein any of the moieties defined forth below may be substituted with a variety of substituents, and that the respective definitions are intended to include such substituted moieties within their scope as set out below. Unless otherwise stated, the term “substituted” is to be defined as set out below. It should be further understood that the terms “groups” and “radicals” can be considered interchangeable when used herein.

[0032] The articles “a” and “an” may be used herein to refer to one or to more than one (i.e. at least one) of the grammatical objects of the article. By way of example “an analogue” means one analogue or more than one analogue.

[0033] ‘Acyl’ or ‘Alkanoyl’ refers to a radical $-C(O)R^{20}$, where R^{20} is hydrogen, C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, C_3 - C_{10} cycloalkylmethyl, 4-10 membered heterocycloalkyl, aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl as defined herein. Representative examples include, but are not limited to, formyl, acetyl, cyclohexylcarbonyl, cyclohexylmethylcarbonyl, benzoyl and benzylcarbonyl. Exemplary ‘acyl’ groups are $-C(O)H$, $-C(O)-C_1$ - C_8 alkyl, $-C(O)-(CH_2)_t(C_6-C_{10}$ aryl), $-C(O)-(CH_2)_t(5-10$ membered heteroaryl), $-C(O)-(CH_2)_t(C_3-C_{10}$ cycloalkyl), and $-C(O)-(CH_2)_t(4-10$ membered heterocycloalkyl), wherein t is an integer from 0 to 4.

[0034] ‘Substituted Acyl’ or ‘Substituted Alkanoyl’ refers to a radical $-C(O)R^{21}$, wherein R^{21} is independently

- C_1 - C_8 alkyl, substituted with halo or hydroxy; or
- C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl, each of which is substituted with unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy.

[0035] ‘Acylamino’ refers to a radical $-NR^{22}C(O)R^{23}$, where R^{22} is hydrogen, C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl and R^{23} is hydrogen, C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl, as defined herein. Exemplary ‘acylamino’ include, but are not limited to, formylamino, acetylamino, cyclohexylcarbonylamino, cyclohexylmethyl-carbonylamino, benzoylamino and benzylcarbonylamino. Exemplary ‘acylamino’ groups are $-NR^{21'}C(O)-C_1$ - C_8 alkyl, $-NR^{21'}C(O)-(CH_2)_t(C_6-C_{10}$ aryl), $-NR^{21'}C(O)-(CH_2)_t(5-10$ membered heteroaryl), $-NR^{21'}C(O)-(CH_2)_t(C_3-C_{10}$ cycloalkyl), and $-NR^{21'}C(O)-(CH_2)_t(4-10$ membered heterocycloalkyl), wherein t is an integer from 0 to 4, each $R^{21'}$ independently represents H or C_1 - C_8 alkyl.

[0036] ‘Substituted Acylamino’ refers to a radical $-NR^{24}C(O)R^{25}$, wherein:

R^{24} is independently

- H, C_1 - C_8 alkyl, substituted with halo or hydroxy; or
- C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl, each of which is substituted with unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy; and

R^{25} is independently

- H, C_1 - C_8 alkyl, substituted with halo or hydroxy; or
- C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, arylalkyl, 5-10 membered heteroaryl or heteroarylalkyl, each of which is substituted with unsubstituted C_1 - C_4 alkyl,

halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxyl;

provided at least one of R²⁴ and R²⁵ is other than H.

[0037] 'Alkoxy' refers to the group -OR²⁶ where R²⁶ is C₁-C₈ alkyl. Particular alkoxy groups are methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, and 1,2-dimethylbutoxy. Particular alkoxy groups are lower alkoxy, i.e. with between 1 and 6 carbon atoms. Further particular alkoxy groups have between 1 and 4 carbon atoms.

[0038] 'Substituted alkoxy' refers to an alkoxy group substituted with one or more of those groups recited in the definition of "substituted" herein, and particularly refers to an alkoxy group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, in particular 1 substituent, selected from the group consisting of amino, substituted amino, C₆-C₁₀ aryl, -O-aryl, carboxyl, cyano, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, halogen, 5-10 membered heteroaryl, hydroxyl, nitro, thioalkoxy, thio-O-aryl, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-S(O)₂- and aryl-S(O)₂-. Exemplary 'substituted alkoxy' groups are -O-(CH₂)_t(C₆-C₁₀ aryl), -O-(CH₂)_t(5-10 membered heteroaryl), -O-(CH₂)_t(C₃-C₁₀ cycloalkyl), and -O-(CH₂)_t(4-10 membered heterocycloalkyl), wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy. Particular exemplary 'substituted alkoxy' groups are OCF₃, OCH₂CF₃, OCH₂Ph, OCH₂-cyclopropyl, OCH₂CH₂OH, OCH₂CH₂NMe₂.

[0039] 'Alkoxy carbonyl' refers to a radical -C(O)-OR²⁷ where R²⁷ represents an C₁-C₈ alkyl, C₃-C₁₀ cycloalkyl, C₃-C₁₀ cycloalkylalkyl, 4-10 membered heterocycloalkylalkyl, aralkyl, or 5-10 membered heteroarylalkyl as defined herein. Exemplary "alkoxy carbonyl" groups are C(O)O-C₁-C₈ alkyl, -C(O)O-(CH₂)_t(C₆-C₁₀ aryl), -C(O)O-(CH₂)_t(5-10 membered heteroaryl), -C(O)O-(CH₂)_t(C₃-C₁₀ cycloalkyl), and -C(O)O-(CH₂)_t(4-10 membered heterocycloalkyl), wherein t is an integer from 1 to 4.

[0040] 'Substituted Alkoxy carbonyl' refers to a radical -C(O)-OR²⁸ where R²⁸ represents:

- C₁-C₈ alkyl, C₃-C₁₀ cycloalkyl, C₃-C₁₀ cycloalkylalkyl, or 4-10 membered heterocycloalkylalkyl, each of which is substituted with halo, substituted or unsubstituted amino, or hydroxy; or
- C₆-C₁₀ aralkyl, or 5-10 membered heteroarylalkyl, each of which is substituted with unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxyl.

[0041] '-O-aryl carbonyl' refers to a radical -C(O)-OR²⁹ where R²⁹ represents an C₆-C₁₀ aryl, as defined herein. Exemplary "-O-aryl carbonyl" groups is -C(O)O-(C₆-C₁₀ aryl).

[0042] 'Substituted -O-aryl carbonyl' refers to a radical -C(O)-OR³⁰ where R³⁰ represents a

- C₆-C₁₀ aryl, substituted with unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxyl.

[0043] 'Hetero-O-arylcarbonyl' refers to a radical $-C(O)-OR^{31}$ where R^{31} represents a 5-10 membered heteroaryl, as defined herein

[0044] 'Substituted Hetero-O-arylcarbonyl' refers to a radical $-C(O)-OR^{32}$ where R^{32} represents a:

- 5-10 membered heteroaryl, substituted with unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxyl.

[0045] 'Alkyl' means straight or branched aliphatic hydrocarbon having 1 to 20 carbon atoms. Particular alkyl has 1 to 12 carbon atoms. More particular is lower alkyl which has 1 to 6 carbon atoms. A further particular group has 1 to 4 carbon atoms. Exemplary straight chained groups include methyl, ethyl n-propyl, and n-butyl. Branched means that one or more lower alkyl groups such as methyl, ethyl, propyl or butyl is attached to a linear alkyl chain, exemplary branched chain groups include isopropyl, iso-butyl, t-butyl and isoamyl.

[0046] 'Substituted alkyl' refers to an alkyl group as defined above substituted with one or more of those groups recited in the definition of "substituted" herein, and particularly refers to an alkyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, in particular 1 substituent, selected from the group consisting of acyl, acylamino, acyloxy ($-O$ -acyl or $-OC(O)R^{20}$), alkoxy, alkoxycarbonyl, alkoxycarbonylamino ($-NR''$ -alkoxycarbonyl or $-NH-C(O)-OR^{27}$), amino, substituted amino, aminocarbonyl (carbamoyl or amido or $-C(O)-NR''_2$), aminocarbonylamino ($-NR''-C(O)-NR''_2$), aminocarbonyloxy ($-O-C(O)-NR''_2$), aminosulfonyl, sulfonylamino, aryl, $-O$ -aryl, azido, carboxyl, cyano, cycloalkyl, halogen, hydroxy, heteroaryl, nitro, thiol, $-S$ -alkyl, $-S$ -aryl, $-S(O)$ -alkyl, $-S(O)$ -aryl, $-S(O)_2$ -alkyl, and $-S(O)_2$ -aryl. In a particular embodiment 'substituted alkyl' refers to a C_1-C_8 alkyl group substituted with halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, azido, $-NR'''SO_2R''$, $-SO_2NR'''R''$, $-C(O)R''$, $-C(O)OR''$, $-OC(O)R''$, $-NR'''C(O)R''$, $-C(O)NR'''R''$, $-NR'''R''$, or $-(CR'''R''')_mOR'''$; wherein each R'' is independently selected from H, C_1-C_8 alkyl, $-(CH_2)_t(C_6-C_{10}$ aryl), $-(CH_2)_t(5-10$ membered heteroaryl), $-(CH_2)_t(C_3-C_{10}$ cycloalkyl), and $-(CH_2)_t(4-10$ membered heterocycloalkyl), wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy. Each of R''' and R'''' independently represents H or C_1-C_8 alkyl.

[0047] 'Amino' refers to the radical $-NH_2$.

[0048] 'Substituted amino' refers to an amino group substituted with one or more of those groups recited in the definition of 'substituted' herein, and particularly refers to the group $-N(R^{33})_2$ where each R^{33} is independently selected from:

- hydrogen, C_1-C_8 alkyl, C_6-C_{10} aryl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, or C_3-C_{10} cycloalkyl; or
- C_1-C_8 alkyl, substituted with halo or hydroxy; or

- $-(CH_2)_t(C_6-C_{10} \text{ aryl})$, $-(CH_2)_t(5-10 \text{ membered heteroaryl})$, $-(CH_2)_t(C_3-C_{10} \text{ cycloalkyl})$ or $-(CH_2)_t(4-10 \text{ membered heterocycloalkyl})$ wherein t is an integer between 0 and 8, each of which is substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy; or
- both R^{33} groups are joined to form an alkylene group.

When both R^{33} groups are hydrogen, $-N(R^{33})_2$ is an amino group. Exemplary 'substituted amino' groups are $-NR^{33'}-C_1-C_8$ alkyl, $-NR^{33'}-(CH_2)_t(C_6-C_{10} \text{ aryl})$, $-NR^{33'}-(CH_2)_t(5-10 \text{ membered heteroaryl})$, $-NR^{33'}-(CH_2)_t(C_3-C_{10} \text{ cycloalkyl})$, and $-NR^{33'}-(CH_2)_t(4-10 \text{ membered heterocycloalkyl})$, wherein t is an integer from 0 to 4, each $R^{33'}$ independently represents H or C_1-C_8 alkyl; and any alkyl groups present, may themselves be substituted by halo, substituted or unsubstituted amino, or hydroxy; and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy. For the avoidance of doubt the term "substituted amino" includes the groups alkylamino, substituted alkylamino, alkylarylamino, substituted alkylarylamino, arylamino, substituted arylamino, dialkylamino and substituted dialkylamino as defined below.

[0049] 'Alkylamino' refers to the group $-NHR^{34}$, wherein R^{34} is C_1-C_8 alkyl.

[0050] 'Substituted Alkylamino' refers to the group $-NHR^{35}$, wherein R^{35} is C_1-C_8 alkyl; and the alkyl group is substituted with halo, substituted or unsubstituted amino, hydroxy, C_3-C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6-C_{10} aryl, 5-10 membered heteroaryl, aralkyl or heteroaralkyl; and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy.

[0051] 'Alkylarylamino' refers to the group $-NR^{36}R^{37}$, wherein R^{36} is C_6-C_{10} aryl and R^{37} is C_1-C_8 alkyl.

[0052] 'Substituted Alkylarylamino' refers to the group $-NR^{38}R^{39}$, wherein R^{38} is C_6-C_{10} aryl and R^{39} is C_1-C_8 alkyl; and the alkyl group is substituted with halo, substituted or unsubstituted amino, hydroxy, C_3-C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6-C_{10} aryl, 5-10 membered heteroaryl, aralkyl or heteroaralkyl; and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, cyano, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy.

[0053] 'Arylamino' means a radical $-NHR^{40}$ where R^{40} is selected from C_6-C_{10} aryl and 5-10 membered heteroaryl as defined herein.

[0054] 'Substituted Arylamino' refers to the group $-NHR^{41}$, wherein R^{41} is independently selected from C_6-C_{10} aryl and 5-10 membered heteroaryl; and any aryl or heteroaryl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, cyano, unsubstituted C_1-C_4 alkoxy,

unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0055] 'Dialkylamino' refers to the group $\text{-NR}^{42}\text{R}^{43}$, wherein each of R⁴² and R⁴³ are independently selected from C₁-C₈ alkyl.

[0056] 'Substituted Dialkylamino' refers to the group $\text{-NR}^{44}\text{R}^{45}$, wherein each of R⁴⁴ and R⁴⁵ are independently selected from C₁-C₈ alkyl; and the alkyl group is independently substituted with halo, hydroxy, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, 5-10 membered heteroaryl, aralkyl or heteroaralkyl; and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0057] 'Diaryl amino' refers to the group $\text{-NR}^{46}\text{R}^{47}$, wherein each of R⁴⁶ and R⁴⁷ are independently selected from C₆-C₁₀ aryl.

[0058] "Aminosulfonyl" or "Sulfonamide" refers to the radical $\text{-S(O}_2\text{)NH}_2$.

[0059] "Substituted aminosulfonyl" or "substituted sulfonamide" refers to a radical such as $\text{-S(O}_2\text{)N(R}^{48}\text{)}_2$ wherein each R⁴⁸ is independently selected from:

- H, C₁-C₈ alkyl, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or
- C₁-C₈ alkyl substituted with halo or hydroxy; or
- C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy;

provided that at least one R⁴⁸ is other than H.

[0060] Exemplary 'substituted aminosulfonyl' or 'substituted sulfonamide' groups are $\text{-S(O}_2\text{)N(R}^{48'}\text{)-C}_1\text{-C}_8$ alkyl, $\text{-S(O}_2\text{)N(R}^{48'}\text{)-(CH}_2\text{)}_t\text{(C}_6\text{-C}_{10}$ aryl), $\text{-S(O}_2\text{)N(R}^{48'}\text{)-(CH}_2\text{)}_t\text{(5-10 membered heteroaryl)}$, $\text{-S(O}_2\text{)N(R}^{48'}\text{)-(CH}_2\text{)}_t\text{(C}_3\text{-C}_{10}$ cycloalkyl), and $\text{-S(O}_2\text{)N(R}^{48'}\text{)-(CH}_2\text{)}_t\text{(4-10 membered heterocycloalkyl)}$, wherein t is an integer from 0 to 4; each R^{48'} independently represents H or C₁-C₈ alkyl; and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

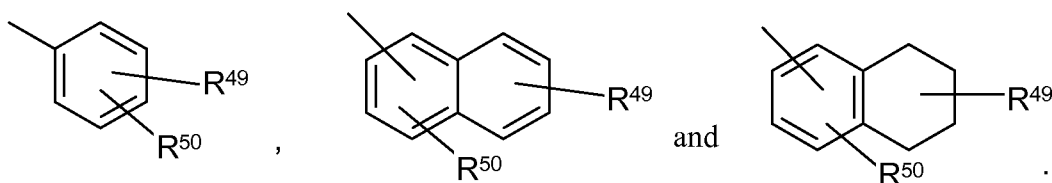
[0061] 'Aralkyl' or 'arylalkyl' refers to an alkyl group, as defined above, substituted with one or more aryl groups, as defined above. Particular aralkyl or arylalkyl groups are alkyl groups substituted with one aryl group.

[0062] 'Substituted Aralkyl' or 'substituted arylalkyl' refers to an alkyl group, as defined above, substituted with one or more aryl groups; and at least one of any aryl group present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, cyano, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0063] 'Aryl' refers to a monovalent aromatic hydrocarbon group derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. In particular aryl refers to an aromatic ring structure, mono-cyclic or poly-cyclic that includes from 5 to 12 ring members, more usually 6 to 10. Where the aryl group is a monocyclic ring system it preferentially contains 6 carbon atoms. Typical aryl groups include, but are not limited to, groups derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorene, hexacene, hexaphene, hexalene, as-indacene, s-indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, penta-2,4-diene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene, rubicene, triphenylene and trinaphthalene. Particularly aryl groups include phenyl, naphthyl, indenyl, and tetrahydronaphthyl.

[0064] 'Substituted Aryl' refers to an aryl group substituted with one or more of those groups recited in the definition of 'substituted' herein, and particularly refers to an aryl group that may optionally be substituted with 1 or more substituents, for instance from 1 to 5 substituents, particularly 1 to 3 substituents, in particular 1 substituent. Particularly, 'Substituted Aryl' refers to an aryl group substituted with one or more of groups selected from halo, C₁-C₈ alkyl, C₁-C₈ haloalkyl, C₁-C₈ haloalkoxy, cyano, hydroxy, C₁-C₈ alkoxy, and amino.

[0065] Examples of representative substituted aryls include the following



[0066] In these formulae one of R⁴⁹ and R⁵⁰ may be hydrogen and at least one of R⁴⁹ and R⁵⁰ is each independently selected from C₁-C₈ alkyl, 4-10 membered heterocycloalkyl, alkanoyl, C₁-C₈ alkoxy, hetero-O-aryl, alkylamino, arylamino, heteroarylamino, NR⁵¹COR⁵², NR⁵¹SOR⁵², NR⁵¹SO₂R⁵², COOalkyl, COOaryl, CONR⁵¹R⁵², CONR⁵¹OR⁵², NR⁵¹R⁵², SO₂NR⁵¹R⁵², S-alkyl, SOalkyl, SO₂alkyl, Saryl, SOaryl, SO₂aryl; or R⁴⁹ and R⁵⁰ may be joined to form a cyclic ring (saturated or unsaturated) from 5 to 8 atoms, optionally containing one or more heteroatoms selected from the group N, O or S. R⁵¹, and R⁵² are independently hydrogen, C₁-C₈ alkyl, C₁-C₄ haloalkyl, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, substituted aryl, 5-10 membered heteroaryl.

[0067] 'Arylalkyloxy' refers to an -O-alkylaryl radical where alkylaryl is as defined herein.

[0068] 'Substituted Arylalkyloxy' refers to an -O-alkylaryl radical where alkylaryl is as defined herein; and any aryl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, cyano, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0069] 'Azido' refers to the radical -N₃.

[0070] 'Carbamoyl or amido' refers to the radical -C(O)NH₂.

[0071] 'Substituted Carbamoyl or substituted amido' refers to the radical -C(O)N(R⁵³)₂ wherein each R⁵³ is independently

- H, C₁-C₈ alkyl, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or
- C₁-C₈ alkyl substituted with halo or hydroxy; or
- C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, each of which is substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy;

provided that at least one R⁵³ is other than H.

Exemplary 'Substituted Amido / Carbamoyl' groups are -C(O) NR⁵³-C₁-C₈ alkyl, -C(O)NR⁵³-(CH₂)_t(C₆-C₁₀ aryl), -C(O)N⁵³-(CH₂)_t(5-10 membered heteroaryl), -C(O)NR⁵³-(CH₂)_t(C₃-C₁₀ cycloalkyl), and -C(O)NR⁵³-(CH₂)_t(4-10 membered heterocycloalkyl), wherein t is an integer from 0 to 4, each R⁵³ independently represents H or C₁-C₈ alkyl and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0072] 'Carboxy' refers to the radical -C(O)OH.

[0073] 'Cycloalkyl' refers to cyclic non-aromatic hydrocarbyl groups having from 3 to 10 carbon atoms. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl.

[0074] 'Substituted cycloalkyl' refers to a cycloalkyl group as defined above substituted with one or more of those groups recited in the definition of 'substituted' herein, and particularly refers to a cycloalkyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, in particular 1 substituent.

[0075] 'Cyano' refers to the radical -CN.

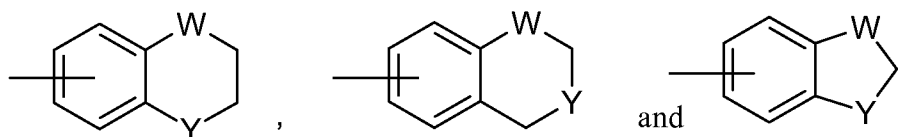
[0076] 'Halo' or 'halogen' refers to fluoro (F), chloro (Cl), bromo (Br) and iodo (I). Particular halo groups are either fluoro or chloro.

[0077] 'Hetero' when used to describe a compound or a group present on a compound means that one or more carbon atoms in the compound or group have been replaced by a nitrogen, oxygen, or sulfur heteroatom. Hetero may be applied to any of the hydrocarbyl groups described above such as alkyl, e.g. heteroalkyl, cycloalkyl, e.g. heterocycloalkyl, aryl, e.g. heteroaryl, cycloalkenyl, e.g. cycloheteroalkenyl, and the like having from 1 to 5, and particularly from 1 to 3 heteroatoms.

[0078] 'Heteroaryl' means an aromatic ring structure, mono-cyclic or polycyclic, that includes one or more heteroatoms and 5 to 12 ring members, more usually 5 to 10 ring members. The heteroaryl group can be, for example, a five membered or six membered monocyclic ring or a bicyclic structure formed from fused five and six membered rings or two fused six membered rings or, by way of a further example, two fused five membered rings. Each ring may contain up to four heteroatoms typically selected from nitrogen, sulphur and oxygen. Typically the heteroaryl ring will contain up to 4 heteroatoms, more typically up to 3 heteroatoms, more usually up to 2, for example a single heteroatom. In one embodiment,

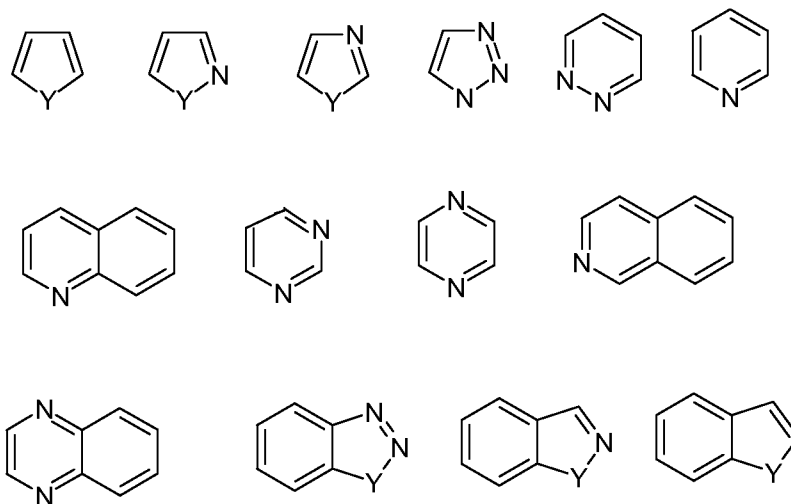
the heteroaryl ring contains at least one ring nitrogen atom. The nitrogen atoms in the heteroaryl rings can be basic, as in the case of an imidazole or pyridine, or essentially non-basic as in the case of an indole or pyrrole nitrogen. In general the number of basic nitrogen atoms present in the heteroaryl group, including any amino group substituents of the ring, will be less than five. Examples of five membered monocyclic heteroaryl groups include but are not limited to pyrrole, furan, thiophene, imidazole, furazan, oxazole, oxadiazole, oxatriazole, isoxazole, thiazole, isothiazole, pyrazole, triazole and tetrazole groups. Examples of six membered monocyclic heteroaryl groups include but are not limited to pyridine, pyrazine, pyridazine, pyrimidine and triazine. Particular examples of bicyclic heteroaryl groups containing a five membered ring fused to another five membered ring include but are not limited to imidazothiazole and imidazoimidazole. Particular examples of bicyclic heteroaryl groups containing a six membered ring fused to a five membered ring include but are not limited to benzfuran, benzthiophene, benzimidazole, benzoxazole, isobenzoxazole, benzisoxazole, benzthiazole, benzisothiazole, isobenzofuran, indole, isoindole, isoindolone, indolizine, indoline, isoindoline, purine (e.g., adenine, guanine), indazole, pyrazolopyrimidine, triazolopyrimidine, benzodioxole and pyrazolopyridine groups. Particular examples of bicyclic heteroaryl groups containing two fused six membered rings include but are not limited to quinoline, isoquinoline, chroman, thiochroman, chromene, isochromene, chroman, isochroman, benzodioxan, quinolizine, benzoxazine, benzodiazine, pyridopyridine, quinoxaline, quinazoline, cinnoline, phthalazine, naphthyridine and pteridine groups. Particular heteroaryl groups are those derived from thiophene, pyrrole, benzothiophene, benzofuran, indole, pyridine, quinoline, imidazole, oxazole and pyrazine.

[0079] Examples of representative aryl having hetero atoms containing substitution include the following:



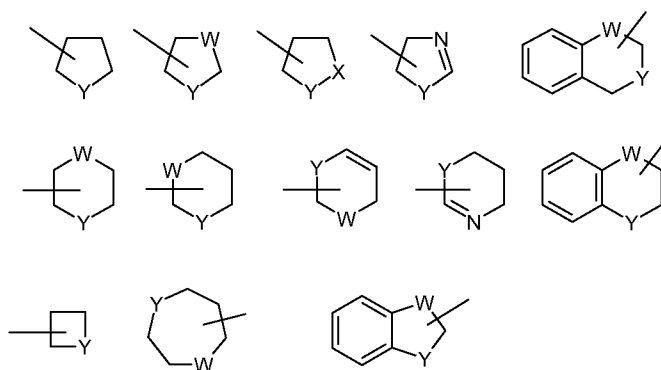
wherein each W is selected from $C(R^{54})_2$, NR^{54} , O and S; and each Y is selected from carbonyl, NR^{54} , O and S; and R^{54} is independently hydrogen, C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, and 5-10 membered heteroaryl.

[0080] Examples of representative heteroaryls include the following:



wherein each Y is selected from carbonyl, N, NR^{55} , O and S; and R^{55} is independently hydrogen, $\text{C}_1\text{-C}_8$ alkyl, $\text{C}_3\text{-C}_{10}$ cycloalkyl, 4-10 membered heterocycloalkyl, $\text{C}_6\text{-C}_{10}$ aryl, and 5-10 membered heteroaryl.

