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(71) Applicants *(for all designated States except US)*: **BAR-ILAN UNIVERSITY** [IL/IL]; Ramat-Gan, 52900 (IL). **UNIVERSITE LAVAL** [CA/CA]; Quebec City, Qu'bec G1V 0A6 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **FISCHER, Bilha** [IL/IL]; 75 Aadulam Street, 73142 Shoham (IL). **SEVIGNY, Jean** [CA/CA]; 2956 rue de Gentilly, Quebec City, Qu'bec G1W 1C4 (CA). **ELIAHU, Shay** [IL/IL]; 34 Ha'arazim Avenue, 52960 Ramat-Efal (IL). **LECKA, Joanna** [CA/CA]; 3328-B, Place de la Monnerie, Ste-Foy, Quebec City, Qu'bec G1X 1Y8 (CA).

(74) Agent: **BEN-AMI & ASSOCIATES**; P.O. Box 94, 76100 Rehovot (IL).

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(54) Title: BORANOPHOSPHATE DERIVATIVES FOR THE TREATMENT OF OSTEOARTHRITIS

(57) Abstract: The present invention provides pharmaceutical compositions and methods for treatment and management of osteoarthritis using certain dinucleotide boranophosphate derivatives or nucleoside boranophosphate derivatives. The invention further provides particular diadenosine penta(Υ-borano)phosphate derivative such as diadenosine 5',5"-P^1,P^5,α,β-methylene-δ,ε-methylene-pentaphosphate-Υ-borano and di-2'-deoxyadenosine 5',5"-P^1,P^5,α,β-methylene-δ,ε-methylene pentaphosphate-Υ-borano, and pharmaceutical compositions thereof.

BORANOPHOSPHATE DERIVATIVES FOR THE TREATMENT OF OSTEOARTHRITIS

TECHNICAL FIELD

[0001] The present invention relates to pharmaceutical compositions and methods for treatment and management of osteoarthritis.

BACKGROUND ART

[0002] Nucleoside triphosphate diphosphohydrolase-1, -2, -3 and -8 (NTPDase1, -2, -3 and -8; EC 3.6.1.5) and nucleotide pyrophosphatase phosphodiesterase-1 and 3 (NPP1 and NPP3; EC 3.1.3.1, EC 3.6.1.9) are the dominant ectonucleotidases that terminate nucleotide signaling through the hydrolysis of nucleotide agonists of the P2X and P2Y receptors (Kukulski *et al.*, 2005; Nahum *et al.*, 2006; Shirley *et al.*, 2009).

[0003] NTPDase1, -2, -3 and -8 are plasma membrane-bound with an extracellular active site, which catalyze the hydrolysis of the terminal phosphate of nucleoside triphosphates, e.g., ATP and UTP, and diphosphates, e.g., ADP and UDP, at different rates. NTPDase1 (CD39/ATPDase/ectoapyrase/ecto-ADPase) hydrolyzes ATP and ADP equally well (Sévigny *et al.*, 1997), while NTPDase2 (ecto-ATPase/CD39L1) is a preferential triphosphonucleosidase (Heine *et al.*, 1999). Both NTPDase3 (CD39L3/HB6) and NTPDase8 are functional intermediates between NTPDase1 and NTPDase2 (Kukulski *et al.*, 2005). NTPDase4-7, are mainly associated with intracellular organelles and are therefore not expected to significantly affect P2 receptor activation. The product of NTPDase activity, AMP, is further hydrolyzed by ecto-5'-nucleotidase (CD73) giving adenosine, which is the natural ligand of P1 receptors (Colgan *et al.*, 2006; Resta *et al.*, 1993).