[0081] As used herein, the term 'heterocycloalkyl' refers to a 4-10 membered, stable heterocyclic non-aromatic ring and/or including rings containing one or more heteroatoms independently selected from N, O and S, fused thereto. A fused heterocyclic ring system may include carbocyclic rings and need only include one heterocyclic ring. Examples of heterocyclic rings include, but are not limited to, morpholine, piperidine (e.g. 1-piperidinyl, 2-piperidinyl, 3-piperidinyl and 4-piperidinyl), pyrrolidine (e.g. 1-pyrrolidinyl, 2-pyrrolidinyl and 3-pyrrolidinyl), pyrrolidone, pyran (2H-pyran or 4H-pyran), dihydrothiophene, dihydropyran, dihydrofuran, dihydrothiazole, tetrahydrofuran, tetrahydrothiophene, dioxane, tetrahydropyran (e.g. 4-tetrahydro pyran), imidazoline, imidazolidinone, oxazoline, thiazoline, 2-pyrazoline, pyrazolidine, piperazine, and N-alkyl piperazines such as N-methyl piperazine. Further examples include thiomorpholine and its S-oxide and S,S-dioxide (particularly thiomorpholine). Still further examples include azetidine, piperidone, piperazone, and N-alkyl piperidines such as N-methyl piperidine. Particular examples of heterocycloalkyl groups are shown in the following illustrative examples:



wherein each W is selected from CR^{56} , $\text{C}(\text{R}^{56})_2$, NR^{56} , O and S; and each Y is selected from NR^{56} , O and S; and R^{56} is independently hydrogen, $\text{C}_1\text{-C}_8$ alkyl, $\text{C}_3\text{-C}_{10}$ cycloalkyl, 4-10 membered heterocycloalkyl, $\text{C}_6\text{-C}_{10}$ aryl, 5-10 membered heteroaryl. These heterocycloalkyl rings may be optionally substituted with one or more groups selected from the group consisting of acyl, acylamino, acyloxy ($-\text{O}-\text{acyl}$ or $-\text{OC}(\text{O})\text{R}^{20}$), alkoxy, alkoxy carbonyl, alkoxy carbonylamino ($-\text{NR}''-\text{alkoxy carbonyl}$ or $-\text{NH}-\text{C}(\text{O})-\text{OR}^{27}$),

amino, substituted amino, aminocarbonyl (amido or $-\text{C}(\text{O})-\text{NR}''_2$), aminocarbonylamino ($-\text{NR}''-\text{C}(\text{O})-\text{NR}''_2$), aminocarbonyloxy ($-\text{O}-\text{C}(\text{O})-\text{NR}''_2$), aminosulfonyl, sulfonylamino, aryl, $-\text{O}$ -aryl, azido, carboxyl, cyano, cycloalkyl, halogen, hydroxy, nitro, thiol, $-\text{S}$ -alkyl, $-\text{S}$ -aryl, $-\text{S}(\text{O})$ -alkyl, $-\text{S}(\text{O})$ -aryl, $-\text{S}(\text{O})_2$ -alkyl, and $-\text{S}(\text{O})_2$ -aryl. Substituting groups include carbonyl or thiocarbonyl which provide, for example, lactam and urea derivatives.

[0082] 'Hydroxy' refers to the radical $-\text{OH}$.

[0083] 'Nitro' refers to the radical $-\text{NO}_2$.

[0084] 'Substituted' refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituent(s). Typical substituents may be selected from the group consisting of:

halogen, $-\text{R}^{57}$, $-\text{O}^-$, $=\text{O}$, $-\text{OR}^{57}$, $-\text{SR}^{57}$, $-\text{S}^-$, $=\text{S}$, $-\text{NR}^{57}\text{R}^{58}$, $=\text{NR}^{57}$, $-\text{CCl}_3$, $-\text{CF}_3$, $-\text{CN}$, $-\text{OCN}$, $-\text{SCN}$, $-\text{NO}$, $-\text{NO}_2$, $=\text{N}_2$, $-\text{N}_3$, $-\text{S}(\text{O})_2\text{O}^-$, $-\text{S}(\text{O})_2\text{OH}$, $-\text{S}(\text{O})_2\text{R}^{57}$, $-\text{OS}(\text{O})_2\text{O}^-$, $-\text{OS}(\text{O})_2\text{R}^{57}$, $-\text{P}(\text{O})(\text{O}^-)_2$, $-\text{P}(\text{O})(\text{OR}^{57})(\text{O}^-)$, $-\text{OP}(\text{O})(\text{OR}^{57})(\text{OR}^{58})$, $-\text{C}(\text{O})\text{R}^{57}$, $-\text{C}(\text{S})\text{R}^{57}$, $-\text{C}(\text{O})\text{OR}^{57}$, $-\text{C}(\text{O})\text{NR}^{57}\text{R}^{58}$, $-\text{C}(\text{O})\text{O}^-$, $-\text{C}(\text{S})\text{OR}^{57}$, $-\text{NR}^{59}\text{C}(\text{O})\text{NR}^{57}\text{R}^{58}$, $-\text{NR}^{59}\text{C}(\text{S})\text{NR}^{57}\text{R}^{58}$, $-\text{NR}^{60}\text{C}(\text{NR}^{59})\text{NR}^{57}\text{R}^{58}$ and $-\text{C}(\text{NR}^{59})\text{NR}^{57}\text{R}^{58}$;

wherein each R^{57} , R^{58} , R^{59} and R^{60} are independently:

- hydrogen, C_1 - C_8 alkyl, C_6 - C_{10} aryl, arylalkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, 5-10 membered heteroaryl, heteroarylalkyl; or
- C_1 - C_8 alkyl substituted with halo or hydroxy; or
- C_6 - C_{10} aryl, 5-10 membered heteroaryl, C_6 - C_{10} cycloalkyl or 4-10 membered heterocycloalkyl substituted by unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy.

In a particular embodiment, substituted groups are substituted with one or more substituents, particularly with 1 to 3 substituents, in particular with one substituent group.

In a further particular embodiment the substituent group or groups are selected from: halo, cyano, nitro, trifluoromethyl, trifluoromethoxy, azido, $-\text{NR}'''\text{SO}_2\text{R}''$, $-\text{SO}_2\text{NR}'''\text{R}''$, $-\text{C}(\text{O})\text{R}''$, $-\text{C}(\text{O})\text{OR}''$, $-\text{OC}(\text{O})\text{R}''$, $-\text{NR}'''\text{C}(\text{O})\text{R}''$, $-\text{C}(\text{O})\text{NR}'''\text{R}''$, $-\text{NR}'''\text{R}''$, $-(\text{CR}'''\text{R}'')_m\text{OR}''$, wherein, each R'' is independently selected from H, C_1 - C_8 alkyl, $-(\text{CH}_2)_t(\text{C}_6$ - C_{10} aryl), $-(\text{CH}_2)_t(5$ -10 membered heteroaryl), $-(\text{CH}_2)_t(\text{C}_3$ - C_{10} cycloalkyl), and $-(\text{CH}_2)_t(4$ -10 membered heterocycloalkyl), wherein t is an integer from 0 to 4; and

- any alkyl groups present, may themselves be substituted by halo or hydroxy; and
- any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy. Each R'' independently represents H or C_1 - C_6 alkyl.

[0085] 'Substituted sulfanyl' refers to the group $-\text{SR}^{61}$, wherein R^{61} is selected from:

- C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or

- C₁-C₈ alkyl substituted with halo, substituted or unsubstituted amino, or hydroxy; or
- C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, each of which is substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0086] Exemplary 'substituted sulfanyl' groups are -S-(C₁-C₈ alkyl) and -S-(C₃-C₁₀ cycloalkyl), -S-(CH₂)_t(C₆-C₁₀ aryl), -S-(CH₂)_t(5-10 membered heteroaryl), -S-(CH₂)_t(C₃-C₁₀ cycloalkyl), and -S-(CH₂)_t(4-10 membered heterocycloalkyl), wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy. The term 'substituted sulfanyl' includes the groups 'alkylsulfanyl' or 'alkylthio', 'substituted alkylthio' or 'substituted alkylsulfanyl', 'cycloalkylsulfanyl' or 'cycloalkylthio', 'substituted cycloalkylsulfanyl' or 'substituted cycloalkylthio', 'arylsulfanyl' or 'arylthio' and 'heteroarylsulfanyl' or 'heteroarylthio' as defined below.

[0087] 'Alkylthio' or 'Alkylsulfanyl' refers to a radical -SR⁶² where R⁶² is a C₁-C₈ alkyl or group as defined herein. Representative examples include, but are not limited to, methylthio, ethylthio, propylthio and butylthio.

[0088] 'Substituted Alkylthio' or 'substituted alkylsulfanyl' refers to the group -SR⁶³ where R⁶³ is a C₁-C₈ alkyl, substituted with halo, substituted or unsubstituted amino, or hydroxy.

[0089] 'Cycloalkylthio' or 'Cycloalkylsulfanyl' refers to a radical -SR⁶⁴ where R⁶⁴ is a C₃-C₁₀ cycloalkyl or group as defined herein. Representative examples include, but are not limited to, cyclopropylthio, cyclohexylthio, and cyclopentylthio.

[0090] 'Substituted cycloalkylthio' or 'substituted cycloalkylsulfanyl' refers to the group -SR⁶⁵ where R⁶⁵ is a C₃-C₁₀ cycloalkyl, substituted with halo, substituted or unsubstituted amino, or hydroxy.

[0091] 'Arylthio' or 'Arylsulfanyl' refers to a radical -SR⁶⁶ where R⁶⁶ is a C₆-C₁₀ aryl group as defined herein.

[0092] 'Heteroarylthio' or 'Heteroarylsulfanyl' refers to a radical -SR⁶⁷ where R⁶⁷ is a 5-10 membered heteroaryl group as defined herein.

[0093] 'Substituted sulfinyl' refers to the group -S(O)R⁶⁸, wherein R⁶⁸ is selected from:

- C₁-C₈ alkyl, C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or
- C₁-C₈ alkyl substituted with halo, substituted or unsubstituted amino, or hydroxy; or
- C₃-C₁₀ cycloalkyl, 4-10 membered heterocycloalkyl, C₆-C₁₀ aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, substituted by unsubstituted C₁-C₄ alkyl, halo, unsubstituted C₁-C₄ alkoxy, unsubstituted C₁-C₄ haloalkyl, unsubstituted C₁-C₄ hydroxyalkyl, or unsubstituted C₁-C₄ haloalkoxy or hydroxy.

[0094] Exemplary 'substituted sulfinyl' groups are -S(O)-(C₁-C₈ alkyl) and -S(O)-(C₃-C₁₀ cycloalkyl), -S(O)-(CH₂)_t(C₆-C₁₀ aryl), -S(O)-(CH₂)_t(5-10 membered heteroaryl), -S(O)-(CH₂)_t(C₃-C₁₀

cycloalkyl), and $-S(O)-(CH_2)_t(4-10 \text{ membered heterocycloalkyl})$, wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy. The term substituted sulfinyl includes the groups 'alkylsulfinyl', 'substituted alkylsulfinyl', 'cycloalkylsulfinyl', 'substituted cycloalkylsulfinyl', 'arylsulfinyl' and 'heteroarylsulfinyl' as defined herein.

[0095] 'Alkylsulfinyl' refers to a radical $-S(O)R^{69}$ where R^{69} is a C_1-C_8 alkyl group as defined herein. Representative examples include, but are not limited to, methylsulfinyl, ethylsulfinyl, propylsulfinyl and butylsulfinyl.

[0096] 'Substituted Alkylsulfinyl' refers to a radical $-S(O)R^{70}$ where R^{70} is a C_1-C_8 alkyl group as defined herein. substituted with halo, substituted or unsubstituted amino, or hydroxy.

[0097] 'Cycloalkylsulfinyl' refers to a radical $-S(O)R^{71}$ where R^{71} is a C_3-C_{10} cycloalkyl or group as defined herein. Representative examples include, but are not limited to, cyclopropylsulfinyl, cyclohexylsulfinyl, and cyclopentylsulfinyl.

[0098] 'Substituted cycloalkylsulfinyl' refers to the group $-S(O)R^{72}$ where R^{72} is a C_3-C_{10} cycloalkyl, substituted with halo, substituted or unsubstituted amino, or hydroxy.

[0099] 'Arylsulfinyl' refers to a radical $-S(O)R^{73}$ where R^{73} is a C_6-C_{10} aryl group as defined herein.

[00100] 'Heteroarylsulfinyl' refers to a radical $-S(O)R^{74}$ where R^{74} is a 5-10 membered heteroaryl group as defined herein.

[00101] 'Substituted sulfonyl' refers to the group $-S(O)_2R^{75}$, wherein R^{75} is selected from:

- C_1-C_8 alkyl, C_3-C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6-C_{10} aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or
- C_1-C_8 alkyl substituted with halo, substituted or unsubstituted amino, or hydroxy; or
- C_3-C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6-C_{10} aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, each of which is substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy.

[00102] Exemplary 'substituted sulfonyl' groups are $-S(O)_2-(C_1-C_8 \text{ alkyl})$ and $-S(O)_2-(C_3-C_{10} \text{ cycloalkyl})$, $-S(O)_2-(CH_2)_t(C_6-C_{10} \text{ aryl})$, $-S(O)_2-(CH_2)_t(5-10 \text{ membered heteroaryl})$, $-S(O)_2-(CH_2)_t(C_3-C_{10} \text{ cycloalkyl})$, and $-S(O)_2-(CH_2)_t(4-10 \text{ membered heterocycloalkyl})$, wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1-C_4 alkyl, halo, unsubstituted C_1-C_4 alkoxy, unsubstituted C_1-C_4 haloalkyl, unsubstituted C_1-C_4 hydroxyalkyl, or unsubstituted C_1-C_4 haloalkoxy or hydroxy. The term substituted sulfonyl includes the groups alkylsulfonyl, substituted alkylsulfonyl, cycloalkylsulfonyl, substituted cycloalkylsulfonyl, arylsulfonyl and heteroarylsulfonyl.

[00103] 'Alkylsulfonyl' refers to a radical $-S(O)_2R^{76}$ where R^{76} is an C_1 - C_8 alkyl group as defined herein. Representative examples include, but are not limited to, methylsulfonyl, ethylsulfonyl, propylsulfonyl and butylsulfonyl.

[00104] 'Substituted Alkylsulfonyl' refers to a radical $-S(O)_2R^{77}$ where R^{77} is an C_1 - C_8 alkyl group as defined herein, substituted with halo, substituted or unsubstituted amino, or hydroxy.

[00105] 'Cycloalkylsulfonyl' refers to a radical $-S(O)_2R^{78}$ where R^{78} is a C_3 - C_{10} cycloalkyl or group as defined herein. Representative examples include, but are not limited to, cyclopropylsulfonyl, cyclohexylsulfonyl, and cyclopentylsulfonyl.

[00106] 'Substituted cycloalkylsulfonyl' refers to the group $-S(O)_2R^{79}$ where R^{79} is a C_3 - C_{10} cycloalkyl, substituted with halo, substituted or unsubstituted amino, or hydroxy.

[00107] 'Arylsulfonyl' refers to a radical $-S(O)_2R^{80}$ where R^{80} is an C_6 - C_{10} aryl group as defined herein.

[00108] 'Heteroarylsulfonyl' refers to a radical $-S(O)_2R^{81}$ where R^{81} is an 5-10 membered heteroaryl group as defined herein.

[00109] 'Sulfo' or 'sulfonic acid' refers to a radical such as $-SO_3H$.

[00110] 'Substituted sulfo' or 'sulfonic acid ester' refers to the group $-S(O)_2OR^{82}$, wherein R^{82} is selected from:

- C_1 - C_8 alkyl, C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, aralkyl, 5-10 membered heteroaryl, and heteroaralkyl; or
- C_1 - C_8 alkyl substituted with halo, substituted or unsubstituted amino, or hydroxy; or
- C_3 - C_{10} cycloalkyl, 4-10 membered heterocycloalkyl, C_6 - C_{10} aryl, aralkyl, 5-10 membered heteroaryl, or heteroaralkyl, each of which is substituted by unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy.

[00111] Exemplary 'Substituted sulfo' or 'sulfonic acid ester' groups are $-S(O)_2O-(C_1-C_8 \text{ alkyl})$ and $-S(O)_2O-(C_3-C_{10} \text{ cycloalkyl})$, $-S(O)_2O-(CH_2)_t(C_6-C_{10} \text{ aryl})$, $-S(O)_2O-(CH_2)_t(5-10 \text{ membered heteroaryl})$, $-S(O)_2O-(CH_2)_t(C_3-C_{10} \text{ cycloalkyl})$, and $-S(O)_2O-(CH_2)_t(4-10 \text{ membered heterocycloalkyl})$, wherein t is an integer from 0 to 4 and any aryl, heteroaryl, cycloalkyl or heterocycloalkyl groups present, may themselves be substituted by unsubstituted C_1 - C_4 alkyl, halo, unsubstituted C_1 - C_4 alkoxy, unsubstituted C_1 - C_4 haloalkyl, unsubstituted C_1 - C_4 hydroxyalkyl, or unsubstituted C_1 - C_4 haloalkoxy or hydroxy.

[00112] 'Thiol' refers to the group $-SH$.

[00113] One having ordinary skill in the art of organic synthesis will recognize that the maximum number of heteroatoms in a stable, chemically feasible heterocyclic ring, whether it is aromatic or non aromatic, is determined by the size of the ring, the degree of unsaturation and the valence of the heteroatoms. In general, a heterocyclic ring may have one to four heteroatoms so long as the heteroaromatic ring is chemically feasible and stable.

[00114] 'Pharmaceutically acceptable' means approved or approvable by a regulatory agency of the Federal or a state government or the corresponding agency in countries other than the United States, or that is listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

[00115] 'Pharmaceutically acceptable salt' refers to a salt of a compound of the invention that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts are non-toxic may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of non toxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. The term "pharmaceutically acceptable cation" refers to an acceptable cationic counter-ion of an acidic functional group. Such cations are exemplified by sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium cations, and the like.

[00116] 'Pharmaceutically acceptable vehicle' refers to a diluent, adjuvant, excipient or carrier with which a compound of the invention is administered.

[00117] 'Prodrugs' refers to compounds, including derivatives of the compounds of the invention, which have cleavable groups and become by solvolysis or under physiological conditions the compounds of the invention which are pharmaceutically active in vivo. Such examples include, but are not limited to, choline ester derivatives and the like, N-alkylmorpholine esters and the like.

[00118] 'Solvate' refers to forms of the compound that are associated with a solvent, usually by a solvolysis reaction. This physical association includes hydrogen bonding. Conventional solvents include water, ethanol, acetic acid and the like. The compounds of the invention may be prepared e.g. in crystalline form and may be solvated or hydrated. Suitable solvates include pharmaceutically acceptable solvates, such as hydrates, and further include both stoichiometric solvates and non-stoichiometric solvates. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. 'Solvate' encompasses

both solution-phase and isolable solvates. Representative solvates include hydrates, ethanlates and methanlates.

[00119] 'Subject' includes humans. The terms 'human', 'patient' and 'subject' are used interchangeably herein.

[00120] 'Therapeutically effective amount' means the amount of a compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" can vary depending on the compound, the disease and its severity, and the age, weight, etc., of the subject to be treated.

[00121] 'Preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset).

[00122] The term 'prophylaxis' is related to 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine, in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

[00123] 'Treating' or 'treatment' of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (i.e., arresting the disease or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment 'treating' or 'treatment' refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In yet another embodiment, 'treating' or 'treatment' refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In a further embodiment, "treating" or "treatment" relates to slowing the progression of the disease.

[00124] 'Compounds of the present invention', and equivalent expressions, are meant to embrace compounds of the Formula(e) as hereinbefore described, which expression includes the prodrugs, the pharmaceutically acceptable salts, and the solvates, e.g., hydrates, where the context so permits. Similarly, reference to intermediates, whether or not they themselves are claimed, is meant to embrace their salts, and solvates, where the context so permits.

[00125] When ranges are referred to herein, for example but without limitation, C₁-C₈ alkyl, the citation of a range should be considered a representation of each member of said range.

[00126] Other derivatives of the compounds of this invention have activity in both their acid and acid derivative forms, but in the acid sensitive form often offers advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (see, Bundgard, H., Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well known to practitioners of the art, such as, for example, esters prepared by reaction of the parent acid with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a substituted or unsubstituted amine, or

acid anhydrides, or mixed anhydrides. Simple aliphatic or aromatic esters, amides and anhydrides derived from acidic groups pendant on the compounds of this invention are particularly useful prodrugs. In some cases it is desirable to prepare double ester type prodrugs such as (acyloxy)alkyl esters or ((alkoxycarbonyl)oxy)alkylesters. Particular such prodrugs are the C₁ to C₈ alkyl, C₂-C₈ alkenyl, aryl, C₇-C₁₂ substituted aryl, and C₇-C₁₂ arylalkyl esters of the compounds of the invention.

[00127] As used herein, the term 'isotopic variant' refers to a compound that contains unnatural proportions of isotopes at one or more of the atoms that constitute such compound. For example, an 'isotopic variant' of a compound can contain one or more non-radioactive isotopes, such as for example, deuterium (²H or D), carbon-13 (¹³C), nitrogen-15 (¹⁵N), or the like. It will be understood that, in a compound where such isotopic substitution is made, the following atoms, where present, may vary, so that for example, any hydrogen may be ²H/D, any carbon may be ¹³C, or any nitrogen may be ¹⁵N, and that the presence and placement of such atoms may be determined within the skill of the art. Likewise, the invention may include the preparation of isotopic variants with radioisotopes, in the instance for example, where the resulting compounds may be used for drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e. ³H, and carbon-14, i.e. ¹⁴C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection. Further, compounds may be prepared that are substituted with positron emitting isotopes, such as ¹¹C, ¹⁸F, ¹⁵O and ¹³N, and would be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

[00128] All isotopic variants of the compounds provided herein, radioactive or not, are intended to be encompassed within the scope of the invention.

[00129] It is also to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed 'isomers'. Isomers that differ in the arrangement of their atoms in space are termed 'stereoisomers'.

[00130] Stereoisomers that are not mirror images of one another are termed 'diastereomers' and those that are non-superimposable mirror images of each other are termed 'enantiomers'. When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a 'racemic mixture'.

[00131] 'Tautomers' refer to compounds that are interchangeable forms of a particular compound structure, and that vary in the displacement of hydrogen atoms and electrons. Thus, two structures may be in equilibrium through the movement of π electrons and an atom (usually H). For example, enols and ketones are tautomers because they are rapidly interconverted by treatment with either acid or base. Another example of tautomerism is the aci- and nitro- forms of phenylnitromethane, that are likewise formed by treatment with acid or base.

[00132] Tautomeric forms may be relevant to the attainment of the optimal chemical reactivity and biological activity of a compound of interest.

[00133] The compounds of this invention may possess one or more asymmetric centers; such compounds can therefore be produced as individual (R)- or (S)- stereoisomers or as mixtures thereof.

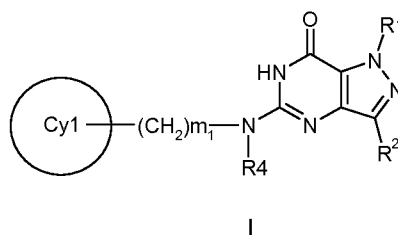
[00134] Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art.

THE COMPOUNDS

[00135] The compounds of the present invention may be described generally as pyrazolo[4,3-d]pyrimidin-7(6H)-ones substituted in the 5-position.

[00136] Accordingly, the present invention relates to, according to formula (I) below.

[00137] Accordingly, the present invention relates to compounds having anti-inflammatory properties, according to formula (I):



wherein:

Cy1 is substituted or unsubstituted cycloalkyl or substituted or unsubstituted heterocycloalkyl;

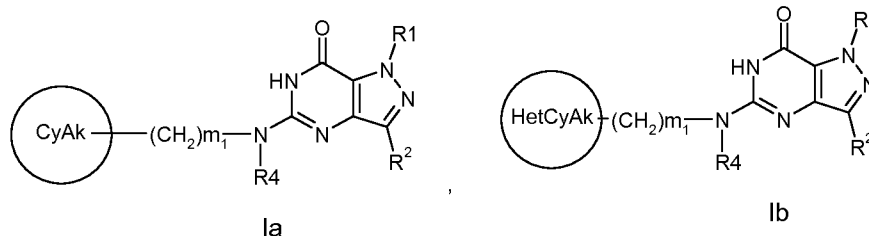
R¹ is H, substituted or unsubstituted alkyl;

R² is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

R⁴ is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl; and m₁ is 0, 1, 2, 3, or 4; provided that when Cy1 is heterocycloalkyl and the heterocycloalkyl group is joined to -(CH₂)m₁- via an N atom of said heterocycloalkyl group then m₁ is 2, 3 or 4;

or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof.

[00138] In one embodiment, with respect to compounds of formula I, the compound is according to formulae Ia or Ib:



wherein R^1 , R^2 , R^4 and m_1 are as described for formula I;

CyAk is substituted or unsubstituted cycloalkyl; and HetCyAk is substituted or unsubstituted heterocycloalkyl.

[00139] In one embodiment, with respect to compounds of formulae Ia-Ib, R^1 is alkyl. In another embodiment R^1 is Me.

[00140] In one embodiment, with respect to compounds of formulae Ia-Ib, R^2 is alkyl or cycloalkyl. In another embodiment R^2 is alkyl.

[00141] In one embodiment, with respect to compounds of formulae Ia-Ib, R^4 is alkyl.

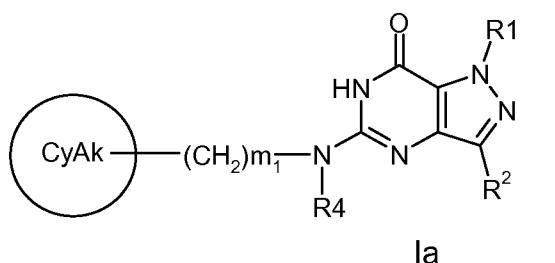
[00142] In one embodiment, with respect to compounds of formulae Ia-Ib, R^1 is alkyl. In another embodiment R^1 is Me, Et or i-Pr. In another embodiment R^1 is Me.

[00143] In one embodiment, with respect to compounds of formulae Ia-Ib, R^4 is substituted alkyl and the substitution is selected from substituted or unsubstituted cycloalkyl and heterocycloalkyl.

[00144] In one embodiment, with respect to compounds of formulae Ia-Ib, m_1 is 0, 1, 2, 3 or 4. In another embodiment m_1 is 1, 2 or 3. In yet another embodiment m_1 is 1.

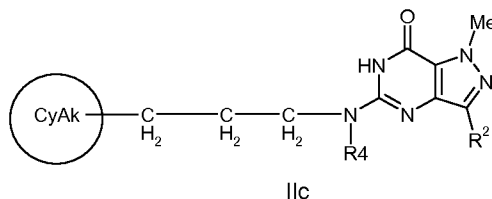
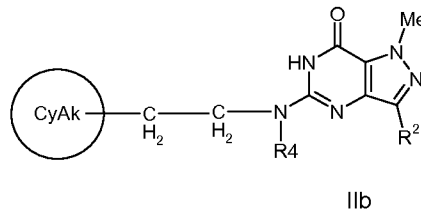
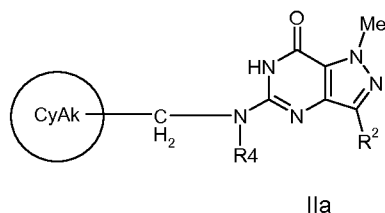
[00145] In one embodiment, with respect to compounds of formulae Ia-Ib, the compound is according to formula Ia.

[00146] In one embodiment, with respect to compounds of formulae Ia-Ib, the compound is according to formula Ia:



wherein R^1 , R^2 , R^3 , R^4 and m_1 are as in claim 1 and CyAk is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[00147] In one embodiment, with respect to compounds of formulae Ia-Ib, the compound is according to formulae IIa, IIb, or IIc:



wherein:

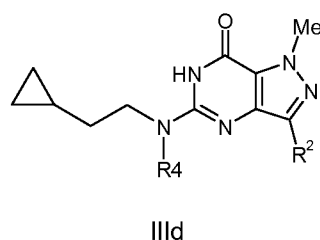
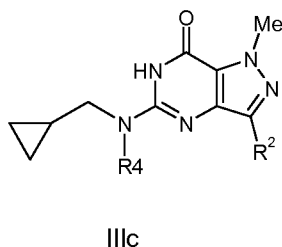
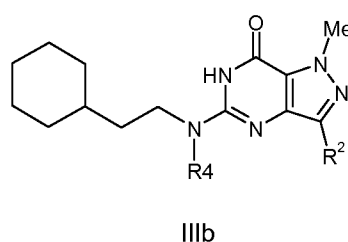
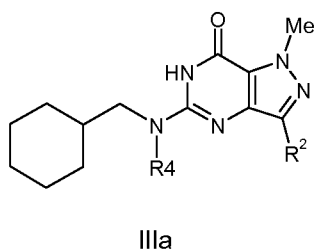
CyAk is substituted or unsubstituted cycloalkyl;

R² and R⁴ are as described for formula I.

[00148] In one embodiment, with respect to compounds of formulae IIa-IIc, CyAk is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[00149] In another embodiment, with respect to compounds of formulae IIa-IIc, CyAk is selected from cyclopropyl and cyclohexyl.

[00150] In another embodiment, with respect to compounds of formula I, the compound is according to formulae IIIa, IIIb, IIIc or IIId:



wherein:

R² and R⁴ are as described for formula I.

[00151] In one embodiment, with respect to compounds of formulae IIIa-IIId, R⁴ is alkyl.

[00152] In another embodiment, with respect to compounds of formulae IIIa-IIId, R⁴ is Me, Et, n-Pr, i-Pr, n-Bu, t-Bu, n-pentyl, CH₂CH(Me)Me, CH₂CH₂-t-Bu, CH₂CH(Me)CH₂Me, CH₂CH(Et)CH₂Me, or CH₂CH(Me)CH₂CH₂Me.

[00153] In another embodiment, with respect to compounds of formulae IIIa-IIId, R⁴ is alkyl substituted with halo, alkoxy, dialkylamino, aryl, cycloalkyl or heterocycloalkyl.

[00154] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is alkyl substituted with halo or alkoxy.

[00155] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is alkyl substituted with Cl, F, OMe, NMe₂, or O-i-Pr.

[00156] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is 4,4,4-trifluoro-n-butyl.

[00157] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is 3-methoxy-n-propyl, 3-isopropoxy-n-propyl, or 3-(N,N-dimethylamino)-n-propyl.

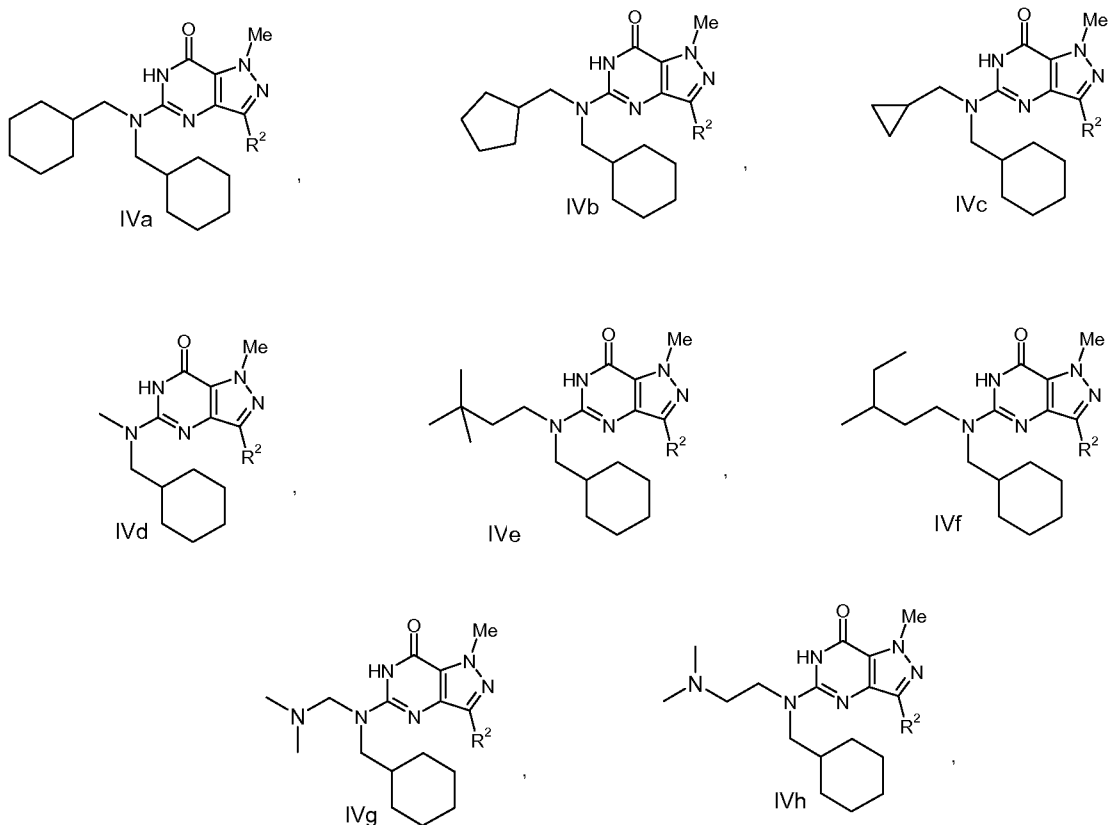
[00158] In one embodiment, with respect to compounds of formulae Ia-Ib, R^4 is substituted alkyl and the substitution is selected from substituted or unsubstituted cycloalkyl and heterocycloalkyl.

[00159] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is alkyl substituted with cycloalkyl.

[00160] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is alkyl substituted with cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl.

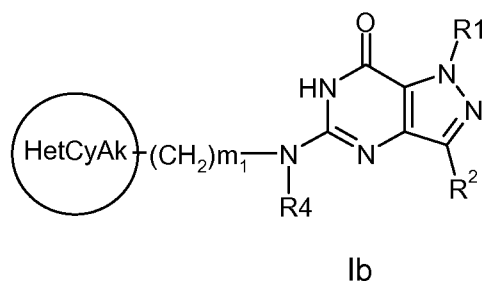
[00161] In another embodiment, with respect to compounds of formulae IIIa-IIIId, R^4 is cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.

[00162] In another embodiment, with respect to compounds of formula I, the compound is according to formulae IVa, IVb, IVc, IVd, IVe, IVf, IVg, or IVh:



wherein R^2 is as described for formula I.

[00163] In another embodiment, with respect to compounds of formula I, the compound is according to formula Ib:

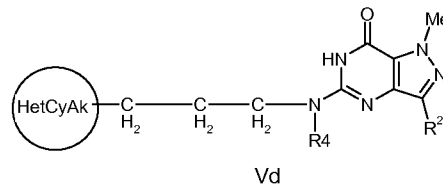
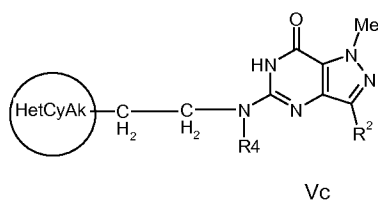
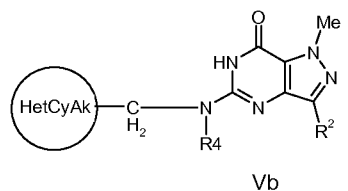
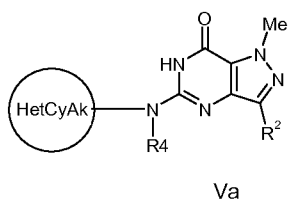


wherein R^1 , R^2 , R^4 and m_1 are described for formula I and HetCyAk is selected from substituted or unsubstituted piperidinyl, piperazinyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and tetrahydro-1,4-dioxanyl.

[00164] In one embodiment, with respect to compounds of formula Ib, R^1 is Me, Et or i-Pr. In another embodiment, R^1 is Me.

[00165] In one embodiment, with respect to compounds of formula Ib, m_1 is 0, 1, 2 or 3.

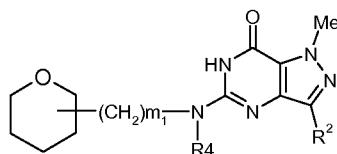
[00166] In one embodiment, with respect to compounds of formula Ib, the compound is according to formulae Va, Vb, Vc or Vd:



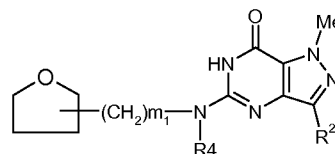
wherein:

HetCyAk is selected from substituted or unsubstituted piperidinyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and tetrahydro-1,4-dioxanyl; and R^2 and R^4 are as described for formula I.

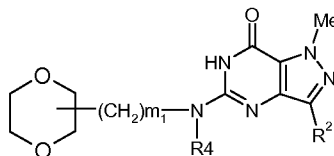
[00167] In one embodiment, with respect to compounds of formula Ib, the compound is according to formulae VIa, VIb, or VIIC:



VIa



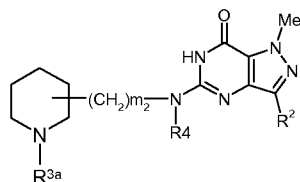
VIb



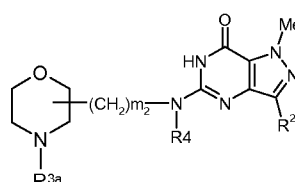
VIc

wherein m_1 , R^2 and R^4 are as described for formula I.

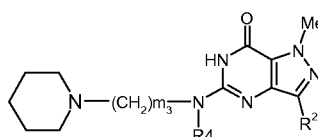
[00168] In one embodiment, with respect to compounds of formula I, the compound is according to formulae VIIa, VIIb, VIIc, VIId, or VIIe:



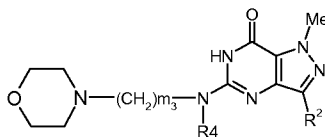
VIIa



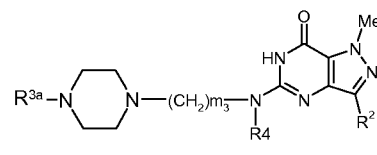
VIIb



VIIc



VIId



or

VIIe

wherein:

R^2 and R^4 are as described for formula I;

m_2 is 0, 1, 2, 3 or 4; m_3 is 2, 3 or 4; and

R^{3a} is

H, substituted or unsubstituted alkyl, substituted or unsubstituted phenyl, or substituted or unsubstituted benzyl;

or

CO-alkyl, CO-cycloalkyl, CO-heterocycloalkyl, CO-aryl, CO-heteroaryl, CO-NH-alkyl, CO-NH-aryl, or CO-NH-heteroaryl;

or

CO-CH₂-cycloalkyl, CO-CH₂-heterocycloalkyl, CO-CH₂-aryl, or CO-CH₂-heteroaryl;

or

SO₂-alkyl, SO₂-cycloalkyl, SO₂-heterocycloalkyl, SO₂-aryl, or SO₂-heteroaryl;

or

SO₂-NH-alkyl, SO₂-NH-aryl, or SO₂-NH-heteroaryl;

or

SO₂-CH₂-cycloalkyl, SO₂-CH₂-heterocycloalkyl, SO₂-CH₂-aryl, or SO₂-CH₂-heteroaryl.

[00169] In one embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is alkyl.

[00170] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is Me, Et, i-Pr, or n-Pr.

[00171] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is substituted or unsubstituted phenyl.

[00172] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is cycloalkyl.

[00173] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is cyclopropyl, cyclohexyl, or cyclopentyl.

[00174] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is alkyl or substituted alkyl.

[00175] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is Me, Et, i-Pr or n-Pr.

[00176] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is cycloalkyl or heterocycloalkyl.

[00177] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.

[00178] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is aryl or heteroaryl.

[00179] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.

[00180] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CONHR^{3b}; and R^{3b} is alkyl or substituted alkyl.

[00181] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CONHR^{3b}; and R^{3b} is Me, Et, i-Pr or n-Pr.

[00182] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CONHR^{3b}; and R^{3b} is cycloalkyl or heterocycloalkyl.

[00183] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CONHR^{3b}; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.

[00184] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CONHR^{3b}; and R^{3b} is aryl or heteroaryl.

[00185] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is R^{3a} is CONHR^{3b}; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.

[00186] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO₂R^{3b} or SO₂CH₂R^{3b}; and R^{3b} is alkyl or substituted alkyl.

- [00187] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is Me, Et, i-Pr or n-Pr.
- [00188] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is cycloalkyl or heterocycloalkyl.
- [00189] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.
- [00190] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is aryl or heteroaryl.
- [00191] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
- [00192] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is alkyl or substituted alkyl.
- [00193] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is Me, Et, i-Pr or n-Pr.
- [00194] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is cycloalkyl or heterocycloalkyl.
- [00195] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.
- [00196] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is aryl or heteroaryl.
- [00197] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is SO_2NHR^{3b} ; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
- [00198] In another embodiment, with respect to compounds of formulae VIIa-VIIe, R^{3a} is CH_2COR^{3b} ; and R^{3b} is alkyl, hydroxyl, alkoxy, or amino.
- [00199] In one embodiment, with respect to compounds of formulae Va-VIIe, R^4 is Me, Et, n-Pr, i-Pr, n-Bu, t-Bu, n-pentyl, $CH_2CH(Me)Me$, CH_2CH_2-t-Bu , $CH_2CH(Me)CH_2Me$, $CH_2CH(Et)CH_2Me$, or $CH_2CH(Me)CH_2CH_2Me$.
- [00200] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with halo, alkoxy, aryl, cycloalkyl or heterocycloalkyl.
- [00201] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with halo or alkoxy.
- [00202] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with Cl, F, OMe, or O-i-Pr.
- [00203] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is 4,4,4-trifluoro-n-butyl.
- [00204] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is 3-methoxy-n-propyl, or 3-isopropoxy-n-propyl.

[00205] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with cycloalkyl.

[00206] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl.

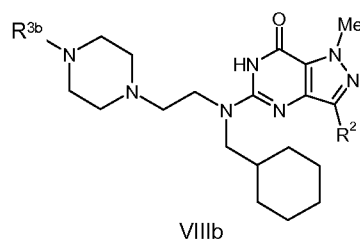
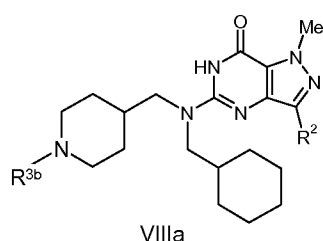
[00207] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.

[00208] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with heterocycloalkyl.

[00209] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is alkyl substituted with piperidinyl, tetrahydrofuranyl, tetrahydropyranyl, or morpholinyl.

[00210] In another embodiment, with respect to compounds of formulae Va-VIIe, R^4 is 2-(morpholin-1-yl)ethyl, 3-(morpholin-1-yl)propyl, 2-(tetrahydropyran-4-yl)ethyl, or (tetrahydrofuran-2-yl)methyl.

[00211] In another embodiment, with respect to compounds of formula Ib, the compound is according to formulae VIIIa, or VIIIb:



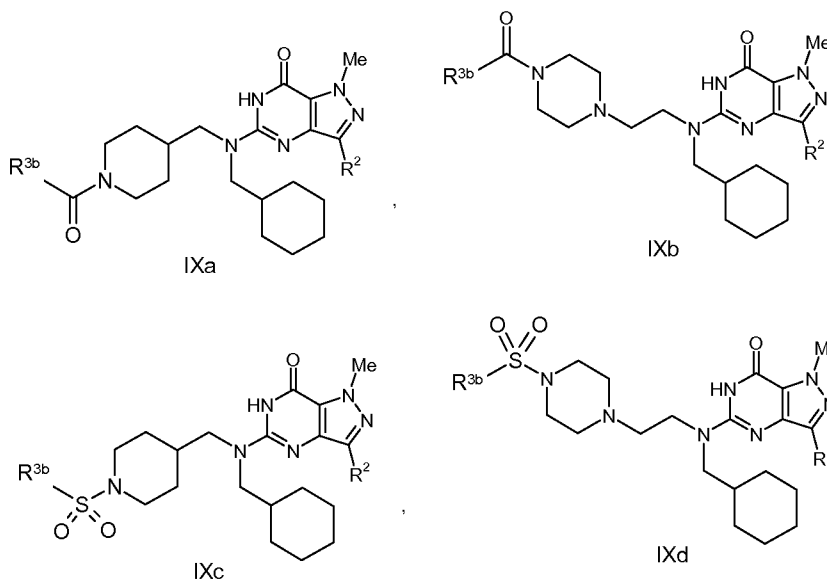
wherein R^2 is as described for formula I; and R^{3b} is alkyl, or substituted alkyl.

[00212] In one embodiment, with respect to compounds of formulae VIIIa-VIIIb, R^{3b} is Me, Et, n-Pr, i-Pr, or t-Bu.

[00213] In another embodiment, with respect to compounds of formulae VIIIa-VIIIb, R^{3b} is alkyl substituted with hydroxyl, CO-alkoxy, heterocycloalkyl, or amino.

[00214] In another embodiment, with respect to compounds of formulae VIIIa-VIIIb, R^{3b} is $\text{CH}_2\text{CO}_2\text{Et}$, $\text{CH}_2\text{CH}_2\text{OH}$, $\text{CH}_2\text{CO}_2\text{H}$, $\text{CH}_2\text{CH}_2\text{NMe}_2$, CH_2CH_2 -morpholin-1-yl.

[00215] In another embodiment, with respect to compounds of formula I, the compound is according to formulae IXa, IXb, IXc, or IXd:



wherein R^2 is as described for formula I; and R^{3b} is alkyl, substituted alkyl, alkoxy, amino, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

[00216] In one embodiment, with respect to compounds of formulae IXa-IXd, R^{3b} is Me, i-Pr, NHEt, NH-i-Pr, NHPh, Ph, cyclopropyl, cyclohexyl, morpholinyl, imidazolyl, piperidinyl, morpholinylmethyl, benzyl, imidazolylmethyl, or tetrazolylmethyl.

[00217] In one embodiment, with respect to compounds of formulae Ia-IXd, R^2 is Me, Et, n-Pr, t-Bu, cyclopropyl, cyclohexyl, or cyclopentyl.

[00218] In another embodiment, with respect to compounds of formulae Ia-IXd, R^2 is t-Bu.

[00219] In another embodiment, with respect to compounds of formulae Ia-IXd, R^2 is substituted or unsubstituted phenyl.

[00220] In another embodiment, with respect to compounds of formulae Ia-IXd, R^2 is substituted or unsubstituted pyridyl.

[00221] In one embodiment, with respect to compounds of formula I, the compound is selected from Table 1; or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof.

[00222] In yet another embodiment, with respect to compounds of formulae Ia-Ib, the compound is selected from all compounds of the invention exemplified specifically herein.

[00223] A compound for use according to the invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. It will be understood by a person of skill in the art that the present invention includes both the racemic mixture and each enantiomer in isolated form. A compound according to an embodiment of the invention may be in trans or cis form.

[00224] The present invention also extends to a prodrug of a compound according to an embodiment of the invention such as an ester or amide thereof. A prodrug is a compound that may be converted under physiological conditions or by solvolysis to a compound according to an embodiment of the invention or to a pharmaceutically acceptable salt of a compound according to an embodiment of the invention. A prodrug may be inactive when administered to a subject but is converted *in vivo* to an active compound of the invention. 'Pharmaceutically acceptable prodrugs' as used herein refers to those

prodrugs of the compounds useful in the present invention, which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients with undue toxicity, irritation, allergic response commensurate with a reasonable benefit/risk ratio, and effective for their intended use of the compounds of the invention. The term 'prodrug' means a compound that is transformed *in vivo* to yield an effective compound useful in the present invention or a pharmaceutically acceptable salt, hydrate or solvate thereof. The transformation may occur by various mechanisms, such as through hydrolysis in blood. The compounds bearing metabolically cleavable groups have the advantage that they may exhibit improved bioavailability as a result of enhanced solubility and/or rate of absorption conferred upon the parent compound by virtue of the presence of the metabolically cleavable group, thus, such compounds act as prodrugs. A thorough discussion is provided in Design of Prodrugs, H. Bundgard, ed., Elsevier (1985); Methods in Enzymology; K. Widder et al, Ed., Academic Press, 42, 309-396 (1985); A Textbook of Drug Design and Development, Krogsgaard-Larsen and H. Bundgard, ed., Chapter 5; "Design and Applications of Prodrugs" 113-191 (1991); Advanced Drug Delivery Reviews, H. Bundgard, 8, 1-38, (1992); J. Pharm. Sci., 77,285 (1988); Chem. Pharm. Bull., N. Nakeya et al, 32, 692 (1984); Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, 14 A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, E.B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, all of which are incorporated herein by reference.

PHARMACEUTICAL COMPOSITIONS

[00225] Compounds of the invention can be incorporated into pharmaceutical compositions suitable for administration to a patient in need thereof. Such compositions typically comprise at least one compound of the invention and at least one pharmaceutically acceptable carrier. As used herein the language 'pharmaceutically acceptable carrier' is intended to include solid carriers such as lactose, magnesium stearate, terra alba, sucrose, talc, stearic acid, gelatin, agar, pectin, acacia or the like; and liquids such as vegetable oils, arachis oil and sterile water, or the like, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. This listing of pharmaceutically acceptable carriers is not to be construed as limiting. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00226] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or

sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00227] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, 'chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum mono stearate and gelatin.

[00228] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a compound according to an embodiment of the invention) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00229] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

[00230] Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide;

a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00231] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[00232] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00233] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00234] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art.

[00235] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00236] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00237] A compound according to an embodiment of the invention may be provided as a salt, preferably as a pharmaceutically acceptable salt of compounds of formula I or Formulae Ia-Ih. Examples of pharmaceutically acceptable salts of these compounds include those derived from organic acids such as acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid, mandelic acid, methanesulphonic acid, benzenesulphonic acid and *p*-toluenesulphonic acid, mineral acids such as hydrochloric and sulphuric acid

and the like, giving methanesulphonate, benzenesulphonate, *p*-toluenesulphonate, hydrochloride and sulphate, and the like, respectively or those derived from bases such as organic and inorganic bases. Examples of suitable inorganic bases for the formation of salts of compounds for this invention include the hydroxides, carbonates, and bicarbonates of ammonia, lithium, sodium, calcium, potassium, aluminium, iron, magnesium, zinc and the like. Salts can also be formed with suitable organic bases. Such bases suitable for the formation of pharmaceutically acceptable base addition salts with compounds of the present invention include organic bases which are nontoxic and strong enough to form salts. Such organic bases are already well known in the art and may include amino acids such as arginine and lysine, mono-, di-, or trihydroxyalkylamines such as mono-, di-, and triethanolamine, choline, mono-, di-, and trialkylamines, such as methylamine, dimethylamine, and trimethylamine, guanidine; *N*-methylglucosamine; *N*-methylpiperazine; morpholine; ethylenediamine; *N*-benzylphenethylamine; tris(hydroxymethyl) aminomethane; and the like.

[00238] Salts of compounds according to an embodiment of the invention may be prepared in a conventional manner using methods well known in the art. Acid addition salts of said basic compounds may be prepared by dissolving the free base compounds according to the first or second aspects of the invention in aqueous or aqueous alcohol solution or other suitable solvents containing the required acid. Where a compound of the invention contains an acidic function, a base salt of said compound may be prepared by reacting said compound with a suitable base. The acid or base salt may separate directly or can be obtained by concentrating the solution *e.g.* by evaporation. The compounds of this invention may also exist in solvated or hydrated forms.

[00239] The following formulation examples illustrate representative pharmaceutical compositions that may be prepared in accordance with this invention. The present invention, however, is not limited to the following pharmaceutical compositions.

Formulation 1 - Tablets

[00240] A compound of the invention is admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ratio. A minor amount of magnesium stearate is added as a lubricant. The mixture is formed into 240-270 mg tablets (80-90 mg of active compound per tablet) in a tablet press.

Formulation 2 - Capsules

[00241] A compound of the invention is admixed as a dry powder with a starch diluent in an approximate 1:1 weight ratio. The mixture is filled into 250 mg capsules (125 mg of active compound per capsule).

Formulation 3 - Liquid

[00242] A compound of the invention (125 mg) may be admixed with sucrose (1.75 g) and xanthan gum (4 mg) and the resultant mixture may be blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of microcrystalline cellulose and sodium carboxymethyl cellulose (11:89, 50 mg) in water. Sodium benzoate (10 mg), flavor, and color are diluted with water and added with stirring. Sufficient water may then added to produce a total volume of 5 mL.

Formulation 4 - Tablets

[00243] A compound of the invention may be admixed as a dry powder with a dry gelatin binder in an approximate 1:2 weight ratio. A minor amount of magnesium stearate is added as a lubricant. The mixture is formed into 450-900 mg tablets (150-300 mg of active compound) in a tablet press.

Formulation 5 - Injection

[00244] A compound of the invention is dissolved or suspended in a buffered sterile saline injectable aqueous medium to a concentration of approximately 5 mg/mL.

Formulation 6 - Topical

[00245] Stearyl alcohol (250 g) and a white petrolatum (250 g) are melted at about 75°C and then a mixture of a compound of the invention (50 g) methylparaben (0.25 g), propylparaben (0.15 g), sodium lauryl sulfate (10 g), and propylene glycol (120 g) dissolved in water (about 370 g) is added and the resulting mixture is stirred until it congeals.

METHODS OF TREATMENT

[00246] The present invention relates also to a method of treatment or prevention of osteoarthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of compound of the invention.

[00247] The present invention relates also to a method of treatment or prevention of osteoarthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of an inhibitor of PDE1A according to any of the formulae herein described.

[00248] The present invention relates also to a method of treatment or prevention of osteoarthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of an inhibitor of PDE1A according to Formulae Ia-Ib.

[00249] Another aspect of the present method invention relates to a method of treatment or prophylaxis of a condition characterized by abnormal PDE1A activity, which comprises administering a therapeutically effective amount of a PDE1A inhibiting compound according to Formula I or Formulae Ia-Ib.

[00250] A further aspect of the present method invention is a method of treatment or prophylaxis of a disease involving degradation of cartilage, which comprises administering a therapeutically effective amount of a compound according to Formula I or Formulae Ia-Ib.

[00251] A special embodiment of the present method invention is a method of treatment or prevention of OA, which comprises administering to a subject in need thereof, a therapeutically effective amount of a compound according to Formula I or Formulae Ia-Ib.

[00252] This invention also relates to the use of the present compounds in the manufacture of a medicament for treatment or prophylaxis of a condition prevented, ameliorated or eliminated by administration of an inhibitor of PDE1A which is a compound of the invention, or a condition selected from diseases involving inflammation, most preferably for the treatment of diseases selected from osteoarthritis, rheumatoid arthritis and osteoporosis.

[00253] Administration of the compound of the present invention to the subject patient includes both self-administration and administration by another person. The patient may be in need of treatment for an existing disease or medical condition, or may desire prophylactic treatment to prevent or reduce the

risk for diseases and medical conditions affected by a disturbance in bone metabolism. The compound of the present invention may be delivered to the subject patient orally, transdermally, via inhalation, injection, nasally, rectally or via a sustained release formulation.

[00254] A preferred regimen of the present method comprises the administration to a subject in suffering from a disease condition characterized by a disturbance in bone and/or cartilage metabolism, of an effective PDEIA-inhibiting amount of a compound of the present invention for a period of time sufficient to reduce the abnormal levels of bone and/or cartilage degradation in the patient, and preferably terminate, the self-perpetuating processes responsible for said degradation. A special embodiment of the method comprises administering of an effective PDEIA inhibiting amount of a compound of the present invention to a subject patient suffering from or susceptible to the development of osteoarthritis, for a period of time sufficient to reduce or prevent, respectively, collagen and bone degradation in the joints of said patient, and preferably terminate, the self-perpetuating processes responsible for said degradation.

[00255] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00256] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00257] A preferred therapeutically effective amount of the compound of the present invention to administer to a subject patient is about 0.1 mg/kg to about 10 mg/kg administered from once to three times a day. For example, an effective regimen of the present method may administer about 5 mg to about 1000 mg of said compound of the present invention from once to three times a day. It will be understood, however, that the specific dose level for any particular subject patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular inflammatory condition. A consideration of these factors is well within the purview of the ordinarily skilled clinician

for the purpose of determining the therapeutically effective or prophylactically effective dosage amount needed to prevent, counter, or arrest the progress of the condition.

[00258] For the prevention and/or treatment of long-term conditions, the regimen for treatment usually stretches over many months or years so oral dosing is preferred for patient convenience and tolerance. With oral dosing, one to five and especially two to four and typically three oral doses per day are representative regimens. Using these dosing patterns, each dose provides from about 0.01 to about 20 mg/kg of the compound of the invention, with preferred doses each providing from about 0.1 to about 10 mg/kg and especially about 1 to about 5 mg/kg.

[00259] Transdermal doses are generally selected to provide similar or lower blood levels than are achieved using injection doses.

[00260] When used to prevent the onset of a condition related to bone and/or cartilage degradation the compounds of this invention will be administered to a patient at risk for developing the condition, typically on the advice and under the supervision of a physician, at the dosage levels described above. Patients at risk for developing a particular condition generally include those who have been identified by genetic testing or screening to be particularly susceptible to developing said condition.

[00261] The compounds of this invention can be administered as the sole active agent or they can be administered in combination with other agents, including other compounds that demonstrate the same or a similar therapeutic activity and that are determined to be safe and efficacious for such combined administration.

[00262] The present invention will now be described in detail with reference to specific examples of compounds and methods for their production. Within this specification embodiments have been described in a way that enables a clear and concise specification to be written, but it will be appreciated that embodiments may be variously combined or separated without parting from the invention.

EXAMPLES

1. Synthetic Preparation of Compounds of the Invention

[00263] The compounds of this invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given; however, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00264] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and P. G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

GENERAL SYNTHETIC PROCEDURES

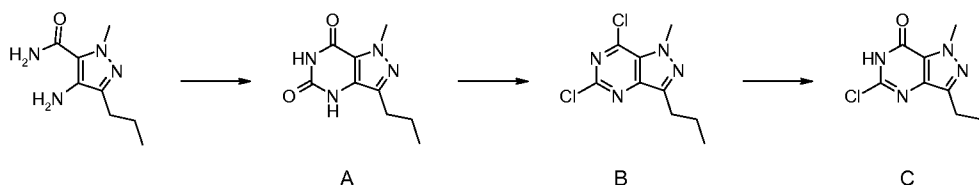
[00267] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and P. G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

[00268] The following representative methods are presented with details as to the preparation of representative pyrazolo[4,3-d]pyrimidinones that have been listed herein above. The compounds of the invention may be prepared from known or commercially available starting materials and reagents by one skilled in the art of organic synthesis.

SYNTHESIS OF INTERMEDIATES

Intermediate 1

5-Chloro-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one



A. 1-Methyl-3-propyl-1,4-dihydro-pyrazolo[4,3-d]pyrimidine-5,7-dione

39

[00270] MS (ES+) 209.1 (M+1); HPLC tr = 0.85min. (Column used for all LCMS analysis: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350)).

B. 5,7-Dichloro-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidine

[00271] 1-Methyl-3-propyl-1,4-dihydro-pyrazolo[4,3-d]pyrimidine-5,7-dione (4.8mmol) is dissolved in phosphorus oxychloride at room temperature, under nitrogen. A trace of DMF is added to the reaction mixture and the solution is heated under reflux for 7h-16h. On complete reaction, the excess of phosphorus oxychloride is removed under reduced pressure. The reaction mixture is poured onto ice/water and extracted with ethyl acetate. The organic layer is dried over anhydrous magnesium sulfate. Removal of solvent under reduced pressure affords a soft solid, which may be used without further purification in the next step.

[00272] MS (ES+) 245.1 (M+1); 247 (M+3); HPLC tr = 1.41 min.

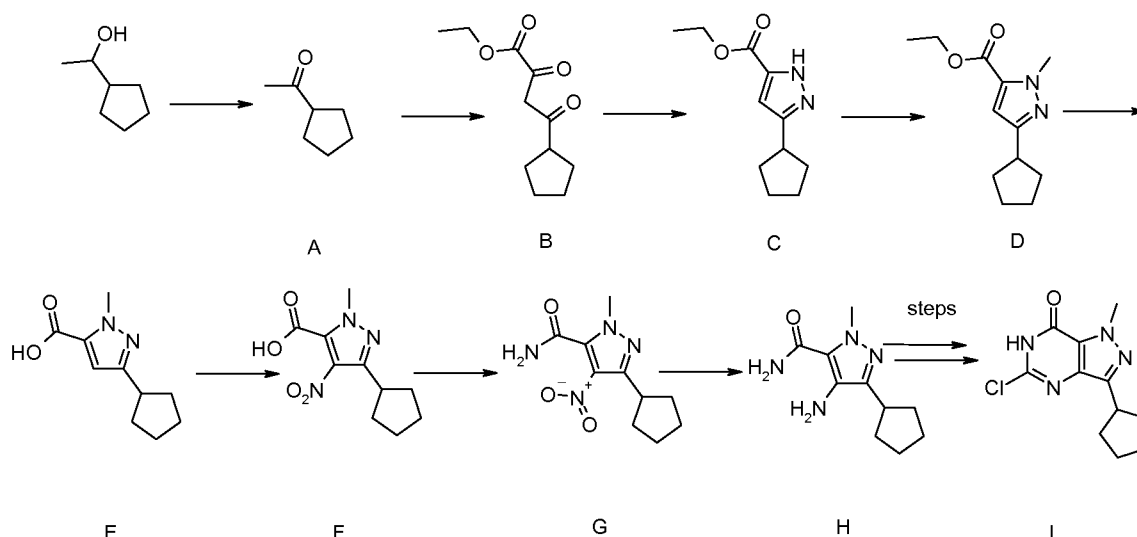
C. 5-Chloro-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one

[00273] Potassium hydroxide (20mmol) is added to a solution of 5,7-dichloro-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidine (4mmol) in dioxane/water (20mL/5mL) at room temperature. The solution is heated under reflux for 1h. After complete reaction, the mixture is allowed to cool to room temperature. The solution is treated with dilute aqueous hydrochloric acid (2M) until a pH of 1 is achieved and then it is extracted with ethyl acetate. The organic phase is dried over anhydrous magnesium sulfate and then the solvent is removed under reduced pressure to afford 5-chloro-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one.

[00274] MS (ES+) 227.1 (M+1); HPLC tr = 1.04 min. NMR (1 H, DMSO) 3.9 (3H, s, CH₃); 2.6 (2H, d, J=7.2Hz, CH₂); 1.69 (2H, q, J=7.2Hz, CH₂), 0.91 (3H, t, J=7.2Hz, CH₃).

Intermediate 2

5-Chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one



A. 1-Cyclopentyl ethanone

[00275] A solution of CrO_3 (0.32mol) in water (50mL) is added to sulfuric acid (28mL, 0.52mmol) dropwise at room temperature for 30 min. This solution is added dropwise to 1-cyclopentylethanol (0.21mol) in acetone (550mL) at 35°C until the orange colour persists for 10min. The reaction is quenched using *iso*-propyl alcohol (25mL) and it is treated with solid sodium hydrogencarbonate (90g) until a pH of 5 is obtained. The mixture is filtered and the filtrate is extracted with methyl *tert*-butyl ether (2 x 75mL). The combined methyl *tert*-butyl ether layer is washed with water (20mL x 3) followed by saturated aqueous sodium chloride (25 mL). The organic phase is dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure to afford 1-cyclopentyl ethanone as a colorless liquid. No further purification is performed, and crude product may be used directly in next step. Yield of the compound: 27g.

B. 4-Cyclopentyl-2,4-dioxobutyric acid ethyl ester

[00276] To a solution of sodium ethoxide in ethanol (5.54g of sodium in 300mL of ethanol) at room temperature, a mixture of 1-cyclopentylethanol (0.241mol) and diethyloxalate (0.265mol) in ethanol (150mL) is added dropwise. The temperature is raised to 60°C and maintained for 2 h. On complete reaction, the mixture is poured onto cold, dilute aqueous hydrochloric acid (2M, 320mL) and the product is extracted with ethyl acetate (75 mL x 3). The combined organic phases are washed with water (20mL x 3), followed by saturated aqueous sodium chloride (30 mL). The organic phase is dried over anhydrous sodium sulfate, filtered and the solvent is removed under reduced pressure. The product is obtained as a dark orange liquid. No further purification is performed, and crude material may be used directly in next step. Weight of the compound: 34.6g, Yield: 77%.

C. 5-Cyclopentyl-1H-pyrazole-3-carboxylic acid ethyl ester

[00277] Hydrazine hydrate (0.070mol) is added to a solution of 4-cyclopentyl-2,4-dioxobutyric acid ethyl ester (0.067mol) in ethanol (50mL). The reaction mixture is stirred at room temperature for 16h. On completion of the reaction, ethanol is removed from the mixture under reduced pressure. The crude material is dissolved in dichloromethane (100 mL) and washed with water (25 mL) followed by saturated aqueous sodium chloride solution (30 mL). The organic phase is dried over anhydrous sodium sulfate, filtered and the solvent is then removed under reduced pressure. The product is obtained as a viscous liquid. Weight of the compound: 8.5g Yield: 77%.

[00278] MS (ES⁺): 209 (M+1).

D. 5-Cyclopentyl-2-methyl-2H-pyrazole-3-carboxylic acid ethyl ester

[00279] To a solution of 5-cyclopentyl-1H-pyrazole-3-carboxylic acid ethyl ester (0.040mol) in toluene, dimethylsulfate (0.040mol) is added and the reaction mixture is stirred at 90°C for 2.5 h. On complete reaction, the mixture is diluted with dichloromethane (100 mL) and washed with water (20mL x 3), followed by saturated aqueous sodium carbonate (20 mL) and saturated aqueous sodium chloride solution. The organic phase is dried over sodium sulfate, filtered and the solvent is removed under reduced pressure. The product is obtained as brown liquid. Weight of the compound: 6.16g Yield: 68%.

[00280] MS (ES+): 223 (M+1). NMR (^1H , CDCl_3): 6.4 (1H, s, CH); 4.3 (2H, q, OCH_2); 4.1 (3H, s, NCH_3); 3.1 (1H, m, CH); 2 (2H, m, CH_2); 1.75 (2H, m, CH_2); 1.65 (4H, s, $2\times\text{CH}_2$); 1.35 (3H, t, CH_3).

E. 5-Cyclopentyl-2-methyl-1H-pyrazole-3-carboxylic acid

[00281] Aqueous sodium hydroxide (3.27g of sodium hydroxide and 14 mL of water) is added to 5-cyclopentyl-2-methyl-1H-pyrazole-3-carboxylic acid ethyl ester (0.027mol) and heated under reflux for about 2.5h. On complete reaction, the mixture is diluted with water (50 mL) and it is treated with aqueous hydrochloric acid (6.97mL, 35%) to precipitate the product. The solid is collected by filtration and dried under reduced pressure to give the product as an off-white solid. Weight of the compound: 3.6 Yield: 70%.

[00282] MS (ES+): 195 (M+1). M.Pt.: 123-128°C

F. 5-Cyclopentyl-2-methyl-4-nitro-1H-pyrazole-3-carboxylic acid

[00283] Oleum (4.24 mL) is added slowly to fuming nitric acid at 0°C. To this is added portionwise, 5-cyclopentyl-2-methyl-1H-pyrazole-3-carboxylic acid (2g, 10mmol) at less than 60°C and thereafter maintained at 60°C for 2.5h. On complete reaction the mixture is allowed to cool and poured on to crushed ice to give a precipitate. The solid is collected by filtration and it can be dried under reduced pressure to give the product as pale yellow solid. Weight of the compound: 1.4g Yield: 58%.

[00284] MS (ES+): 240 (M+1). M.Pt: 134-138°C.

G. 5-Cyclopentyl-2-methyl-4-nitro-2H-pyrazole-3-carboxylic acid amide

[00285] Thionyl chloride (60mmol) and 5-cyclopentyl-2-methyl-4-nitro-1H-pyrazole-3-carboxylic acid (3mmol) are heated under reflux for 3h. On complete reaction, thionyl chloride is distilled out from the reaction mixture and the residue is dissolved in acetone (7 mL) and ice cold aqueous ammonia (10 mL) is added dropwise. The solid is collected by filtration and it is dried under reduced pressure to give the product as an off-white solid. Weight of the compound: 830mg Yield: 59%.

[00286] MS (ES+): 239 (M+1). M.Pt: 133-135 °C.

H. 4-Amino-5-cyclopentyl-2-methyl-2H-pyrazole-3-carboxylic acid amide

[00287] Palladium (10% on charcoal) is added to 5-cyclopentyl-2-methyl-4-nitro-1H-pyrazole-3-carboxamide (18mmol) in methanol (50 mL). The reaction mixture is stirred at room temperature for 5h under hydrogen atmosphere. On complete reaction, the catalyst is removed by filtration through a pad of celite and the filtrate is concentrated under reduced pressure. The resulting crude product (4.6g) is purified by column chromatography eluting initially with hexane followed by 2% of ethyl acetate in hexane. The product may be isolated as a light pink solid. Weight of the compound: 1.6g Yield: 95%.

[00288] M.Pt: 116-120°C.

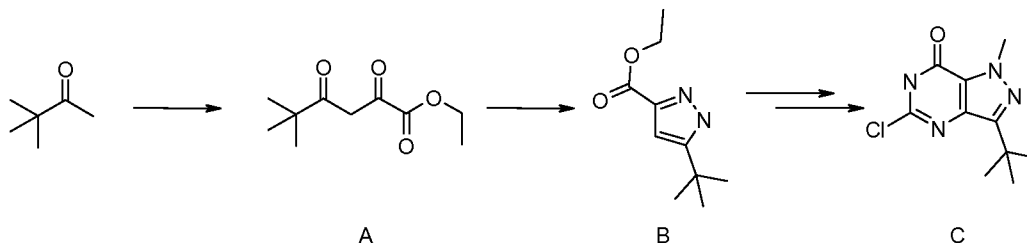
I. 5-Chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one

[00289] This intermediate may be obtained using the same reaction sequence as that described for 5-chloro-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one starting with 4-amino-3-cyclopentyl-1-methyl-1H-pyrazole-5-carboxamide instead of 4-amino-3-propyl-1-methyl-1H-pyrazole-5-carboxamide.

[00290] MS (ES⁺): 253 (M+1), 255. ¹H NMR (400 MHz, CDCl₃) 10.39 (1H, brs), 4.23 (3H, s), 3.37 (1H, pent, J = 8 Hz), 2.11 (2H, m), 1.91-1.83 (4H, m), 1.69 (2H, m).

Intermediate 3

5-Chloro-1-methyl-3-*tert*-butyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one



A. 5,5-Dimethyl-2,4-dioxohexanoic acid ethyl ester

[00291] To a solution of sodium ethoxide (740mmol of sodium in 450mL ethanol), a mixture of methyl *tert*-butyl ketone (740mmol) and diethyl oxalate (820mmol) are added dropwise at room temperature and stirred for 1h at 78°C. On complete reaction, the mixture is allowed to cool to room temperature and it is poured onto cold aqueous hydrochloric acid (5M, 800 mL). Following extraction with methyl *tert*-butyl ether (300 mL x 3), the combined organic phases are washed with saturated aqueous sodium chloride (500 mL) and dried over anhydrous sodium sulfate. After filtration, removal of the solvent under reduced pressure affords the product as a red liquid. Compound wt: 140g, Yield: 93.3%.

[00292] MS (ES⁺): 199 (M+1). NMR (¹H, CDCl₃): 6.5 (1H, s, CH); 4.3 (2H, q, OCH₂); 1.35 (3H, t, CH₃); 1.15 (9H, s, 3xCH₃).

B. 5- *tert*-butyl-1H-pyrazole-3-carboxylic acid ethyl ester

[00293] To a solution of methyl 5,5-dimethyl-2,4-dioxohexanoic acid ethyl ester (720mmol) in ethanol (500 mL) is added dropwise, at 0°C, hydrazine hydrate (640 mmol) and it is stirred for 3h at room temperature. On complete reaction, the mixture is diluted with water (1 L) and stirred for 10min. The precipitate is collected by filtration and dried at room temperature for 5 hours. The product is isolated as an off-white solid. Compound wt: 107g Yield: 75%.

[00294] MS (ES⁻): 195 (M-1). NMR (¹H, CDCl₃): 6.6 (1H, s, CH); 4.3 (2H, q, OCH₂); 1.2 (12H, m, 4xCH₃). M.Pt.: 138-139°C.

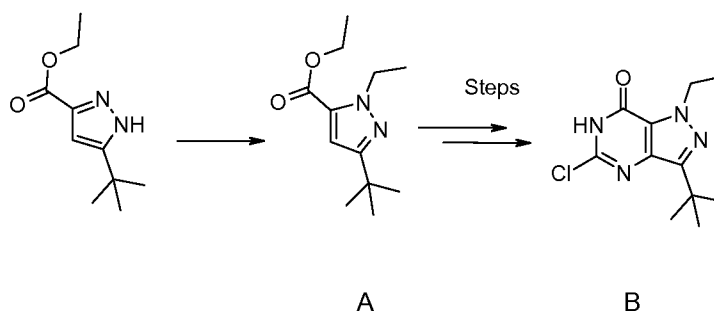
C. 5-Chloro-1-methyl-3- *tert*-butyl -1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one

[00295] This intermediate may be obtained using same reaction sequence as that described for 5-chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one starting with 5-*tert*-butyl-1H-pyrazole-3-carboxylic acid ethyl ester instead of 5-cyclopentyl-1H-pyrazole-3-carboxylic acid ethyl ester.

[00296] MS (ES⁺): 223 (M+1). NMR (¹H, CDCl₃): 13.15 (1H, s, NH); 4.08 (3H, s, CH₃); 1.37 (3H, s, CH₃).

Intermediate 4

5-Chloro-1-ethyl-3- *tert*-butyl -1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one



A. 5-*tert*-butyl –2-ethyl-pyrazole-3-carboxylic acid ethyl ester

[00297] To a suspension of sodium hydride (20mmol) in dimethylformamide (5ml) at 0°C is added a solution of 5- *tert*-butyl-1H-pyrazole-3-carboxylic acid ethyl ester. The resulting mixture is stirred for 30min at room temperature before it is cooled to 0°C. Ethyl bromide (40mmol) is added dropwise. The resulting mixture is heated to 55°C for 18h. On complete reaction, the mixture is cooled to room temperature and diluted with water (50 mL). Extraction of the mixture with methyl *tert*-butyl ether is performed (50 mL x 2). The combined organic phases are washed with saturated aqueous sodium chloride (50 mL) and dried over anhydrous sodium sulfate. Following filtration, the solvent is removed under reduced pressure. Crude compound (2.2g) is purified by column chromatography using silica gel, eluting with 1%ethyl acetate, hexane. The product is isolated as a yellow liquid. Compound wt: 1.3g. Yield: 57%.

[00298] NMR (¹H, CDCl₃): 6.6 (1H, s, CH); 4.55 (2H, q, CH₂); 4.55 (2H, q, CH₂); 1.2-1.4 (15H, m, 5xCH₃).

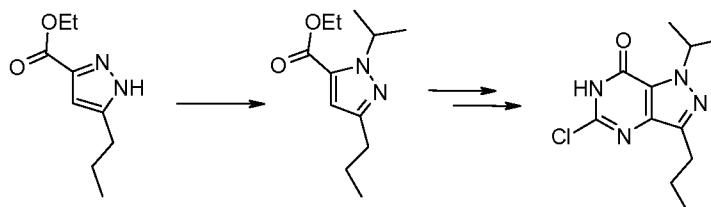
B. 5-Chloro-1-ethyl-3- *tert*-butyl -1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one

[00299] This intermediate may be obtained using same reaction sequence as that described for 5-chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one, starting with 2-ethyl-5-*tert*-butyl –pyrazole-3-carboxylic acid ethyl ester instead of 2-methyl-5-cyclopentyl –pyrazole-3-carboxylic acid ethyl ester.

[00300] MS (ES⁺): 255 (M+1), HPLC rt: 1.33min. NMR (¹H, CDCl₃) 4.46 (2H, q, J= 7.2, NCH₂); 1.38 (9H, s, 3xCH₃); 1.35 (2H, t, J =7.2, CH₃).

Intermediate 5

5-Chloro-1-isopropyl-3- *tert*-butyl -1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one



A. 2-*Iso*-propyl-5-*tert*-butyl –pyrazole-3-carboxylic acid ethyl ester

[00301] To a suspension of sodium hydride (20mmol) in dimethylformamide (5mL) a solution of 5-*tert*-butyl pyrazole-3-carboxylic acid ethyl ester (10mmol) in dimethylformamide (5mL) is added at 0°C. The resulting mixture is stirred for 30 min at room temperature and then it is cooled to 0°C. To this is then added dropwise *iso*-propyl bromide (40mmol). The resulting mixture is heated to 55°C for 18h. On complete reaction, the mixture is allowed to cool to room temperature and diluted with water (50 mL). Following extraction with methyl *tert*-butyl ether (50 mL x 2), the combined organic phases are washed with saturated aqueous sodium chloride (50 mL) and it is dried over anhydrous sodium sulfate. After filtration, the solvent is removed under reduced pressure and the crude product is used in subsequent steps without further purification.

B. 5-Chloro-1-*iso*-propyl-3- propyl -1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one

[00302] This intermediate may be obtained using same reaction sequence as the one described for 5-chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one starting with 2-*iso*-propyl-5-propyl-pyrazole-3-carboxylic acid ethyl ester instead of 2-methyl-5-*tert*-butyl –pyrazole-3-carboxylic acid ethyl ester.

[00303] MS (ES⁺): 255 (M+1), HPLC rt: 1.26min. NMR (¹H, DMSO): 5.31 (1H, q, J=6.4Hz, CH); 2.77 (2H, t, J=7.9Hz, CH₂); 1.75 (2H, t, J=7.6Hz, CH₂); 1.48 (6H, d, J=6.4Hz, 2*CH₃); 0.97 (3H, t, J=7.2Hz, CH₃).

REPRESENTATIVE METHODS

Method A

[00304] The corresponding amine (0.26mmol) is added to a solution of the corresponding Cl-derivative (intermediates 1 to 6) (8.84.10⁻²mmol) in t-BuOH (0.5 mL). The reaction is then heated in a sealed tube (100°C to 120°C) for 12-72h. After complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature. The solvent is removed under reduced pressure to give the crude product. The final compound is isolated by preparative HPLC (yield 10-60%).

[00305] Analytical: Waters Acquity UPLC BEH C18 1.7μm, 2.1mm ID x 50mm L (Part No.186002350).

[00306] Preparative HPLC: Waters XBridge Prep C18 5μm ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method B

[00307] To the corresponding primary amine (4.8mmol) in THF (25 mL) at room temperature are added aldehyde (5.6mmol) and anhydrous magnesium sulfate (0.75g). After stirring for 1.5h at room temperature, sodium borohydride (4.8mmol) is added and the mixture is stirred for a further 2h. Water (10-15 mL) is added to the mixture and stirring is resumed for 30-60min. Additional water is added (20-30 mL) and the mixture is extracted with CH₂Cl₂ (40 mL x 3). After being dried over anhydrous magnesium sulfate, the solvent is then removed under reduced pressure to give the crude product. This material may then be used in subsequent steps without further purification.

[00308] The amine obtained using the methods of the previous step (0.26mmol) is added to a solution of corresponding chloro-derivative ($8.84 \cdot 10^{-2}$ mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 12-72h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is then removed under reduced pressure. The final compound is then isolated by preparative HPLC (yield 10-60%).

[00309] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00310] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method C

[00311] The appropriate compound obtained with Method B (0.26mmol) is dissolved in dichloromethane (2 mL) and trifluoroacetic acid (1 mL) is added to the solution. Stirring is then maintained for 1-24h at room temperature. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC (yield 90%) or used crude, in next reaction.

[00312] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00313] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L (Part No.186002978). All the methods used MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method D

[00314] The appropriate *iso*-cyanate (0.119mmol) is added to a solution of the amine obtained following Method C (0.099mmol) in dichloromethane (1 mL) and stirred at room temperature for 3-24h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC (yield 40-70%).

[00315] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00316] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method E

[00317] The appropriate sulfonyl chloride (0.119mmol) is added to a solution of the amine obtained following Method C (0.099mmol) and N,N-diisopropylethylamine (0.199mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 3-24h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC (yield 40-70%).

[00318] Analytical: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350).

[00319] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods used MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method F

[00320] The appropriate acid chloride (0.119mmol) is added to a solution of the amine obtained following Method C (0.099mmol) and N,N-diisopropylethylamine (0.199mmol) in dichloromethane (1 mL) and stirred at room temperature for 3-24h. After completion of the reaction (monitored by LCMS), the solvent is then removed under reduced pressure and the final compound is then isolated by preparative HPLC (yield 40-70%).

[00321] Analytical: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350).

[00322] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method G

[00323] Ethyl bromoacetate (0.280mmol) is added to a solution of the amine obtained following Method C (0.233mmol) and N,N-diisopropylethylamine (0.466mmol) in tetrahydrofuran (3 mL) and stirred at room temperature for 12-24h. After completion of the reaction (monitored by LCMS), the solvent is then removed under reduced pressure and the final compound may be isolated by preparative HPLC (yield 40-70%).

[00324] Analytical: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350).

[00325] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method H

[00326] Lithium hydroxide (0.372mmol) is added to a solution of the ester obtained following Method G (0.186mmol) in dioxane (1.5 mL) and water (0.5 mL) and the mixture is heated to 90°C for 24-72h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC (yield 25-60%).

[00327] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00328] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method I

[00329] Chloroacetyl chloride (0.722mmol) is added to a solution of the amine obtained following Method C (0.241mmol) and N,N-diisopropylethylamine (0.722mmol) in dichloromethane (4 mL) and stirred at room temperature for 3-24h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound may be used in the next reaction without further purification.

[00330] The corresponding nucleophile (0.480mmol) is added to a solution of the alkyl chloride obtained in the previous step (0.080mmol) and N,N-diisopropylethylamine (0.48mmol) in DMF (1ml) and the reaction is heated to 120°C for 24-72h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound may be isolated by preparative HPLC (yield 50-90%).

[00331] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00332] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L (Part No.186002978). All the methods used MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method J

[00333] The corresponding amine (0.053mmol) is added to a solution of the acid obtained following Method H (0.041mmol), benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoroborate (PyBOP, 0.160mmol) and N,N-diisopropylethylamine (0.480mmol) in DMF (1ml) and heated to 120°C for 24-72h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound may be isolated by preparative HPLC (yield 50-80%).

[00334] Analytical: Waters Acquity UPLC BEH C18 1.7µm, 2.1mm ID x 50mm L (Part No.186002350).

[00335] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods used MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method K

[00336] The corresponding aldehyde (0.210mmol) is added to a solution of the amine obtained following Method C (0.070mmol), N,N-diisopropylethylamine (0.140mmol) and anhydrous magnesium sulfate in tetrahydrofuran (1 mL) and stirred at room temperature for 20h. To this is added sodium borohydride (0.070mmol) and stirring continues for a further 24h. Water (2 mL) is added to the mixture and the crude product is extracted with dichloromethane (3x10 mL). The solvent is then removed under reduced pressure and the final compound may be isolated by preparative HPLC (yield 10-20%). Analytical: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350).

[00337] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Method L

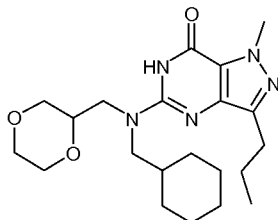
[00338] Thionyl chloride (0.096mmol) is added to a solution of an hydroxylamine obtained following Method B (0.048mmol) in dichloromethane (0.5 mL) and it is stirred at room temperature for 18h. The corresponding amine or ammonia (2 mmol) is added and it is stirred at room temperature for 24h. The solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC (yield 10-20%).

[00339] Analytical: Waters Acquity UPLC BEH C18 1.7 μ m, 2.1mm ID x 50mm L (Part No.186002350).

[00340] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L (Part No.186002978). All the methods use MeCN/H₂O gradients. H₂O contained either 0.1% Trifluoroacetic acid (TFA) or 0.1% Ammonia.

Example 7

5-(((1,4-dioxan-2-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



[00341] To C-[1,4]Dioxan-2-yl-methylamine (0.5mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6 mmol)) and anhydrous magnesium sulfate (60 mg).

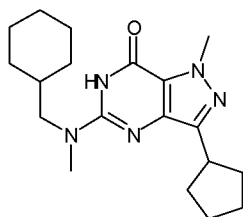
After stirring for 1.5h at room temperature, sodium borohydride (0.5 mmol) is added and the mixture stirred for a further 2h. Water (3 mL) is added to the mixture and stirring resumed for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material is used in subsequent steps without further purification.

[00342] The amine obtained in the previous step (0.26mmol) is added to a solution of 5-chloro-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.13 mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is isolated by preparative HPLC.

[00343] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contained 0.1% Trifluoroacetic acid (TFA).

Example 25

5-((cyclohexylmethyl)(methyl)amino)-3-cyclopentyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

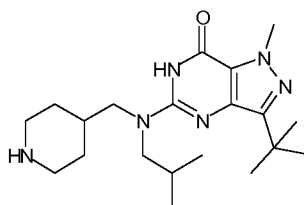


[00344] To methylamine hydrochloride (0.5mmol) and triethylamine (0.8mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6mmol) and anhydrous magnesium sulfate (60mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5mmol) is added and the mixture is stirred for a further 2h. Water (3 mL) is added to the mixture and stirring is resumed for a further 10min. Additional water is added (1 mL) and the mixture is extracted with CH₂Cl₂ (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material may be used in subsequent steps without further purification.

[00345] The amine obtained in the previous step (0.26mmol) is added to a solution of 5-chloro-1-methyl-3-cyclopentyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.13mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The crude product is purified by gradient column chromatography, eluting with 0-5 % methanol in dichloromethane, to give the title compound.

Example 31

3-tert-butyl-5-(isobutyl(piperidin-4-ylmethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

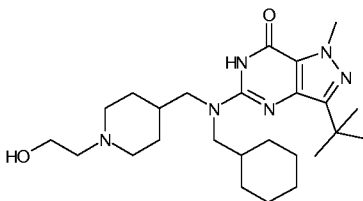


[00346] 4- {[(3-tert-Butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-isobutyl-amino]-methyl}-piperidine-1-carboxylic acid tert-butyl ester (0.26mmol) is dissolved in dichloromethane (2 mL) and trifluoroacetic acid (1 mL) is added to the solution. Stirring is maintained for 12h at room temperature. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC (yield 90%).

[00347] The method uses MeCN/H₂O 25-50% gradients. H₂O contained 0.1% Trifluoroacetic acid (TFA).

Example 35

3-tert-butyl-5-((cyclohexylmethyl)((1-(2-hydroxyethyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



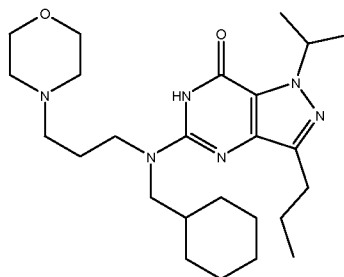
[00348] To 2-(4-Aminomethyl-piperidin-1-yl)-ethanol (0.5 mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6 mmol) and anhydrous magnesium sulfate (60 mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5 mmol) is added and the mixture is then stirred for a further 2h. Water (3 mL) is added to the mixture and stirring resumed for 10 min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material is used in subsequent steps without requiring further purification.

[00349] The amine obtained in the previous step (0.26mmol) is added to a solution of 5-chloro-1-methyl-3-tert-Butyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.13mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is then isolated by preparative HPLC.

[00350] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 35-60% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA)..

Example 37

5-((cyclohexylmethyl)(3-morpholinopropyl)amino)-1-isopropyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



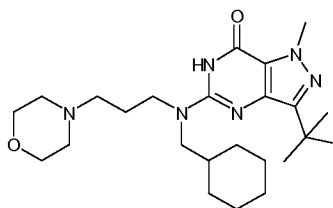
[00351] To N-(3-Aminopropyl)morpholine (0.5 mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6mmol)) and anhydrous magnesium sulfate (60mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5mmol) is added and the mixture is stirred for a further 2h. Water (3 mL) is added to the mixture and stirring is then resumed for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material is then used in subsequent steps without further purification.

[00352] The amine obtained according to the methods described in the previous step (0.26mmol) is added to a solution of 5-chloro-1-isopropyl-3-propyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.13 mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is isolated by preparative HPLC.

[00353] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 35-60% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 38

3-tert-butyl-5-((cyclohexylmethyl)(3-morpholinopropyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



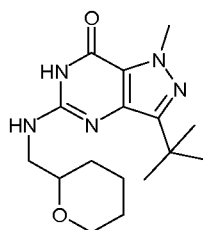
[00354] To N-(3-aminopropyl)morpholine (0.5mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6 mmol) and anhydrous magnesium sulfate (60mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5 mmol) is added and the mixture is stirred for a further 2h. Water (3 mL) is added to the mixture and stirring then resumes for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material may be used in subsequent steps without further purification.

[00355] The amine obtained according to the methods described in the previous step (0.26 mmol) is added to a solution of 5-chloro-1-methyl-3-*tert*-Butyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (0.13mmol) in *t*-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and then the solvent is removed under reduced pressure. The final compound is isolated by preparative HPLC.

[00356] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 35-60% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 50

3-*tert*-butyl-1-methyl-5-((tetrahydro-2H-pyran-2-yl)methylamino)-1H-pyrazolo[4,3-*d*]pyrimidin-7(6H)-one

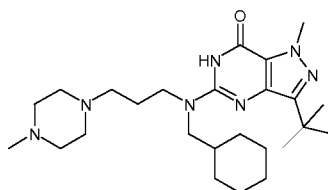


[00357] Tetrahydropyran-2-ylmethylamine (0.26mmol) is added to a solution of chloro-1-methyl-3-*tert*-butyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one ($8.84 \cdot 10^{-2}$ mmol) in *t*-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is purified by preparative HPLC.

[00358] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses: MeCN/H₂O 35-55% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 89

3-*tert*-butyl-5-((cyclohexylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-*d*]pyrimidin-7(6H)-one



[00359] To 1-(3-Aminopropyl)-4-methylpiperazine (0.5 mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6 mmol) and anhydrous magnesium sulfate (60mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5mmol) is added and the mixture stirred for a further 2h. Water (3 mL) is added to the mixture and stirring resumes for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried

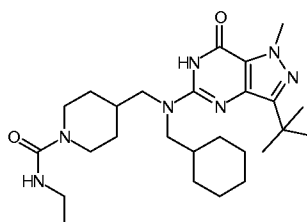
over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material may then be used in subsequent steps without further purification.

[00360] The amine obtained in the previous step (0.26mmol) is added to a solution of 5-chloro-1-methyl-3-*tert*-Butyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (0.13mmol) in *t*-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is isolated by preparative HPLC.

[00361] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 45-70% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 104

4-(((3-*tert*-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-*d*]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)-N-ethylpiperidine-1-carboxamide

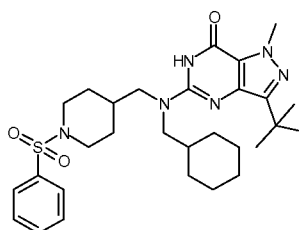


[00362] Ethyl *iso*-cyanate (0.119mmol) is added to a solution of 3-*tert*-butyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (0.099mmol) in dichloromethane (1 mL) and stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC.

[00363] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 65-90% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 107

3-*tert*-butyl-5-((cyclohexylmethyl)((1-(phenylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-*d*]pyrimidin-7(6H)-one



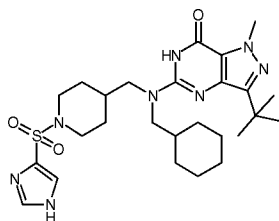
[00364] Benzenesulfonyl chloride (0.119mmol) is added to a solution of 3-*tert*-butyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (0.099mmol) and *N,N*-diisopropylethylamine (0.199mmol) in dichloromethane (1 mL) and stirred at room

temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC.

[00365] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 75-100% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 110

5-(((1-(1H-imidazol-4-ylsulfonyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

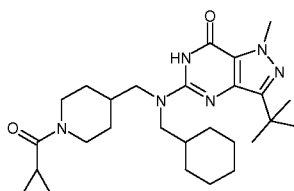


[00366] 1H-Imidazole-4-sulfonyl chloride (0.119mmol) is added to a solution of 3-tert-butyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099mmol) and N,N-diisopropylethylamine (0.199mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC.

[00367] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 112

3-tert-butyl-5-((cyclohexylmethyl)((1-(cyclopropanecarbonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

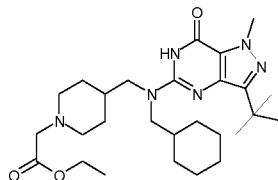


[00368] Cyclopropanecarbonyl chloride (0.119 mmol) is added to a solution of 3-tert-butyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099 mmol) and N,N-diisopropylethylamine (0.199 mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound may be isolated by preparative HPLC.

[00369] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 75-100% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 113

Ethyl 2-(4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)piperidin-1-yl)acetate

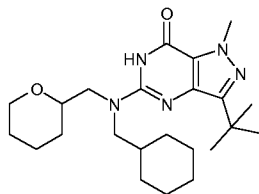


[00370] Ethyl bromoacetate (0.280 mmol) is added to a solution of 3-tert-butyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.233 mmol) and N,N-diisopropylethylamine (0.466 mmol) in tetrahydrofuran (3 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC.

[00371] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 45-70% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 119

3-tert-butyl-5-((cyclohexylmethyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



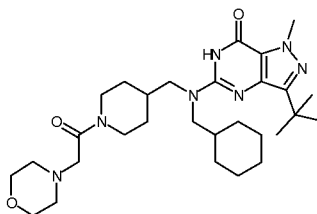
[00372] To tetrahydropyran-2-ylmethylamine (0.5 mmol) in tetrahydrofuran (2 mL) at room temperature are added cyclohexylmethylaldehyde (0.6 mmol) and anhydrous magnesium sulfate (60 mg). After stirring for 1.5h at room temperature, sodium borohydride (0.5mmol) is added and the mixture is then stirred for a further 2h. Water (3 mL) is added to the mixture and stirring resumed for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. This material may be used in subsequent steps without further purification.

[00373] The amine obtained using the methods described in the previous step (0.26mmol) is added to a solution of 5-chloro-1-methyl-3-tert-Butyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.13 mmol) in t-BuOH (0.5 mL). The reaction is heated in a sealed tube to 100°C for 24h. On complete reaction (monitored by LCMS), the mixture is allowed to cool to room temperature and the solvent is removed under reduced pressure. The final compound is isolated by preparative HPLC.

[00374] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 75-100% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 124

3-tert-butyl-5-((cyclohexylmethyl)((1-(2-morpholinoacetyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



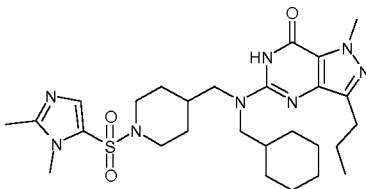
[00375] Chloroacetyl chloride (0.722 mmol) is added to a solution of 3-tert-butyl-5-((cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.241 mmol) and N,N-diisopropylethylamine (0.722 mmol) in dichloromethane (4 mL) and stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound may be used without further purification in the next reaction.

[00376] Morpholine (0.480 mmol) is added to a solution of the alkyl chloride obtained using the methods described in the previous step (0.080 mmol) and N,N-diisopropylethylamine (0.48 mmol) in N,N-dimethylformamide (1 mL) and the reaction is heated to 120°C for 24h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC.

[00377] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 125

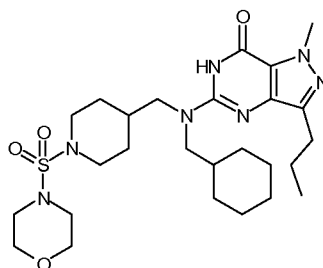
5-((cyclohexylmethyl)((1-(1,2-dimethyl-1H-imidazol-5-ylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



[00378] 1,2-Dimethylimidazole-4-sulfonyl chloride (0.119 mmol) is added to a solution of 3-propyl-5-((cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099 mmol) and N,N-diisopropylethylamine (0.199 mmol) in dichloromethane (1 mL) and stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC. Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 140

5-((cyclohexylmethyl)((1-(morpholinosulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

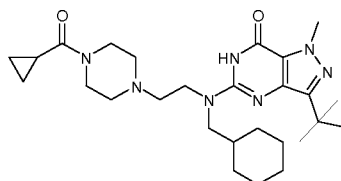


[00379] Morpholine-4-sulfonyl chloride (0.119 mmol) is added to a solution of 3-propyl-5-(cyclohexylmethyl-piperidin-4-ylmethyl-amino)-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099 mmol) and N,N-diisopropylethylamine (0.199 mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC.

[00380] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 146

3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(cyclopropanecarbonyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

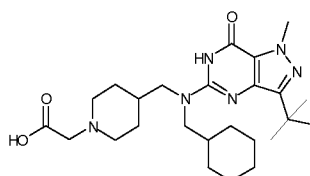


[00381] Cyclopropanecarbonyl chloride (0.119 mmol) is added to a solution of 3-tert-Butyl-5-[cyclohexylmethyl-(2-piperazin-1-yl-ethyl)-amino]-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099 mmol) and N,N-diisopropylethylamine (0.199 mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is evaporated and the final compound is isolated by preparative HPLC.

[00382] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 35-60% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 148

2-(4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)piperidin-1-yl)acetic acid

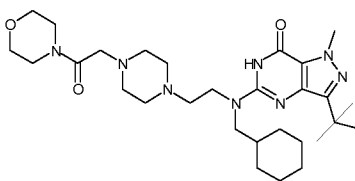


[00383] Lithium hydroxide (0.372 mmol) is added to a solution of (4-{[(3-*tert*-Butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-cyclohexylmethyl-amino]-methyl}-piperidin-1-yl)-acetic acid ethyl ester (0.186mmol), dioxane (1.5 mL) and water (0.5 mL). The mixture is heated to 90°C for 24h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC.

[00384] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 35-60% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 160

3-*tert*-butyl-5-((cyclohexylmethyl)(2-(4-(2-morpholino-2-oxoethyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

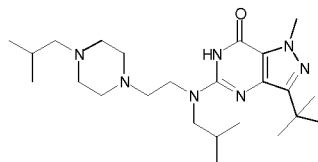


[00385] Morpholine (0.053 mmol) is added to a solution of (4-{2-[(3-*tert*-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-cyclohexylmethyl-amino]-ethyl}-piperazin-1-yl)-acetic acid (0.041 mmol), benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoroborate (PyBOP, 0.160 mmol) and N,N-diisopropylethylamine (0.480 mmol) in N,N-dimethylformamide (1 mL) and it is heated to 120°C for 18h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound may be isolated by preparative HPLC.

[00386] Preparative HPLC: Waters XBridge Prep C18 5µm ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 45-70% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 167

3-*tert*-butyl-5-((*iso*-butyl)(2-(4-*isobutyl*piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

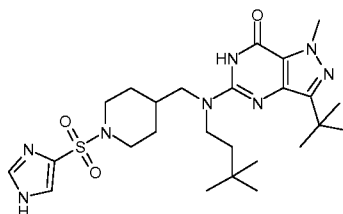


[00387] To 3-*tert*-butyl-5-[*iso*-butyl-(2-piperazin-1-yl-ethyl)-amino]-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.070 mmol) in tetrahydrofuran (1 mL) at room temperature are added 2-methyl-propionaldehyde (0.210 mmol), N,N-diisopropylethylamine (0.140 mmol) and anhydrous magnesium sulfate (60 mg). After stirring for 24h at room temperature, sodium borohydride (0.07 mmol) is added and the mixture is then stirred for a further 12h. Water (3 mL) is added to the mixture and stirring is resumed for 10min. Additional water is added (1 mL) and the mixture is extracted with dichloromethane (10 mL x 3). After being dried over anhydrous magnesium sulfate the solvent is removed under reduced pressure to give the crude product. The final compound is isolated by preparative HPLC.

[00388] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 169

5-(((1-(1H-imidazol-4-ylsulfonyl)piperidin-4-yl)methyl)(3,3-dimethylbutyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one

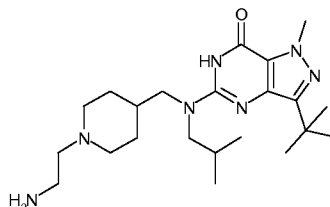


[00389] 1H-Imidazole-4-sulfonyl chloride (0.119 mmol) is added to a solution of 3-tert-Butyl-5-[(3,3-dimethyl-butyl)-piperidin-4-ylmethyl-amino]-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.099 mmol) and N,N-diisopropylethylamine (0.199 mmol) in dichloromethane (1 mL) and it is stirred at room temperature for 18h. After completion of the reaction (monitored by LCMS), the solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC.

[00390] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method uses MeCN/H₂O 55-80% gradients. H₂O contains 0.1% Trifluoroacetic acid (TFA).

Example 171

5-(((1-(2-aminoethyl)piperidin-4-yl)methyl)(isobutyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one



[00391] Thionyl chloride (0.096 mmol) is added to a solution of 3-tert-Butyl-5-{[1-(2-hydroxyethyl)-piperidin-4-ylmethyl]-isobutyl-amino}-1-methyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one (0.048 mmol) in dichloromethane (0.5 mL) and stirred at room temperature for 18h. Then methanolic ammonia (2 mmol) is added and it is then stirred at room temperature for 24h. The solvent is removed under reduced pressure and the final compound is isolated by preparative HPLC.

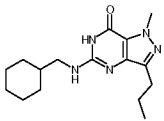
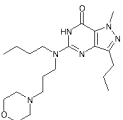
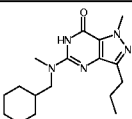
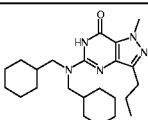
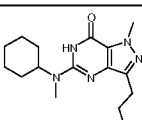
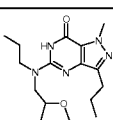
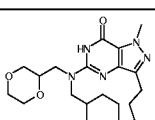
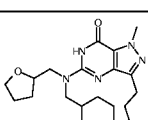
[00392] Preparative HPLC: Waters XBridge Prep C18 5 μ m ODB 19mm ID x 100mm L. The method used MeCN/H₂O 25-50% gradients. H₂O contained 0.1% Trifluoroacetic acid (TFA).

[00393] In addition to the compounds exemplified above, various other pyrazolo[4,3-d]pyrimidinone compounds of this invention may be prepared using the procedure and synthetic methods described here, or via routine modification of the methods described here, and the corresponding starting

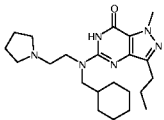
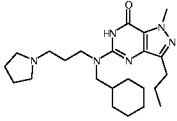
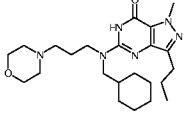
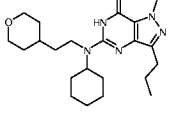
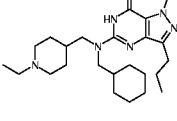
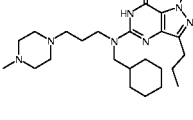
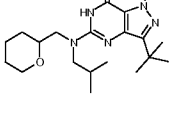
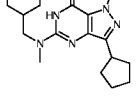
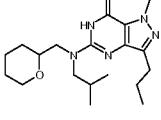
materials, appropriate reagents, and purification methods known to those skilled in the art. The compounds prepared along with their analytical data and synthetic method information are listed in the Table 1, below.

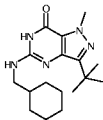
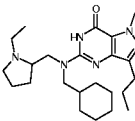
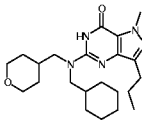
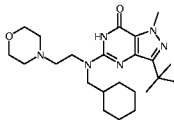
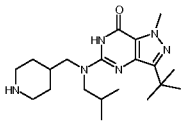
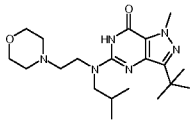
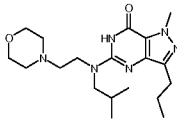
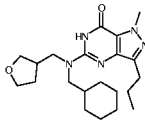
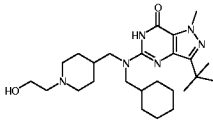
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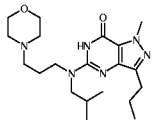
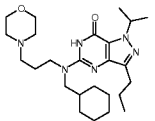
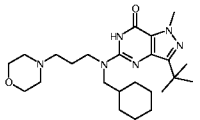
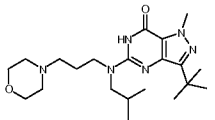
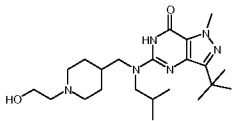
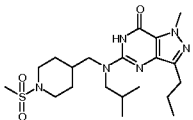
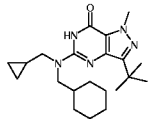
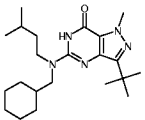
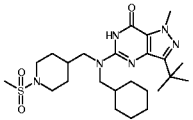
TABLE 1: pyrazolo[4,3-d]pyrimidin-7(6H)-one derivatives

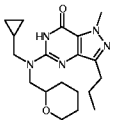
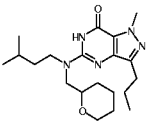
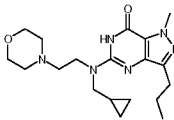
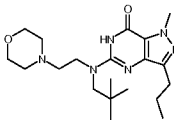
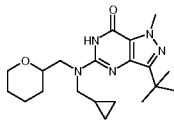
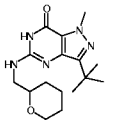
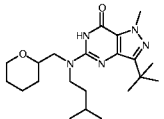
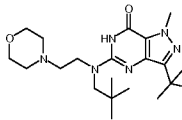
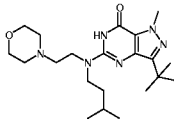
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
1		303.41	304.1	Method A
2		390.53	391.2	Method B
3		317.44	318.2	Method A
4		399.58	400.3	Method B
5		303.41	304.2	Method A
6		333.44	334.1	Method A
7		403.53	404.2	Method B (See Ex. 7)
8		387.53	388.3	Method B

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
9		347.46	457.2	Method B
10		400.57	401.3	Method B
11		414.60	415.3	Method B
12		444.63	445.3	Method B
13		388.56	389.3	Method B
14		374.53	375.2	Method B
15		416.57	417.3	Method B
16		331.46	332.2	Method A
17		414.60	415.3	Method B

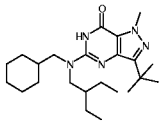
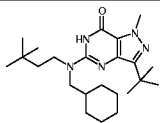
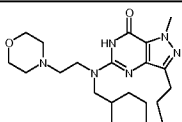
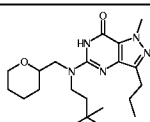
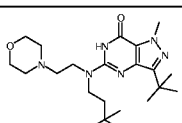
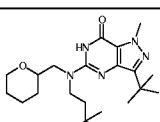
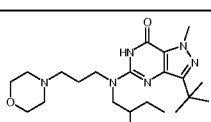
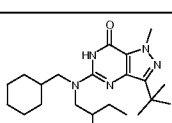
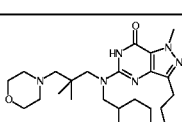
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
18		400.57	401.3	Method B
19		414.60	415.3	Method B
20		430.60	432.1	Method B
21		401.56	402.3	Method B
22		428.63	430.1	Method B
23		443.64	444.2	Method B
24		375.52	376.1	Method B
25		343.48	344(Xterra).	Method A (See Ex. 25)
26		361.49	362.1	Method B

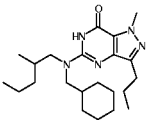
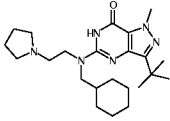
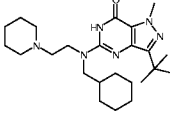
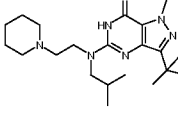
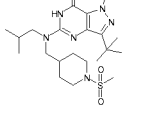
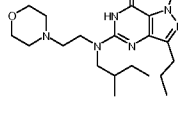
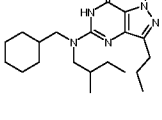
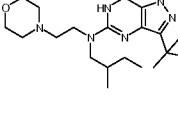
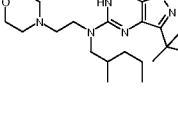
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
27		317.44	318.1	Method A
28		414.60	415.1	Method B
29		401.56	402.1	Method B
30		430.60	431.1	Method B
31		374.53	375.1	Method C
32		390.53	391.1	Method B
33		376.51	377.1	Method B
34		387.53	388	Method B
35		458.65	459.2	Method B (See Ex. 35)

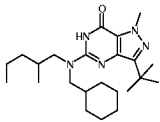
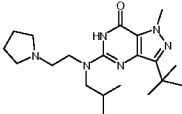
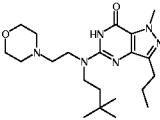
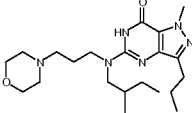
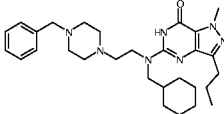
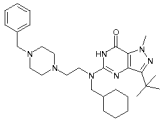
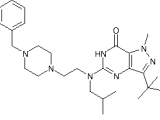
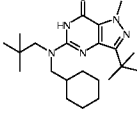
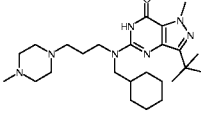
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
36		390.53	391.1	Method B
37		458.65	459.1	Method B (See Ex. 37)
38		444.63	445.2	Method B (See Ex. 38)
39		404.56	405.1	Method B
40		418.59	419.2	Method B
41		438.60	439.1	Method E
42		371.53	372.1	Method B
43		387.57	388.1	Method B
44		492.69	493.2	Method E

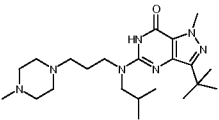
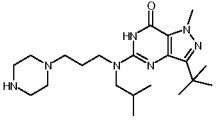
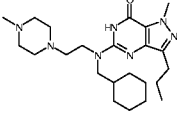
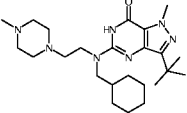
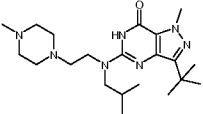
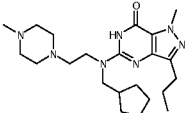
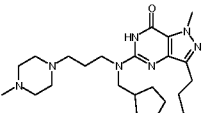
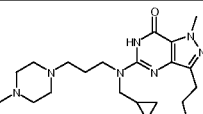
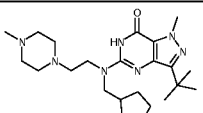
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
45		359.48	360.1	Method B
46		375.52	376.1	Method B
47		374.49	375.1	Method B
48		390.53	391.1	Method B
49		373.50	374.1	Method B
50		319.41	320.1	Method A (See Ex. 50)
51		389.55	390.1	Method B
52		404.56	405.2	Method B
53		404.56	405.2	Method B

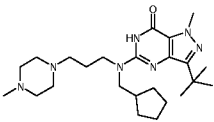
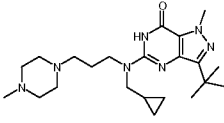
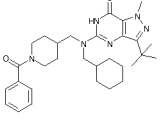
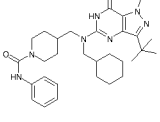
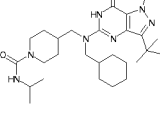
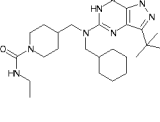
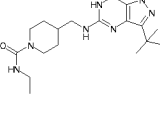
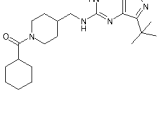
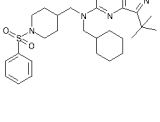
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
54		389.55	390.1	Method B
55		403.57	404.1	Method B
56		390.53	391.1	Method B
57		388.52	389.1	Method B
58		404.56	405.1	Method B
59		389.55	390.1	Method B
60		387.57	388.1	Method B
61		418.59	419.1	Method B
62		403.57	404.1	Method B

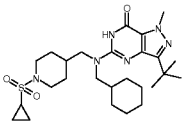
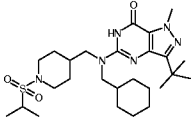
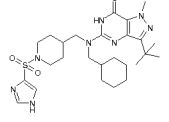
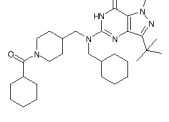
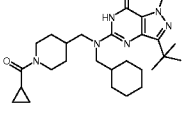
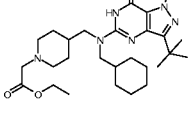
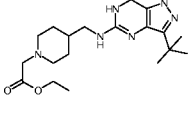
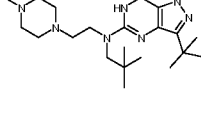
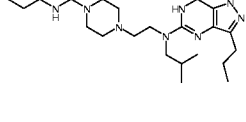
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63		401.60	402.1	Method B
64		401.60	402.1	Method B
65		404.56	405.1	Method B
66		389.55	390.2	Method B
67		418.59	419.2	Method B
68		403.57	404.2	Method B
69		418.59	419.2	Method B
70		387.57	388.1	Method B
71		458.65	459.2	Method B

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
72		387.57	388.1	Method B
73		414.60	415.2	Method B
74		428.63	429.2	Method B
75		388.56	389.2	Method B
76		452.62	453.1	Method E
77		390.53	391.1	Method B
78		373.55	374.1	Method B
79		404.56	405.1	Method B
80		418.59	419.2	Method B

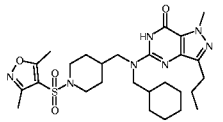
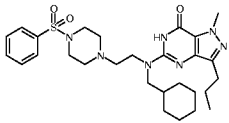
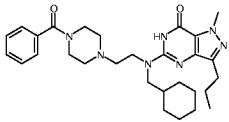
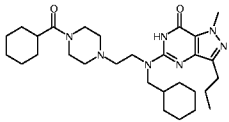
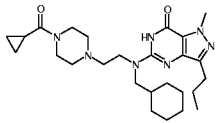
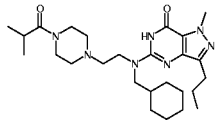
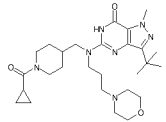
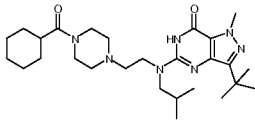
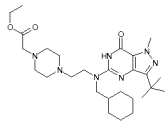
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
81		401.60	402.2	Method B
82		374.53	375.1	Method B
83		404.56	405.2	Method B
84		404.56	405.2	Method B
85		505.71	506.3	Method B
86		519.74	520.3	Method B
87		479.67	480.2	Method B
88		387.57	388.1	Method B
89		457.67	458.2	Method B (See Ex. 89)

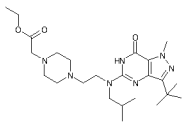
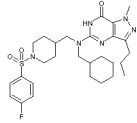
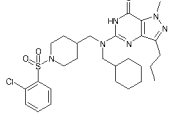
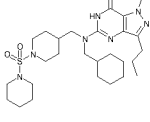
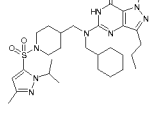
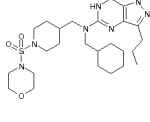
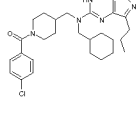
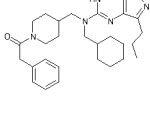
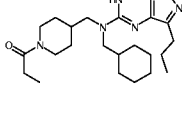
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
90		417.60	418.2	Method B
91		403.58	404.2	Method C
92		429.61	430.2	Method B
93		443.64	444.2	Method B
94		403.58	404.2	Method B
95		415.59	416.3	Method B
96		429.61	430.3	Method B
97		401.56	402.3	Method B
98		429.61	430.3	Method B

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
99		443.64	444.3	Method B
100		415.59	416.2	Method B
101		518.71	519.3	Method F
102		533.72	534.3	Method D
103		499.71	500.2	Method D
104		485.68	486.3	Method D (See Ex. 104)
105		389.50	390.2	Method D
106		428.58	429.2	Method F
107		554.76	555.3	Method E (See Ex. 107)

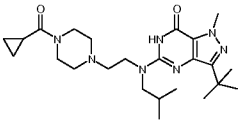
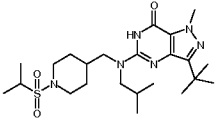
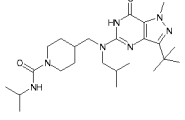
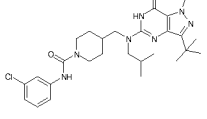
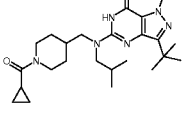
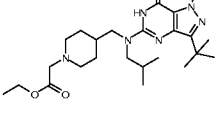
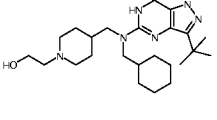
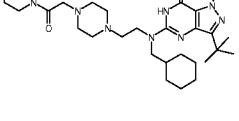
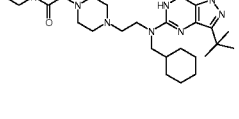
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
108		518.73	519.2	Method E
109		520.74	521.2	Method E
110		544.72	545.2	Method E (See Ex. 110)
111		524.76	525.4	Method F
112		482.67	483.3	Method F (See Ex. 112)
113		500.69	501.2	Method G
114		404.52	405.1	Method G
115		417.60	418.2	Method B
116		500.69	501.3	Method D

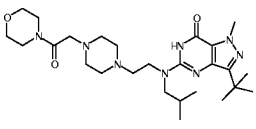
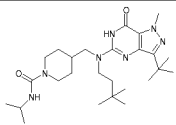
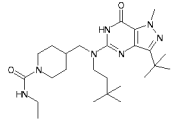
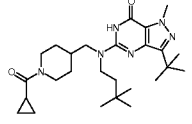
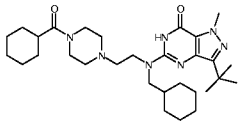
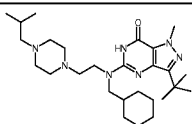
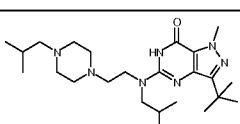
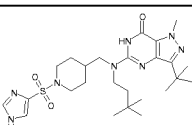
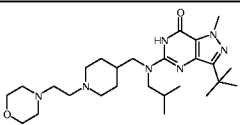
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
117		479.65	480.2	Method E
118		514.72	515.3	Method D
119		415.58	416.2	Method B (See Ex. 119)
120		401.56	402.2	Method B
121		519.71	520.3	Method E
122		521.73	522.2	Method E
123		545.71	546.2	Method E
124		541.74	542.3	Method I
125		558.75	559.2	Method E (See Ex. 125)

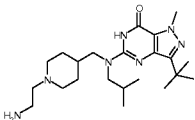
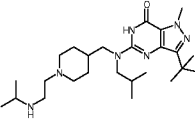
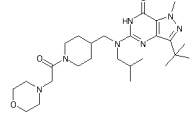
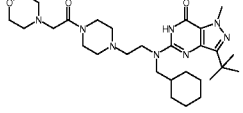
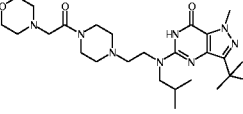
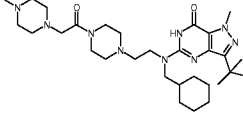
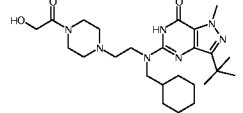
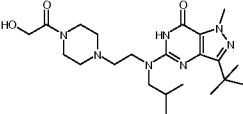
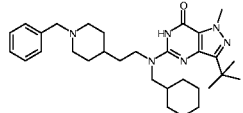
Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
126		559.74	560.2	Method E
127		555.75	556.2	Method E
128		519.70	520.3	Method F
129		525.74	526.3	Method F
130		483.66	484.2	Method F
131		485.68	486.2	Method F
132		513.69	514.2	Method F
133		499.71	500.2	Method F
134		515.70	516.3	Method G

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
135		475.64	476.2	Method G
136		558.72	559.2	Method E
137		575.18	575.2	Method E
138		547.77	548.2	Method E
139		586.81	587.3	Method E
140		549.74	550.2	Method E (See Ex. 140)
141		539.13	539.2	Method F
142		518.71	519.3	Method F
143		456.64	457.2	Method F

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
144		542.73	543.3	Method I
145		473.62	474.3	Method I
146		497.69	498.3	Method F (See Ex. 146)
147		459.64	460.2	Method F
148		472.64	473.2	Method H
149		554.14	554.2	Method D
150		510.65	511.2	Method F
151		528.75	529.3	Method I
152		499.71	500.3	Method F

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
153		457.62	458.2	Method F
154		480.68	481.2	Method E
155		459.64	460.2	Method D
156		528.10	528.2	Method D
157		442.61	443.2	Method F
158		460.62	461.3	Method G
159		472.68	473.3	Method B
160		556.76	557.3	Method J (See Ex. 160)
161		542.77	543.3	Method J

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
162		516.69	517.3	Method J
163		487.69	488.3	Method D
164		473.67	474.2	Method D
165		470.66	471.2	Method F
166		539.77	540.3	Method F
167		485.72	486.3	Method K (See Ex. 167)
168		445.66	446.2	Method K
169		532.71	533.2	Method E (See Ex. 169)
170		487.69	488.3	Method L

Pat ID	STRUCTURE	MW	MS (observed)	Synthetic Method
171		417.60	418.2	Method L (See Ex. 171)
172		459.68	460.3	Method L
173		501.68	502.3	Method I
174		556.76	557.3	Method I
175		516.69	517.3	Method I
176		569.80	570.4	Method I
177		487.65	488.3	Method I
178		447.59	448.2	Method I
179		518.75	519.3	Method B

[00395]

TABLE 2: NMR Data for Exemplary Compounds

Pat ID	NMR (¹ H, DMSO- <i>d</i> ₆ , 400MHz, <i>d</i> 6) (unless specified)
7	4.0 (3H, s, CH ₃); 3.4 (4H, m, 2xCH ₂); 3.6-3.2 (7H, m); 2.62 (2H, t, J 7, CH ₂); 1.7-1.5 (8H, m); 1.15 (3H, m); 0.9 (5H, m, CH ₃ +CH ₂).
25	NMR (¹ H, 400MHz, CDCl ₃): 8.65 (1H, s), 4.12 (3H, s), 3.29 (3H, m), 3.11 (3H, s), 2.07 (2H, m), 1.96 (2H, m), 1.85 (2H, m), 1.76-1.68 (8H, m), 1.20 (3H, m), 0.96 (2H, m).
31	4.17 (2H, m, CH ₂); 4.0 (3H, s, CH ₃); 3.6-3.2 (water peak, 2H beneath+NH); 2.85 (2H, m, CH ₂); 2.75 (2H, m, CH ₂); 1.96 (2H, m, 2xCH); 1.78 (2H, m, CH ₂); 1.38 (9H, s, 3xCH ₃); 1.25 (2H, m, CH ₂); 0.95 (6H, d, J 6.8, 2xCH ₃).
35	4.0 (3H, s, CH ₃); 3.7 (2H, t, J 5.2, CH ₂); 3.5 (2H, m, CH ₂); 3.42 (2H, d, J 7.6, CH ₂) 3.34 (2H, d, J 7.6, CH ₂); 3.24 (1H, m, CH); 3.08 (2H, m, CH ₂); 2.86 (2H, m, CH ₂); 1.9 (1H, m, CH); 1.8-1.4 (9H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH ₂); 0.9 (2H, m, CH ₂).
37	5.18 (1H, q, J 3, CH); 3.97 (2H, m, CH ₂); 3.65 (2H, m, CH ₂); 3.49 (2H, t, J 7.6, CH ₂); 3.43 (2H, m, CH ₂); 3.34 (2H, d, J 7.2, CH ₂); 3.2-2.9 (4H, m, CH ₂); 2.64 (2H, t, J 7.6, CH ₂); 1.93 (2H, m, CH ₂); 1.8-1.6 (8H, m, 4xCH ₂); 1.39 (6H, d, J 6.8; 2xCH ₃); 1.15 (3H, m, CH ₂); 0.9 (5H, m, CH ₃ +CH ₂).
38	4.0 (3H, s, CH ₃); 3.9 (2H, m, CH ₂); 3.9-3.6 (water peak+CH ₂); 3.49 (2H, t, J 7.6, CH ₂) 3.43 (2H, m, CH ₂); 3.32 (2H, d, J 7.2, CH ₂); 3.2-2.9 (4H, m, 2xCH ₂); 2.86 (2H, m, CH ₂); 2.05-1.82 (2H, m, CH ₂); 1.8-1.6 (4H, m, 2xCH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (2H, m, CH ₂); 0.9 (2H, m, CH ₂).
50	6.0 (1H, s, NH); 4.0 (3H, s, CH ₃); 3.83 (2H, dd, J 11.3, J 2.8, CH ₂); 3.27 (2H, td, J 11.6, J 2, CH ₂); 3.17 (2H, t, J 6, CH ₂); 1.83 (1H, m, CH); 1.57 (2H, dd, J 13.2, J 2.4, CH ₂); 1.39 (9H, s, 3*CH ₃); 1.23 (2H, qd, J 13.2, 8.8, CH ₂).
89	11.19 (1H, b, NH); 4.09 (8H, bm, 4xCH ₂); 4.00 (3H, s, CH ₃); 3.43 (4H, m, 2xCH ₂); 3.27 (2H, d, J 8, CH ₂); 2.81 (2H, s, CH ₂); 2.09 (3H, s, CH ₃); 1.81 (4H, m, 2xCH ₂); 1.64 (3H, m, CH, CH ₂); 1.38 (9H, s, 3xCH ₃); 1.12 (2H, m, CH ₂); 0.95 (2H, m, CH ₂).
104	6.4 (1H, m, NH); 4.0 (3H, s, CH ₃); 3.7 (4H, dd, J 11.6, J 1.2, 2xCH ₂); 3.4, (4H, m, 2xCH ₂); 3.0 (2H, q, J 7.2, CH ₂); 1.9-1.4 (11H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH+CH ₂); 1.1 (3H, t, J 7.2, CH ₃); 0.9 (2H, m, CH ₂).
107	6.4 (1H, m, NH); 4.0 (3H, s, CH ₃); 3.7 (4H, dd, J 11.6, J 1.2, 2xCH ₂); 3.4, (4H, m, 2xCH ₂); 3.0 (2H, q, J 7.2, CH ₂); 1.9-1.4 (11H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH, CH ₂); 1.1 (3H, t, J 7.2, CH ₃); 0.9 (2H, m, CH ₂).
110	7.83 (1H, s, Ar); 7.73 (1H, s, Ar); 4.0 (3H, s, CH ₃); 3.6 (2H, m, CH ₂); 3.5 (water peak, 6H beneath); 1.7-1.5 (11H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.11 (3H, m, CH, CH ₂); 0.9 (2H, m, CH ₂).

Pat ID	NMR (¹ , DMSO- δ H, 400MHz, <i>d</i> 6) (unless specified)
112	10.9 (1H, m, NH); 4.3 (H, m, CH ₂); 4.25 (H, m, CH ₂); 4.0 (3H, s, CH ₃); 3.5 (water peak, 6H beneath); 2.97 (1H, m, CH); 1.93 (2H, m, 2xCH); 1.8-1.5 (9H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH ₂); 0.9 (2H, m, CH ₂); 0.72 (4H, m, 2xCH ₂).
113	10.9 (1H, m, NH); 4.4 (4H, m, 2CH ₂); 4.16 (2H, s, CH ₂); 4.0 (3H, s, CH ₃); 3.8-3.5 (water peak, 4H beneath); 2.92 (2H, m, CH ₂); 1.78 (1H, m, CH); 1.85-1.45 (10H, m, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.24 (3H, t, J 7.2, CH ₃); 1.15 (3H, m, CH ₂); 0.9 (2H, m, CH ₂).
119	4.0 (3H, s, CH ₃); 3.83 (2H, dd, J 10, J 3, CH ₂); 3.5 (water peak, 1H beneath); 3.4 (2H, m, CH ₂); 3.17 (2H, td, J 8, 1, CH ₂); 1.96 (1H, m, CH); 1.8-1.5 (7H, m, CH, 3xCH ₂); 1.48 (2H, dd, J 13, 1, CH ₂); 1.39 (9H, s, 3xCH ₃); 1.23 (2H, qd, J 13, 8, CH ₂); 1.15 (3H, m, CH ₂); 0.9 (2H, m, CH ₂).
124	10.9 (1H, m, NH); 4.37 (2H, m, CH ₂); 4.0 (3H, s, CH ₃); 3.77 (4H, m, CH ₂); 3.6-3.25 (water peak, 8H beneath); 2.97 (2H, m, CH ₂); 2.02 (H, m, CH); 1.8-1.5 (10H, m, 5xCH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH, CH ₂); 0.9 (2H, m, CH ₂).
125	7.66 (1H, s, Ar); 4.0 (3H, s, CH ₃); 3.57 (3H, s, CH ₃); 3.6 (2H, m, CH ₂); 3.5-3.3 (water peak, 6H beneath); 2.5 (2H, t, J 7.2, CH ₂); 2.32 (1H, m, CH); 2.27 (3H, s, CH ₃); 1.7-1.5 (10H, m, 5xCH ₂); 1.34-1.11 (3H, m, CH, CH ₂); 0.9 (2H, m, CH ₂); 0.85 (3H, t, J 7.2, CH ₃);
140	4.0 (3H, s, CH ₃); 3.6 (8H, m, CH ₂); 3.45-3.3 (water peak, 2H beneath); 3.06 (4H, m, CH ₂); 2.77 (2H, m, CH ₂); 2.62 (2H, t, J 7.2, CH ₂); 1.85 (1H, m, CH); 1.7-1.5 (12H, m, 6xCH ₂); 1.17 (3H, m, CH ₂); 0.92 (2H, m, CH ₂); 0.9 (3H, t, J 7.2, CH ₃)
146	4.01 (3H, s, CH ₃); 3.35 (8H, bm, 4xCH ₂); 3.85 (2H, m, CH ₂); 3.57 (2H, d, J 8, CH ₂); 2.64 (2H, m, CH ₂); 2.09 (1H, m, CH); 1.68 (4H, m, 2xCH ₂); 1.63 (3H, m, CH+CH ₂); 1.38 (9H, s, 3xCH ₃); 1.15 (2H, m, CH ₂); 0.98 (2H, m, CH ₂); 0.76 (4H, m, 2xCH ₂).
148	7.2 (m, OH); 4.14 (2H, m, CH ₂); 3.99 (3H, s, CH ₃); 3.5-3.2 (water peak, 8H beneath); 1.82 (1H, m, CH); 1.75-1.55 (10H, m, 5xCH ₂); 1.39 (9H, s, 3xCH ₃); 1.15 (3H, m, CH+CH ₂); 0.9 (2H, m, CH ₂).
160	4.01 (3H, s, CH ₃); 3.60 (4H, m, 2xCH ₂); 3.49 (4H, m, CH ₂); 3.35 (8H, bm, 4xCH ₂); 2.64 (2H, m, CH ₂); 1.66 (4H, m, CH ₂); 1.63 (3H, m, CH+CH ₂); 1.38 (9H, s, 3xCH ₃); 1.15 (2H, m, CH ₂); 0.97 (2H, m, CH ₂).
168	4.01 (3H, s, CH ₃); 3.54 (6H, bm, 3xCH ₂); 3.14 (2H, m, CH ₂); 2.96 (2H, d, J 8, CH ₂); 2.09 (1H, m, CH); 1.37 (9H, s, 3xCH ₃); 0.09 (6H, d, J 6.4, 2xCH ₃); 0.87 (6H, d, J 6.8, 2xCH ₃).
169	7.66 (1H, s, Ar); 7.83 (1H, s, Ar); 7.73 (1H, s, Ar); 3.98 (3H, s, CH ₃); 3.60 (2H, m, CH ₂); 3.29 (2H, m, CH ₂); 2.37 (2H, m, CH ₂); 1.63 (4H, m, 2xCH ₂); 1.44 (2H, m, CH ₂); 1.37 (1H, m, CH); 1.35 (9H, s, 3xCH ₃); 1.26 (2H, m, CH ₂); 0.90 (9H, s, 3xCH ₃).
171	8.0 (1H, m, NH); 3.99 (3H, s, CH ₃); 3.45 (2H, s, CH ₂); 3.5-3.0 (water peak, 10H beneath); 3.0-2.8 (m, NH ₂); 2.1-1.75 (3H, m, CH+CH ₂); 1.75-1.65 (3H, m, CH+CH ₂); 1.37 (9H, s, 3xCH ₃); 0.85 (6H, d, J 6.4, 2xCH ₃).

Biological Assays

[00396] For the primary screening, an assay using the cAMP dynamic htrf kit from Cisbio (catnr 62AM2PEB) is used. Its principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). The method is a competitive immunoassay between native cAMP and the cAMP labeled with XL665. The tracer binding is visualized by a monoclonal antibody against cAMP, labeled with Cryptate. The specific signal (i.e. energy transfer) is inversely proportional to the concentration of cAMP in the sample (see Figure 1).

[00397] For the enzymatic reaction we make a mix of 20µL with purified PDE1A enzyme, 100nM cAMP and the compound (10µM in a final concentration of 1% DMSO) in a black 384-plate. The reaction buffer is Tris 20mM pH 7.4, 4µg/mL calmodulin, 3mM MgCl₂, 1.5mM CaCl₂, 0.2mg/mL BSA and 0.001% Brij-35. After an incubation of 30 minutes at room temperature, the reaction is stopped by the addition of 10µL labelled cAMP-XL665 and 10 µL anti-cAMP-Cryptate. After 1 hour incubation at room temperature, the readout is performed on the Envision (excitation 360nm; emission donor 615nm ; emission acceptor 665nm).

[00398] PDE1A hydrolyses cAMP into 5'AMP; this low cAMP concentration will result in a high signal. A PDE1A inhibitor will result in a decrease of the signal.

[00399] As a positive control we used 10µM Vardenafil (100% inhibition) , as negative control we used 1% DMSO (0% inhibition), as variable control 10µM Zaprinast (+/- 50% inhibition) and as negative control compound 10µM Ro-20-1724 (0% inhibition). The positive and negative control are used to calculate z' and PIN values.

[00400] All compounds are screened in single at 10µM. The hit criterium is set at PIN 50 (50% inhibition).

[00401] For the dose response and further screening, we used the Cyclic Nucleotide Phosphodiesterase Assay Kit from Biomol, a colorimetric, non-radioactive assay. The basis for the assay is the cleavage of cAMP by PDE1A. The 5'AMP is further cleaved into the nucleoside and phosphate by the enzyme 5'-nucleotidase (catnr KI-307). The phosphate released due to enzymatic cleavage is quantified using BIOMOL GREENTM reagent (catnr AK-111) in a modified Malachite Green assay^{1,2}. A PDE1A inhibitor will result in a decrease of the signal.

[00402] For the enzymatic reaction a mix of 25µL with purified PDE1A enzyme, 100µM cAMP, 5'Nucleotidase and the compound is made in a clear 384-plate. The reaction buffer is Tris 20mM pH 7.4, 4µg/mL calmodulin, 3mM MgCl₂, 1.5mM CaCl₂, 0.2mg/mL BSA and 0.001% Brij-35. After an incubation of 45 minutes at 37°C, the reaction is stopped by the addition of 50µL BIOMOL GREENTM reagent. After 30 minutes incubation at room temperature, the readout is performed on the Envision (absorption at 615nm).

[00403] All compounds are tested in duplicate starting from 20µM followed by a 1/3 serial dilution, 8 points (20µM - 6.67µM - 2.22µM - 740nM - 247nM - 82nM - 27nM - 9nM) in a final concentration of 1% DMSO. As the positive dose response control compound Vardenafil is used. For the calculation of z' and PIN values 10µM Vardenafil is used as positive control (100% inhibition) and 1% DMSO as negative control (0% inhibition).

Exemplary Compounds of the Invention

[00404] The following compounds have been or can be prepared according to the synthetic methods described above. For the purpose of Table 3 below, activity of each compound, which can be determined using the PDE1A assay method described in above, is expressed as follows:

“+”	IC ₅₀ >500 nM
“++”	IC ₅₀ 101-500 nM
“+++”	IC ₅₀ 0.001 - 100 nM

[00405] TABLE 3: PDE1A IC₅₀ Data for Exemplary Compounds

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
1	5-(cyclohexylmethylamino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
2	5-(butyl(3-morpholinopropyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
3	5-((cyclohexylmethyl)(methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
4	5-(bis(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
5	5-(cyclohexyl(methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+
6	1-methyl-3-propyl-5-(propyl((tetrahydrofuran-2-yl)methyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
7	5-(((1,4-dioxan-2-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
8	5-((cyclohexylmethyl)((tetrahydrofuran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
9	3-tert-butyl-1-methyl-5-(propyl((tetrahydrofuran-2-yl)methyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
10	5-((cyclohexylmethyl)(1-methylpiperidin-4-yl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+
11	5-((cyclohexylmethyl)((1-methylpiperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
12	5-((cyclohexylmethyl)((1-(2-hydroxyethyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
13	5-((cyclohexylmethyl)(3-(dimethylamino)propyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
14	5-((cyclohexylmethyl)(2-(dimethylamino)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
15	5-((cyclohexylmethyl)(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
16	3-tert-butyl-5-((cyclohexylmethyl)(methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
17	5-((cyclohexylmethyl)(2-(piperidin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
18	5-((cyclohexylmethyl)(2-(pyrrolidin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
19	5-((cyclohexylmethyl)(3-(pyrrolidin-1-yl)propyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
20	5-((cyclohexylmethyl)(3-morpholinopropyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
21	5-(cyclohexyl(2-(tetrahydro-2H-pyran-4-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
22	5-((cyclohexylmethyl)((1-ethylpiperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
23	5-((cyclohexylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
24	3-tert-butyl-5-(isobutyl((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
25	5-((cyclohexylmethyl)(methyl)amino)-3-cyclopentyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
26	5-(isobutyl((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
27	3-tert-butyl-5-(cyclohexylmethylamino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
28	5-((cyclohexylmethyl)((1-ethylpyrrolidin-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
29	5-((cyclohexylmethyl)((tetrahydro-2H-pyran-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
30	3-tert-butyl-5-((cyclohexylmethyl)(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
31	3-tert-butyl-5-(isobutyl(piperidin-4-ylmethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+
32	3-tert-butyl-5-(isobutyl(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
33	5-(isobutyl(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
34	5-((cyclohexylmethyl)((tetrahydrofuran-3-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
35	3-tert-butyl-5-((cyclohexylmethyl)((1-(2-hydroxyethyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
36	5-(isobutyl(3-morpholinopropyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
37	5-((cyclohexylmethyl)(3-morpholinopropyl)amino)-1-isopropyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
38	3-tert-butyl-5-((cyclohexylmethyl)(3-morpholinopropyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
39	3-tert-butyl-5-(isobutyl(3-morpholinopropyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
40	3-tert-butyl-5-(((1-(2-hydroxyethyl)piperidin-4-yl)methyl)(isobutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
41	5-(isobutyl((1-(methylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
42	3-tert-butyl-5-((cyclohexylmethyl)(cyclopropylmethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
43	3-tert-butyl-5-((cyclohexylmethyl)(isopentyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
44	3-tert-butyl-5-((cyclohexylmethyl)((1-(methylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
45	5-((cyclopropylmethyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
46	5-(isopentyl((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
47	5-((cyclopropylmethyl)(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
48	1-methyl-5-((2-morpholinoethyl)(neopentyl)amino)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
49	3-tert-butyl-5-((cyclopropylmethyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
50	3-tert-butyl-1-methyl-5-((tetrahydro-2H-pyran-2-yl)methylamino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+
51	3-tert-butyl-5-(isopentyl((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
52	3-tert-butyl-1-methyl-5-((2-morpholinoethyl)(neopentyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
53	3-tert-butyl-5-(isopentyl(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
54	1-methyl-5-((2-methylpentyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
55	3-tert-butyl-1-methyl-5-((2-methylpentyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
56	5-(isopentyl(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
57	3-tert-butyl-5-((cyclopropylmethyl)(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
58	5-((2-ethylbutyl)(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
59	5-((2-ethylbutyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
60	5-((cyclohexylmethyl)(2-ethylbutyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
61	3-tert-butyl-5-((2-ethylbutyl)(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
62	3-tert-butyl-5-((2-ethylbutyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
63	3-tert-butyl-5-((cyclohexylmethyl)(2-ethylbutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
64	3-tert-butyl-5-((cyclohexylmethyl)(3,3-dimethylbutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
65	1-methyl-5-((2-methylpentyl)(2-morpholinoethyl)amino)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
66	5-((3,3-dimethylbutyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
67	3-tert-butyl-5-((3,3-dimethylbutyl)(2-morpholinoethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
68	3-tert-butyl-5-((3,3-dimethylbutyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
69	3-tert-butyl-1-methyl-5-((2-methylbutyl)(3-morpholinopropyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
70	3-tert-butyl-5-((cyclohexylmethyl)(2-methylbutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
71	5-((cyclohexylmethyl)(2,2-dimethyl-3-morpholinopropyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
72	5-((cyclohexylmethyl)(2-methylpentyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
73	3-tert-butyl-5-((cyclohexylmethyl)(2-(pyrrolidin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
74	3-tert-butyl-5-((cyclohexylmethyl)(2-(piperidin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
75	3-tert-butyl-5-(isobutyl(2-(piperidin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
76	3-tert-butyl-5-(isobutyl((1-(methylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
77	1-methyl-5-((2-methylbutyl)(2-morpholinoethyl)amino)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
78	5-((cyclohexylmethyl)(2-methylbutyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
79	3-tert-butyl-1-methyl-5-((2-methylbutyl)(2-morpholinoethyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
80	3-tert-butyl-1-methyl-5-((2-methylpentyl)(2-morpholinoethyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
81	3-tert-butyl-5-((cyclohexylmethyl)(2-methylpentyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
82	3-tert-butyl-5-(isobutyl(2-(pyrrolidin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
83	5-((3,3-dimethylbutyl)(2-morpholinoethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
84	1-methyl-5-((2-methylbutyl)(3-morpholinopropyl)amino)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
85	5-((2-(4-benzylpiperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
86	5-((2-(4-benzylpiperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
87	5-((2-(4-benzylpiperazin-1-yl)ethyl)(isobutyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
88	3-tert-butyl-5-((cyclohexylmethyl)(neopentyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
89	3-tert-butyl-5-((cyclohexylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
90	3-tert-butyl-5-(isobutyl(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
91	3-tert-butyl-5-(isobutyl(3-(piperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
92	5-((cyclohexylmethyl)(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
93	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
94	3-tert-butyl-5-(isobutyl(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
95	5-((cyclopentylmethyl)(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
96	5-((cyclopentylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
97	5-((cyclopropylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
98	3-tert-butyl-5-((cyclopentylmethyl)(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
99	3-tert-butyl-5-((cyclopentylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
100	3-tert-butyl-5-((cyclopropylmethyl)(3-(4-methylpiperazin-1-yl)propyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
101	5-(((1-benzoylpiperidin-4-yl)methyl)(cyclohexylmethyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
102	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)-N-phenylpiperidine-1-carboxamide	+++
103	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)-N-isopropylpiperidine-1-carboxamide	+++
104	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)-N-ethylpiperidine-1-carboxamide	+++
105	4-((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-ylamino)methyl)-N-ethylpiperidine-1-carboxamide	++
106	3-tert-butyl-5-((1-(cyclohexanecarbonyl)piperidin-4-yl)methylamino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
107	3-tert-butyl-5-((cyclohexylmethyl)((1-(phenylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
108	3-tert-butyl-5-((cyclohexylmethyl)((1-(cyclopropylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
109	3-tert-butyl-5-((cyclohexylmethyl)((1-(isopropylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
110	5-(((1-(1H-imidazol-4-ylsulfonyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
111	3-tert-butyl-5-(((1-(cyclohexanecarbonyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
112	3-tert-butyl-5-((cyclohexylmethyl)((1-(cyclopropanecarbonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
113	ethyl 2-(4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)piperidin-1-yl)acetate	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
114	ethyl 2-(4-((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-ylamino)methyl)piperidin-1-yl)acetate	++
115	3-tert-butyl-1-methyl-5-((2-(4-methylpiperazin-1-yl)ethyl)(neopentyl)amino)-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
116	N-cyclohexyl-4-(2-(isobutyl(1-methyl-7-oxo-3-propyl-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)amino)ethyl)piperazine-1-carboxamide	+
117	5-((2-(4-(cyclopropylsulfonyl)piperazin-1-yl)ethyl)(isobutyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
118	4-(2-((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)ethyl)-N-isopropylpiperazine-1-carboxamide	+++
119	3-tert-butyl-5-((cyclohexylmethyl)((tetrahydro-2H-pyran-2-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
120	3-tert-butyl-5-((cyclopropylmethyl)(2-(4-methylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
121	5-((cyclohexylmethyl)(2-(4-(cyclopropylsulfonyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
122	5-((cyclohexylmethyl)(2-(4-(isopropylsulfonyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
123	5-((2-(4-(1H-imidazol-4-ylsulfonyl)piperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
124	3-tert-butyl-5-((cyclohexylmethyl)((1-(2-morpholinoacetyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
125	5-((cyclohexylmethyl)((1-(1,2-dimethyl-1H-imidazol-5-ylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
126	5-((cyclohexylmethyl)((1-(3,5-dimethylisoxazol-4-ylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
127	5-((cyclohexylmethyl)(2-(4-(phenylsulfonyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
128	5-((2-(4-benzoylpiperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
129	5-((2-(4-(cyclohexanecarbonyl)piperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
130	5-((cyclohexylmethyl)(2-(4-(cyclopropanecarbonyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
131	5-((cyclohexylmethyl)(2-(4-isobutyroylpiperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
132	3-tert-butyl-5-(((1-(cyclopropanecarbonyl)piperidin-4-yl)methyl)(3-morpholinopropyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+
133	3-tert-butyl-5-((2-(4-(cyclohexanecarbonyl)piperazin-1-yl)ethyl)(isobutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
134	ethyl 2-(4-(2-((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)ethyl)piperazin-1-yl)acetate	+++
135	ethyl 2-(4-(2-((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(isobutyl)amino)ethyl)piperazin-1-yl)acetate	+++
136	5-((cyclohexylmethyl)((1-(4-fluorophenylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
137	5-(((1-(2-chlorophenylsulfonyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
138	5-((cyclohexylmethyl)((1-(piperidin-1-ylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
139	5-((cyclohexylmethyl)((1-(1-isopropyl-3-methyl-1H-pyrazol-5-ylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
140	5-((cyclohexylmethyl)((1-(morpholinosulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
141	5-(((1-(4-chlorobenzoyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
142	5-((cyclohexylmethyl)((1-(2-phenylacetyl)piperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
143	5-((cyclohexylmethyl)((1-propionoylpiperidin-4-yl)methyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
144	5-((cyclohexylmethyl)(2-(4-(2-morpholinoacetyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
145	5-((cyclohexylmethyl)(2-(4-(2-hydroxyacetyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
146	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(cyclopropanecarbonyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
147	3-tert-butyl-5-(isobutyl(2-(4-isobutyroylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
148	2-(4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)methyl)piperidin-1-yl)acetic acid	+++
149	N-(3-chlorophenyl)-4-(((cyclohexylmethyl)(1-methyl-7-oxo-3-propyl-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)amino)methyl)piperidine-1-carboxamide	+++
150	5-(((1-(2-(2H-tetrazol-5-yl)acetyl)piperidin-4-yl)methyl)(cyclohexylmethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
151	5-((cyclohexylmethyl)(2-(4-(2-(diethylamino)acetyl)piperazin-1-yl)ethyl)amino)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
152	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-isobutyroylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
153	3-tert-butyl-5-((2-(4-(cyclopropanecarbonyl)piperazin-1-yl)ethyl)(isobutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
154	3-tert-butyl-5-(isobutyl((1-(isopropylsulfonyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
155	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(isobutyl)amino)methyl)-N-isopropylpiperidine-1-carboxamide	+++
156	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(isobutyl)amino)methyl)-N-(3-chlorophenyl)piperidine-1-carboxamide	+++
157	3-tert-butyl-5-(((1-(cyclopropanecarbonyl)piperidin-4-yl)methyl)(isobutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
158	ethyl 2-(4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(isobutyl)amino)methyl)piperidin-1-yl)acetate	+++
159	3-tert-butyl-5-((cyclohexylmethyl)((1-(2-hydroxyethyl)piperidin-4-yl)methyl)amino)-1-ethyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
160	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(2-morpholino-2-oxoethyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
161	2-(4-(2-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(cyclohexylmethyl)amino)ethyl)piperazin-1-yl)-N,N-diethylacetamide	+++
162	3-tert-butyl-5-(isobutyl(2-(4-(2-morpholino-2-oxoethyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
163	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(3,3-dimethylbutyl)amino)methyl)-N-isopropylpiperidine-1-carboxamide	+++
164	4-(((3-tert-butyl-1-methyl-7-oxo-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)(3,3-dimethylbutyl)amino)methyl)-N-ethylpiperidine-1-carboxamide	+++
165	3-tert-butyl-5-(((1-(cyclopropanecarbonyl)piperidin-4-yl)methyl)(3,3-dimethylbutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
166	3-tert-butyl-5-((2-(4-(cyclohexanecarbonyl)piperazin-1-yl)ethyl)(cyclohexylmethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
167	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-isobutylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
168	3-tert-butyl-5-(isobutyl(2-(4-isobutylpiperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
169	5-(((1-(1H-imidazol-4-ylsulfonyl)piperidin-4-yl)methyl)(3,3-dimethylbutyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
170	3-tert-butyl-5-(isobutyl((1-(2-morpholinoethyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
171	5-(((1-(2-aminoethyl)piperidin-4-yl)methyl)(isobutyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	++
172	3-tert-butyl-5-(isobutyl((1-(2-(isopropylamino)ethyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
173	3-tert-butyl-5-(isobutyl((1-(2-morpholinoacetyl)piperidin-4-yl)methyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
174	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(2-morpholinoacetyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
175	3-tert-butyl-5-(isobutyl(2-(4-(2-morpholinoacetyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
176	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(2-(4-methylpiperazin-1-yl)acetyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
177	3-tert-butyl-5-((cyclohexylmethyl)(2-(4-(2-hydroxyacetyl)piperazin-1-yl)ethyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++
178	3-tert-butyl-5-((2-(4-(2-hydroxyacetyl)piperazin-1-yl)ethyl)(isobutyl)amino)-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

Pat ID	IUPAC NAME	PDE1A IC ₅₀ (nM)
179	5-((2-(1-benzylpiperidin-4-yl)ethyl)(cyclohexylmethyl)amino)-3-tert-butyl-1-methyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one	+++

2.2 PDE1A cellular assay

[00406] As an alternative assay to the *in vitro* assay described above, a cellular PDE1A assay was developed in order to determine PDE1A inhibitor activity on cAMP levels in forskolin (NHK477) stimulated HEK293 cells transiently transfected with PDE1A with HTRF® (Homogeneous Time-Resolved Fluorescence) cAMP dynamic 2 bulk kit (from Cisbio).

[00407] HEK 293T are routinely maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal calf serum, 100 U/mL Penicillin and 100 µg/ml Streptomycin.

[00408] 70 % confluent HEK 293T are used for reverse transfection that involves simultaneously transfecting and plating cells. 60 000 cells are transiently transfected with 100ng of pcDNA3.1(+)PDE1A DNA using 0.2µL Jet-PEI (Polyplus) as transfection reagent per well for 96 well plate format. Transfected cells are seeded in poly-D-lysine coated 96-well plates. After overnight incubation at 37°C, 10% CO₂, transfection medium is removed and 100µL of cell culture medium are added.

[00409] 48 hour after transfection, the medium is removed, 20µL DMEM is added, then 20 µL of compound dilution in DMEM, 50 mM Hepes (25mM f.c.), 2*10⁻⁴M Forskolin (10⁻⁴M f.c.; NHK477; to activate adenylate cyclase) and 2*10⁻³M Rolipram (10⁻³M f.c.). By pharmacologically inhibiting PDE4 endogenous activity with Rolipram, transiently expressed PDE1A becomes the main PDE using cAMP as substrate in transfected HEK293T cells and this allows a specific PDE1A readout.

[00410] All compounds are tested in duplicate starting from 100µM followed by a 1/3 serial dilution, 8 points (100µM – 33.3µM – 11.1µM - 3.7µM - 1.2µM - 411nM - 137nM - 46nM - 15nM) in a final concentration of 5% DMSO.

[00411] After 1h incubation at 37°C, cells are lysed by addition of 40µL lysis buffer (50 mM KH₂PO₄, 50 mM Na₂HPO₄, 0.8 M Potassium Fluoride, 0.2 % BSA, 1% Triton-X100). Cell lysates are diluted 1/100 (2x 1/10) in lysis buffer.

[00412] Htrf reagents are added to 20µL diluted cell lysate: 10µL cAMP-d2 (cAMP labelled with d2 dye), then 10µL anti cAMP-Cryptate (monoclonal antibody against cAMP, labeled with cryptate). The plates are incubated at room temperature for 1 hour and read on the Envision ((excitation 360nm; emission donor 615nm; emission acceptor 665nm). Results are calculated from the 665nm / 615nm ratio and expressed in Delta F% as described by manufacturer.

[00413] Appropriate positive and negative control may be selected by a person of skill in the art and used to calculate z' and PIN values.

2.3 PDE Selectivity Panel

[00414] In one aspect the compounds of the invention are more potent against PDE1A than against other PDE isoforms. In a particular embodiment, the compounds are 2 fold more potent against PDE1A than against one or more of the other isoforms. In an alternative embodiment, the compounds of the invention are 5 fold, particularly 10-fold, particularly 20-fold more potent against PDE1A than against one or more of the other isoforms of PDE. In particular, the compounds of the invention are more potent against PDE1A than against at least one of PDE1B, PDE2A, PDE4A or PDE5A. In particular the compounds are more potent against PDE1A than against at least two of PDE1B, PDE2A, PDE4A or PDE5A. In particular the compounds are more potent against PDE1A than against all of the other PDE isoforms. Methods for testing the selectivity of the compounds against a range of PDE isoforms will be familiar to those of skill in the art, and for example, may measure comparative IC₅₀ values or percentage inhibition values at a set concentration. Typical methods are described below.

[00415] To test the selectivity of the compounds against a panel of PDE's, lysate derived from transiently transfected HEK293 cells (transfected with PDE5A, PDE1B, PDE2A or PDE4A for 48h) is used as the enzyme source.

[00416] The dose response of compounds on PDE5A lysate is performed using the cGMP bulk htrf kit from Cisbio (catnr 62GM2PEC). The principle of this kit is based on the HTRF® technology (Homogeneous Time-Resolved Fluorescence). The method is based on the competition between native cGMP and the cGMP labeled with d2. The tracer binding is visualized by a monoclonal antibody against cGMP, labeled with Cryptate. The specific signal (i.e. energy transfer) is inversely proportional to the concentration of cGMP in the sample. PDE5A hydrolyses cGMP into 5'GMP; the decrease in cGMP concentration upon PDE5A activity will result in an increased signal. A PDE5A inhibitor will cause a decrease of this signal.

[00417] For the enzymatic reaction, a mix of 20µL with PDE5A lysate, 400nM cGMP, and the compound is made in a black 384-plate. The reaction buffer consists of Tris 20mM pH 7.4, 3mM MgCl₂, 1.5mM CaCl₂, 0.2mg/mL BSA and 0.001% Brij-35. After an incubation of 25 minutes at room temperature, the reaction is stopped by the addition of 10µL labeled cGMP-d2 and 10 µL anti-cGMP-Cryptate. After 1 hour incubation at room temperature, the readout is performed on the Envision (excitation 360nm; emission donor 615nm; emission acceptor 665nm).

[00418] All compounds are tested in duplicate starting from 20µM and 20nM followed by a 1/3 serial dilution, 8 points (20µM - 6.67µM - 2.22µM - 740nM - 247nM - 82nM - 27nM - 9nM and 20nM - 6.67nM - 2.22nM - 740pM - 247pM - 82pM - 27pM - 9pM) in a final concentration of 1% DMSO. As positive control the compound Vardenafil is also added in dose response. For the calculation of z' and PIN values 1% DMSO is used as positive control (100% inhibition) and lysate in 1% DMSO as negative control (0% inhibition).

[00419] For the single dose screening on PDE1B, PDE2A and PDE4A lysates an assay using the cAMP dynamic 2 bulk htrf kit from Cisbio (catnr 62AM4PEC) is used. The principle of this kit is based on the HTRF® technology (Homogeneous Time-Resolved Fluorescence). The method is based on the competition between native cAMP and the cAMP labeled with d2. The tracer binding is visualized by a

monoclonal antibody against cAMP, labeled with Cryptate. The specific signal (i.e. energy transfer) is inversely proportional to the concentration of cAMP in the sample.

[00420] For the enzymatic reaction, a mixture is made of 10µL with PDE1B, PDE2A or PDE4A lysate, 100nM cAMP and the compound (50nM in a final concentration of 1% DMSO) in a black 384-plate. The reaction buffer is Tris 20mM pH 7.4, 37.5 U/ml calmodulin, 3mM MgCl₂, 1.5mM CaCl₂, 0.2mg/mL BSA and 0.001% Brij-35[®]. After an incubation of 25 minutes at room temperature, the reaction is stopped by the addition of 5µL labelled cAMP-d2 and 5 µL anti-cAMP-Cryptate. After 1 hour incubation at room temperature, the readout is performed on the Envision (excitation 360nm; emission donor 615nm; emission acceptor 665nm).

[00421] PDE1B, PDE2A and PDE4A hydrolyse cAMP into 5'AMP; this decrease in cAMP concentration will result in an increase in signal. A PDE1A, PDE2A or PDE4A inhibitor will result in a decrease of this signal.

[00422] 1% DMSO (100% inhibition) may be used as a positive control, lysate with 1% DMSO (0% inhibition) may be used as a negative control. The positive and negative control are used to calculate z' and PIN values.

[00423] All compounds may be screened at a single concentration of 50nM. The hit criteria is set at PIN 50 (50% inhibition).

[00424] It should be understood that factors such as the differential cell penetration capacity of the various compounds can contribute to discrepancies between the activity of the compounds in the *in vitro* biochemical and cellular assays.

2.4 *Development of a high-throughput screening method for the detection of endogenous collagen type II, alpha-1 (col2 a1)*

Principle of the assay:

[00425] The mouse embryonic cell line ATDC5 is a cell line that can be induced to a chondrogenic fate by certain culturing conditions such as high cell density or certain growth factors. Anabolic compounds can be tested in this cell line for their capacity to induce or enhance the chondrogenic differentiation by measuring a typical chondrocyte markers such as collagen type II, alpha-1 (col2a1), a major constituent of normal cartilage. ATDC5 cells are seeded in 384 well plates and 3 days after plating treated with compounds. Col2a1 deposition is determined 14 days after the start of the infection.

Control compounds

[00426] FK506 was described to induce chondrogenic differentiation in ATDC5 cells and increase collagen II production (Nishigaki *et al*, 2002, Eur J Pharmacol. FK506 induces chondrogenic differentiation of clonal mouse embryonic carcinoma cells, ATDC5).

Assay description

[00427] ATDC5 cells are seeded on day 0 at 1000 cells/well in 50 µL of DMEM/F12 (Invitrogen), containing 5% heat-inactivated fetal calf serum, 1% Pen/Strep in a 384-well plate previously coated with a 0.1% solution of gelatin in PBS. After 3 days the medium is replaced by the same medium

containing 12.5% cell differentiation medium (CDM, Cell applications) and either 0.1 % DMSO or a compound solution in DMSO such that DMSO levels are 0.1%. At day 7 medium and compound solutions are refreshed, and at day 10 60 µg/mL 2-Phospho-L-ascorbic acid is added.

[00428] Up-regulation of Col2a1 is read at day 13: The medium is removed with a VacuSafe; 50 µL ice-cold MeOH is added and removed immediately by inverting the plate; 50 µL of ice-cold MeOH is added to fix the cells, and plates are incubated for 20 min at -20°C; MeOH is removed and plates are air-dried for 20 min, followed by 2x washing with 80 µL of phosphate buffered saline (PBS); 75 µL of blocking buffer (0.1% casein in PBS) is added and plates are incubated for at least 2 h at room temperature (RT). After the incubation the blocking buffer is removed; cells are washed with 25 µL of EC buffer (20 mM sodium phosphate, 2mM EDTA, 400 mM NaCl, 0.2% BSA, 0.05% CHAPS, 0.4% casein, 0.05% NaN₃, pH 7) and 35 µL of the primary antibody (Collagen II Ab-2, Neomarkers MS-235-P) diluted 1/400 in buffer C (20 mM sodium phosphate, 2 mM EDTA, 400 mM NaCl, 1% BSA, pH 7) is added; plates are incubated overnight at 4°C. After the incubation the primary antibody is removed; cells are washed once with 80 µL of PBST (0.5% Tween 20 in PBS) and once with 80 µL PBS; 35 µL of the secondary antibody (Goat-anti-mouse Immunoglobulins/HRP. DAKO, P0477; diluted 1/2000 in buffer C) is added; plates are incubated at RT for at least 45min but no longer than 1 h. After this incubation, the secondary antibody is removed and cells are washed twice with 80 µL PBST and once with 80 µL PBS; 50 µL of luminol substrate is added and after 5 minutes read-out is determined on a luminometer.

[00429] It should be understood that factors such as the differential cell penetration capacity of the various compounds can contribute to discrepancies between the activity of the compounds in the *in vitro* biochemical and cellular assays.

[00430] It will be appreciated by those skilled in the art that the foregoing description is exemplary and explanatory in nature, and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, an artisan will recognise apparent modifications and variations that may be made without departing from the spirit of the invention. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

[00431] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application are specifically and individually indicated to be incorporated by reference.

[00432] The chemical names of compounds given in this application were generated using various commercially available chemical naming software tools including MDL's ISIS Draw Autonom Software tool, and were not verified. Preferably, in the event of inconsistency, the depicted structure governs.

[00433] REFERENCES

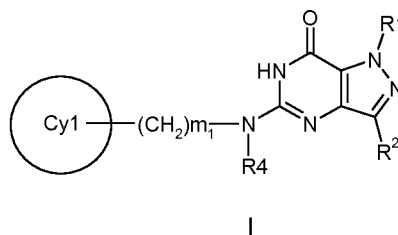
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WHAT IS CLAIMED IS:

1. A compound according to formula I:



wherein:

Cy1 is substituted or unsubstituted cycloalkyl or substituted or unsubstituted heterocycloalkyl;

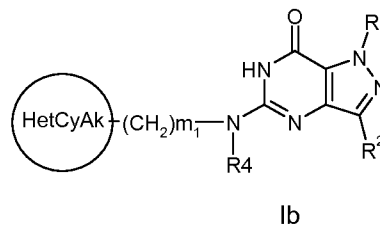
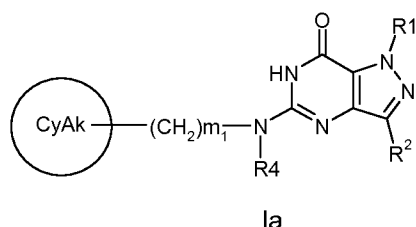
R¹ is H, substituted or unsubstituted alkyl;

R² is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

R⁴ is H, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkylalkyl; and m₁ is 0, 1, 2, 3, or 4; provided that when Cy1 is heterocycloalkyl and the heterocycloalkyl group is joined to -(CH₂)m₁- via a N atom of heterocycloalkyl group then m₁ is 2, 3 or 4;

or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof.

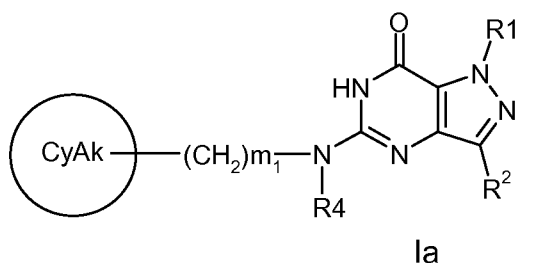
2. The compound according to claim 1 of formulae Ia or Ib:



wherein R¹, R², R⁴ and m₁ are as in claim 1;

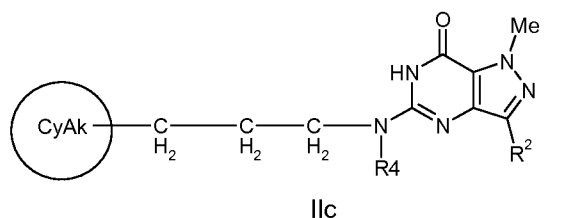
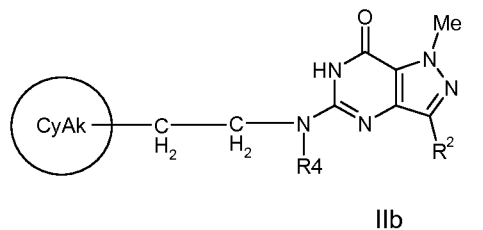
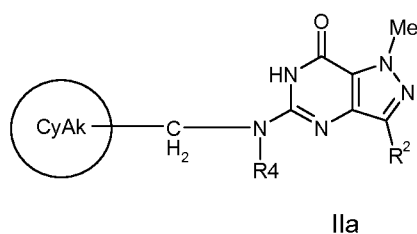
CyAk is substituted or unsubstituted cycloalkyl; and HetCyAk is substituted or unsubstituted heterocycloalkyl.

3. The compound according to claim 2 wherein R¹ is H or alkyl.
4. The compound according to claim 2 wherein R¹ is methyl, ethyl or i-Pr.
5. The compound according to claim 2 wherein m₁ is 0, 1, 2, 3 or 4.
6. The compound according to claim 2 wherein m₁ is 1, 2 or 3.
7. The compound according to claim 1 wherein the compound is according to formula Ia:



wherein R^1 , R^2 , R^3 , R^4 and m_1 are as in claim 1 and CyAk is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

8. The compound according to claim 1 of formulae IIa, IIb, or IIc:

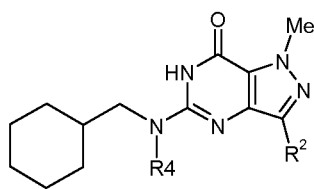


wherein:

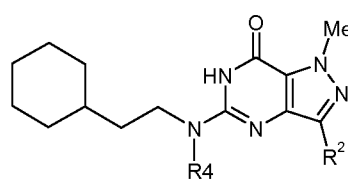
CyAk is substituted or unsubstituted cycloalkyl;

R^2 and R^4 are as in claim 1.

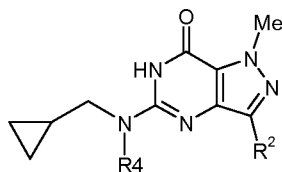
9. The compound according to claim 8 wherein CyAk is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.
10. The compound according to claim 8 wherein CyAk is selected from cyclopropyl and cyclohexyl.
11. The compound according to claim 1 wherein the compound is according to formulae IIIa, IIIb, IIIc or IIId:



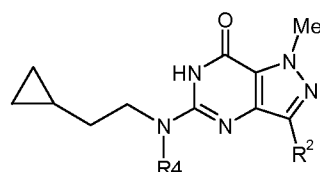
IIIa



IIIb



IIIc

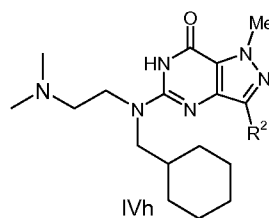
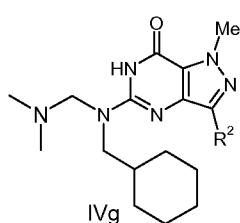
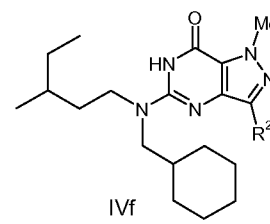
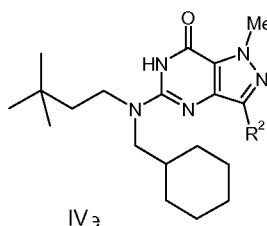
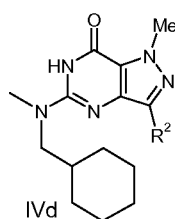
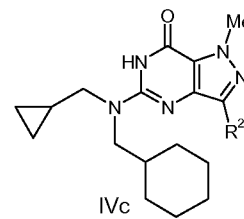
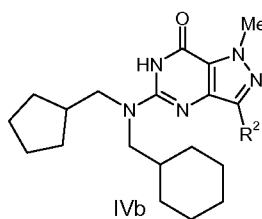
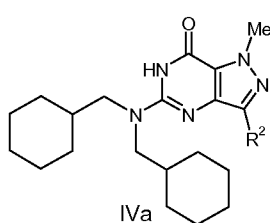


IIId

wherein:

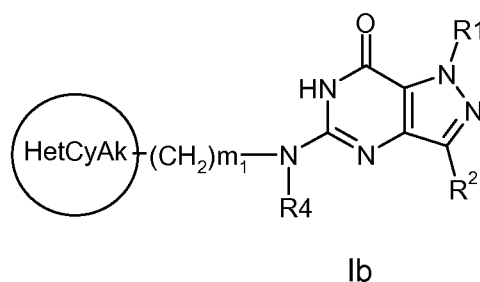
R^2 and R^4 are as in claim 1.

12. The compound according to any one of claims 7-11 wherein R^4 is alkyl.
13. The compound according to any one of claims 7-11 wherein R^4 is Me, Et, n-Pr, i-Pr, n-Bu, t-Bu, n-pentyl, $\text{CH}_2\text{CH}(\text{Me})\text{Me}$, $\text{CH}_2\text{CH}_2\text{-t-Bu}$, $\text{CH}_2\text{CH}(\text{Me})\text{CH}_2\text{Me}$, $\text{CH}_2\text{CH}(\text{Et})\text{CH}_2\text{Me}$, or $\text{CH}_2\text{CH}(\text{Me})\text{CH}_2\text{CH}_2\text{Me}$.
14. The compound according to any one of claims 7-11 wherein R^4 is alkyl substituted with halo, alkoxy, dialkylamino, aryl, cycloalkyl or heterocycloalkyl.
15. The compound according to any one of claims 7-11 wherein R^4 is alkyl substituted with halo or alkoxy.
16. The compound according to any one of claims 7-11 wherein R^4 is alkyl substituted with Cl, F, OMe, NMe_2 , or O-i-Pr.
17. The compound according to any one of claims 7-11 wherein R^4 is 4,4,4-trifluoro-n-butyl.
18. The compound according to any one of claims 7-11 wherein R^4 is 3-methoxy-n-propyl, 3-isopropoxy-n-propyl, or 3-(N,N-dimethylamino)-n-propyl.
19. The compound according to any one of claims 7-11 wherein R^4 is alkyl substituted with cycloalkyl.
20. The compound according to any one of claims 7-11 wherein R^4 is alkyl substituted with cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl.
21. The compound according to any one of claims 7-11 wherein R^4 is cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.
22. The compound according to claim 1 wherein the compound is according to formulae IVa, IVb, IVc, IVd, IVe, IVf, IVg, or IVh:



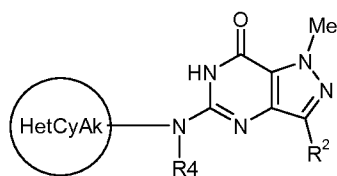
wherein R^2 is as in claim 1.

23. The compound according to claim 1 wherein the compound is according to formula Ib:

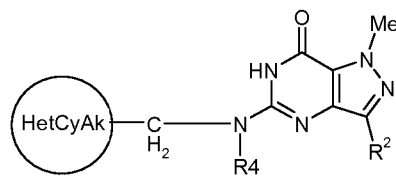


wherein R^1 , R^2 , R^4 and m_1 are as in claim 1 and HetCyAk is selected from substituted or unsubstituted piperidiny, piperaziny, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and tetrahydro-1,4-dioxanyl.

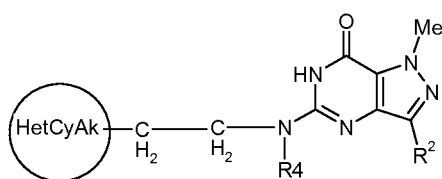
24. The compound according to claim 23 wherein R^1 is Me, Et or i-Pr.
 25. The compound according to claim 23 wherein m_1 is 0, 1, 2 or 3.
 26. The compound according to claim 3 wherein the compound is according to formulae Va, Vb, Vc or Vd:



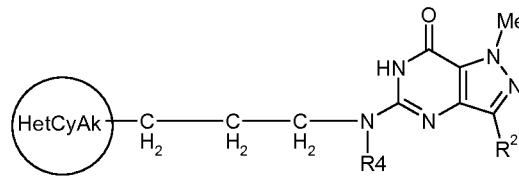
Va



Vb



Vc



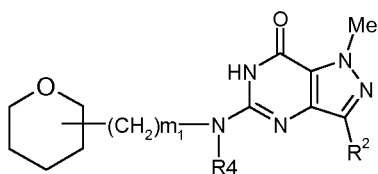
Vd

wherein:

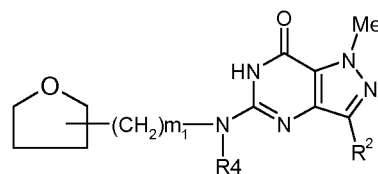
HetCyAk is selected from substituted or unsubstituted piperidiny, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and tetrahydro-1,4-dioxanyl; and

R² and R⁴ are as in claim 1.

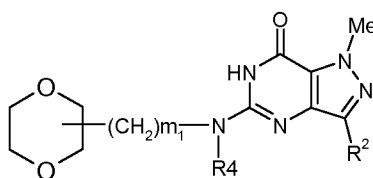
27. The compound according to claim 1 wherein the compound is according to formulae VIa, VIb, or VIc:



VIa



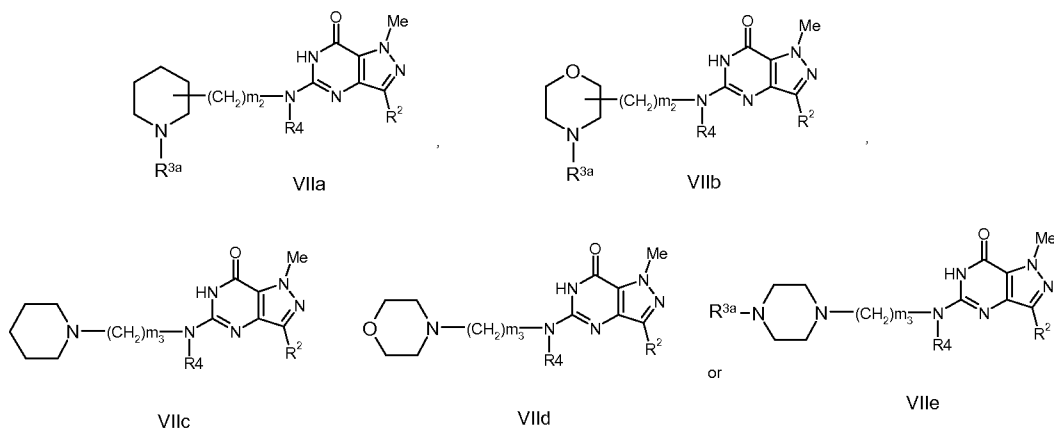
VIb



VIc

wherein m₁, R² and R⁴ are as in claim 1.

28. The compound according to claim 1 wherein the compound is according to formulae VIIa, VIIb, VIIc, VIId, or VIIe:



wherein:

R^2 and R^4 are as in claim 1;

m_2 is 0, 1, 2, 3 or 4; m_3 is 2, 3 or 4; and

R^{3a} is

H, substituted or unsubstituted alkyl, substituted or unsubstituted phenyl, or substituted or unsubstituted benzyl;

or

CO-alkyl, CO-cycloalkyl, CO-heterocycloalkyl, CO-aryl, CO-heteroaryl, CO-NH-alkyl, CO-NH-aryl, or CO-NH-heteroaryl;

or

CO-CH₂-cycloalkyl, CO-CH₂-heterocycloalkyl, CO-CH₂-aryl, or CO-CH₂-heteroaryl;

or

SO₂-alkyl, SO₂-cycloalkyl, SO₂-heterocycloalkyl, SO₂-aryl, or SO₂-heteroaryl;

or

SO₂-NH-alkyl, SO₂-NH-aryl, or SO₂-NH-heteroaryl;

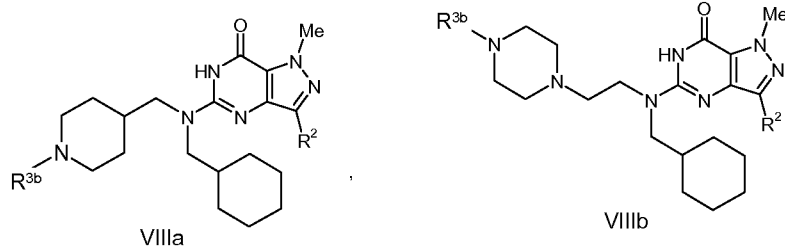
or

SO₂-CH₂-cycloalkyl, SO₂-CH₂-heterocycloalkyl, SO₂-CH₂-aryl, or SO₂-CH₂-heteroaryl.

29. The compound according to claim 28 wherein R^{3a} is alkyl.
30. The compound according to claim 28 wherein R^{3a} is Me, Et, i-Pr, or n-Pr.
31. The compound according to claim 28 wherein R^{3a} is substituted or unsubstituted phenyl.
32. The compound according to claim 28 wherein R^{3a} is cycloalkyl.
33. The compound according to claim 28 wherein R^{3a} is cyclopropyl, cyclohexyl, or cyclopentyl.
34. The compound according to claim 28 wherein R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is alkyl or substituted alkyl.
35. The compound according to claim 28 wherein R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is Me, Et, i-Pr or n-Pr.
36. The compound according to claim 28 wherein R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is cycloalkyl or heterocycloalkyl.
37. The compound according to claim 28 wherein R^{3a} is COR^{3b} or COCH₂R^{3b}; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.

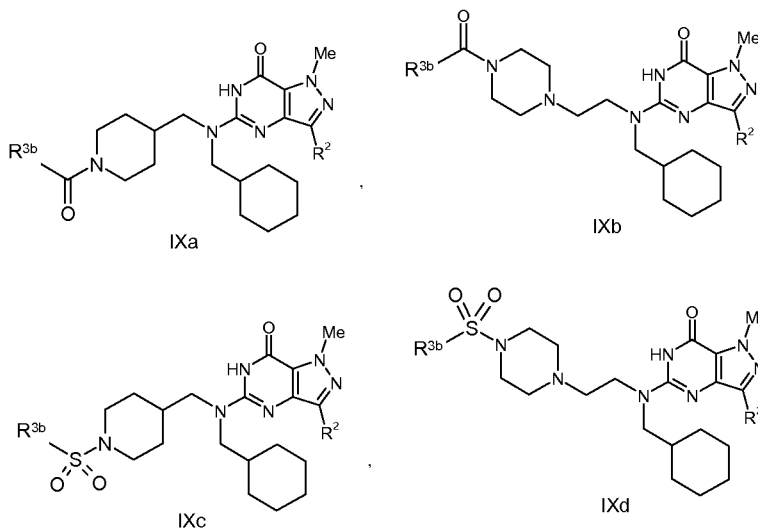
38. The compound according to claim 28 wherein R^{3a} is COR^{3b} or $COCH_2R^{3b}$; and R^{3b} is aryl or heteroaryl.
39. The compound according to claim 28 wherein R^{3a} is COR^{3b} or $COCH_2R^{3b}$; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
40. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is alkyl or substituted alkyl.
41. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is Me, Et, i-Pr or n-Pr.
42. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is cycloalkyl or heterocycloalkyl.
43. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.
44. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is aryl or heteroaryl.
45. The compound according to claim 28 wherein R^{3a} is $CONHR^{3b}$; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
46. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is alkyl or substituted alkyl.
47. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is Me, Et, i-Pr or n-Pr.
48. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is cycloalkyl or heterocycloalkyl.
49. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.
50. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is aryl or heteroaryl.
51. The compound according to claim 28 wherein R^{3a} is SO_2R^{3b} or $SO_2CH_2R^{3b}$; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
52. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is alkyl or substituted alkyl.
53. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is Me, Et, i-Pr or n-Pr.
54. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is cycloalkyl or heterocycloalkyl.
55. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is cyclopropyl, cyclohexyl, morpholinyl or piperidinyl.
56. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is aryl or heteroaryl.
57. The compound according to claim 28 wherein R^{3a} is SO_2NHR^{3b} ; and R^{3b} is phenyl, pyridyl, pyrazolyl, pyrrolyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, or thiazolyl.
58. The compound according to claim 28 wherein R^{3a} is CH_2COR^{3b} ; and R^{3b} alkyl, hydroxyl, alkoxy, or amino.

59. The compound according to any one of claims 23-58 wherein R^4 is Me, Et, n-Pr, i-Pr, n-Bu, t-Bu, n-pentyl, $\text{CH}_2\text{CH}(\text{Me})\text{Me}$, $\text{CH}_2\text{CH}_2\text{-t-Bu}$, $\text{CH}_2\text{CH}(\text{Me})\text{CH}_2\text{Me}$, $\text{CH}_2\text{CH}(\text{Et})\text{CH}_2\text{Me}$, or $\text{CH}_2\text{CH}(\text{Me})\text{CH}_2\text{CH}_2\text{Me}$.
60. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with halo, alkoxy, aryl, cycloalkyl or heterocycloalkyl.
61. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with halo or alkoxy.
62. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with Cl, F, OMe, or O-i-Pr.
63. The compound according to any one of claims 23-58 wherein R^4 is 4,4,4-trifluoro-n-butyl.
64. The compound according to any one of claims 23-58 wherein R^4 is 3-methoxy-n-propyl, or 3-isopropoxy-n-propyl.
65. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with cycloalkyl.
66. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl.
67. The compound according to any one of claims 23-58 wherein R^4 is cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl or cyclohexylmethyl.
68. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with heterocycloalkyl.
69. The compound according to any one of claims 23-58 wherein R^4 is alkyl substituted with piperidinyl, tetrahydrofuranyl, tetrahydropyranyl, or morpholinyl.
70. The compound according to any one of claims 23-58 wherein R^4 is 2-(morpholin-1-yl)ethyl, 3-(morpholin-1-yl)propyl, 2-(tetrahydropyran-4-yl)ethyl, or (tetrahydrofuran-2-yl)methyl.
71. The compound according to claim 1 of formulae VIIIa, or VIIIb:



wherein R^2 is as in claim 1; and R^{3b} is alkyl, or substituted alkyl.

72. The compound according to claim 71 wherein R^{3b} is Me, Et, n-Pr, i-Pr, or t-Bu.
73. The compound according to claim 71 wherein R^{3b} is alkyl substituted with hydroxyl, CO-alkoxy, heterocycloalkyl, or amino.
74. The compound according to claim 71 wherein R^{3b} is $\text{CH}_2\text{CO}_2\text{Et}$, $\text{CH}_2\text{CH}_2\text{OH}$, $\text{CH}_2\text{CO}_2\text{H}$, $\text{CH}_2\text{CH}_2\text{NMe}_2$, $\text{CH}_2\text{CH}_2\text{-morpholin-1-yl}$.
75. The compound according to claim 1 wherein the compound is according to formulae IXa, IXb, IXc, or IXd:



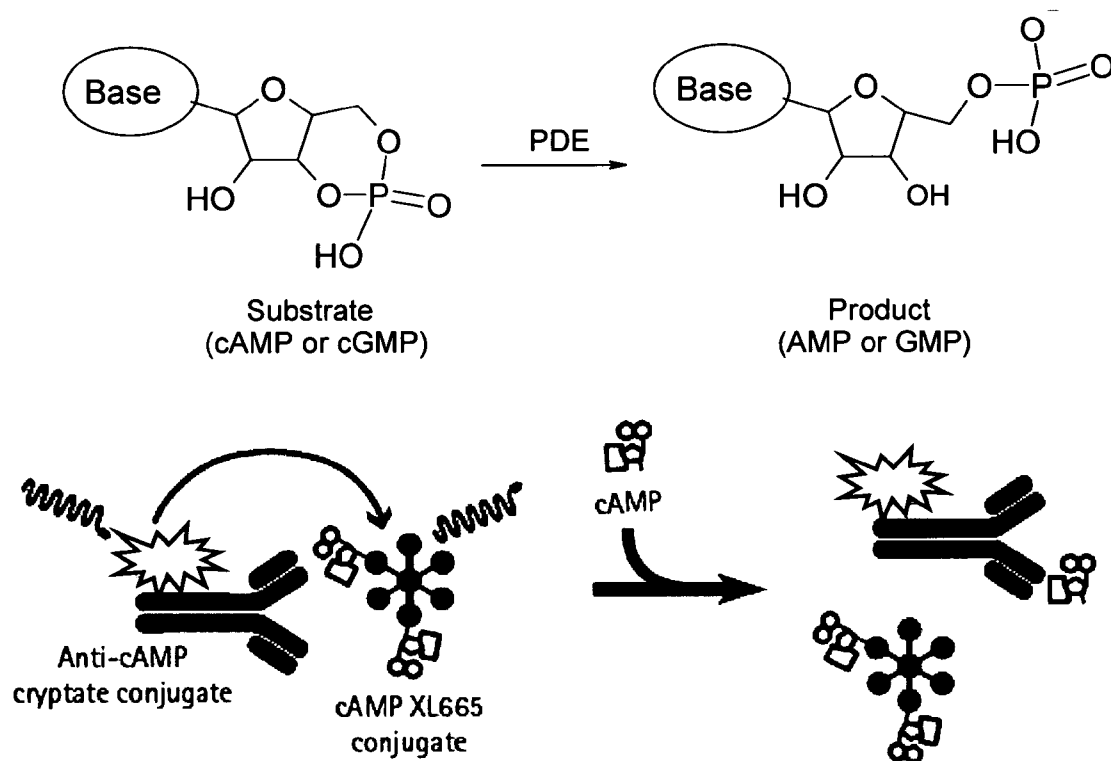
wherein R^2 is as in claim 1; and R^{3b} is alkyl, substituted alkyl, alkoxy, amino, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl.

76. The compound according to claim 75 wherein R^{3b} is Me, i-Pr, NHEt, NH-i-Pr, NHPH, Ph, cyclopropyl, cyclohexyl, morpholinyl, imidazolyl, piperidinyl, morpholinylmethyl, benzyl, imidazolylmethyl, or tetrazolylmethyl.
77. The compound according to any one of claims 2-76 wherein R^2 is Me, Et, n-Pr, t-Bu, cyclopropyl, cyclohexyl, or cyclopentyl.
78. The compound according to any one of claims 2-76 wherein R^2 is t-Bu.
79. The compound according to any one of claims 2-76 wherein R^2 is unsubstituted or substituted phenyl.
80. The compound according to any one of claims 2-76 wherein R^2 is unsubstituted or substituted pyridyl.
81. The compound according to claim 1 wherein the compound is selected from compounds exemplified in Table 1;
or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof or isotopic variants thereof, stereoisomers or tautomers thereof.
82. A pharmaceutical composition comprising an effective therapeutically effective amount of a compound according to any one of claims 1 to 81, in admixture with a pharmaceutically acceptable carrier.
83. The pharmaceutical composition of claim 82, wherein the carrier is a parenteral carrier.
84. The pharmaceutical composition of claim 82, wherein the carrier is an oral carrier.
85. The pharmaceutical composition of claim 82, wherein the carrier is a topical carrier.
86. Use of a compound according to any one of claims 1 to 81, in the manufacture of a medicament for treatment or prophylaxis of a condition characterized by cartilage or joint degradation.
87. Use of a compound according to any one of claims 1-81, in the manufacture of a medicament for treatment or prophylaxis of a condition selected from diseases involving inflammation.
88. Use according to claim 87, wherein said disease is osteoarthritis.

89. Use of a compound according to any one of claims 1 to 81 in the manufacture of a medicament for treatment or prophylaxis of a condition prevented, ameliorated or eliminated by administration of an inhibitor of phosphodiesterase 1A.
90. A method of treatment of a condition characterized by abnormal PDE1A activity, which comprises administering a therapeutically effective amount of a PDE1A inhibiting compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.
91. A method of treatment or prophylaxis of a disease involving degradation of cartilage, which comprises administering a therapeutically effective a compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.
92. A method of treatment or prevention of osteoarthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of a compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.
93. A composition useful for treating a condition characterized by abnormal PDE1A activity, comprising an effective amount of a compound of any of claims 1-81.
94. A method of treatment or prevention of diseases associated with bone and/or cartilage degradation, which comprises administering to a subject in need thereof, a therapeutically effective amount of a compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.
95. A method of treatment or prevention of rheumatoid arthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of a compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.
96. A method of treatment or prevention of osteoarthritis, which comprises administering to a subject in need thereof, a therapeutically effective amount of a compound according to any one of claims 1 to 81, or a pharmaceutical composition according to claims 82-85.

1/1

Figure 1



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/067048

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/04 A61K31/519 A61P19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 294 612 A (BACON EDWARD R [US] ET AL) 15 March 1994 (1994-03-15) See compounds on tables B-D, columns 39-54.	1-96
P,X	WO 2008/071650 A (GALAPAGOS N V [BE]; FLETCHER STEPHEN ROBERT [BE]; MENET CHRISTEL JEANN) 19 June 2008 (2008-06-19) See the examples; in particular, examples 8 and 37, pages 51-52.	1-96

☐ 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 document 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.

Z document member of the same patent family

Date of the actual completion of the international search

9 February 2009

Date of mailing of the international search report

24/02/2009

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/067048

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5294612	A	15-03-1994	US 5541187 A	30-07-1996
WO 2008071650	A	19-06-2008	US 2008242661 A1	02-10-2008