[0004] The NPP family members are conserved eukaryotic enzymes which, as for NTPDases, exist as membrane glycoproteins with an extracellular active site. Three members of this family, in particular, NPP1-3, are capable of hydrolyzing phosphodiester and pyrophosphate bonds found in a variety of endogenous nucleotides and their derivatives, e.g., nucleotide triphosphates (NTPs), nucleotide diphosphates (NDPs), dinucleotides, oligonucleotides, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) and uracil diphosphate (UDP) sugars (Bollen *et al.*, 2000), and thus control purinergic signaling. NPP1 can hydrolyze both phosphodiester, e.g., cAMP, and pyrophosphate (PPi), e.g., ATP, bonds (Belli *et al.*, 1993), and in the latter case hydrolysis

could be performed between phosphate-α and phosphate-β (Stefan *et al.*, 2005). NPP1 and NPP3 are closely related, with ~50% identity, and share 39% and 41% identity, respectively, with NPP2 (Deissler *et al.*, 1995). NPP2 has a much lower capacity to hydrolyze nucleotides than NPP1 and NPP3, and therefore may not play an important role in the regulation of P2 receptor activation.

[0005] NPP1 is a membrane protein consisting of 925 amino acids organized into six main domains including an N-terminus cytoplasmic tail, a transmembrane domain, an extracellular region, a phosphodiesterase domain and a nuclease domain (Stefan *et al.*, 2005). The catalytic site of NPP1 is located in the extracellular phosphodiesterase domain. Gijsbers *et al.* (2001) proposed a structural model and a catalytic reaction mechanism for mouse NPPs based on secondary structure similarities to known crystallographic structures of alkaline phosphatase, independent phosphoglycerate mutase and arylsulfatase. According to this mechanism, when a substrate, e.g., ATP, approaches the active site, one of its negatively charged oxygens can partially coordinate both binding site di-valent metal ions, thereby bringing the phosphate group into close proximity with the nucleophilic oxygen of Thr238, which can then hydrolyze the ATP molecule to generate a protein-nucleoside mono-phosphate adduct and release PPi. At the second step of the catalysis, AMP can be easily released from the protein-AMP adduct through hydrolysis occurring by an active site water molecule. In a later study, Zalatan *et al.* (2006) determined the structure of the bacterial NPP Xanthomonas axonopodis pv. citri (Xac) in the apo form (PDB code 2GSN), in complex with vanadate (PDB code 2GSO) and in complex with AMP (PDB codes 2GSU and 2RH6). These structures contributed to the understanding of the NPP active site and its catalytic reaction.

[0006] NPP1 is expressed in different tissues, especially in bone (osteoblasts) and cartilage (chondrocytes), and has a role in regulating skeletal remodeling and calcification. Studies have shown that deficiency in bone NPP1 in mice leads to hyper calcification (Okawa *et al.*, 1998), whereas over expression is linked with reduced bone calcification (Johnson *et al.*, 1999). NPP1 affects skeletal remodeling and calcification by regulating processes such as bone mineralization and soft tissue calcification. The primary role of NPP1 is to regulate extracellular PPi levels thereby contributing to the balance between the extracellular levels of phosphate (Pi) and PPi that is a key factor in mineralization process (Stefan *et al.*, 2005). Examples of other proteins that contribute to the mineralization process through the regulation of Pi and PPi levels include the tissue-nonspecific alkaline

phosphatase (TNAP) that hydrolyzes PPi into Pi, the progressive ankylosis protein (ANK) that is responsible for the intracellular-to-extracellular channeling of PPi, and possibly also ATPases (specific for ATP) and pyrophosphatases (specific for PPi) (Terkeltaub *et al.*, 2006).

[0007] Under normal physiological conditions, extracellular PPi levels are balanced leading to a normal mineralization process whereby crystals of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ are generated in their expected locations, such as bone and cartilage, through the matrix vesicles (Stefan *et al.*, 2005). However, under pathological conditions, deficiencies in NPP1 and ANK lead to low levels of extracellular PPi and consequently to ectopic calcification. In contrast, over expression of NPP1 elevates extracellular PPi levels and thus leads to the deposition of calcium pyrophosphate dihydrate crystals $Ca_2(P_2O_7)\cdot 2H_2O$, a condition known as the calcium pyrophosphate dehydrate (CPPD) disease. Crystal deposition often occurs in the articular cartilage and is therefore termed chondrocalcinosis. This phenomenon often occurs in aging chondrocytes and accompanies age-related osteoarthritis, a degenerative joint disease (Stefan *et al.*, 2005). These CPPD crystals can be detected in the synovial fluid causing stiffness and severe pain and eventually leading to cartilage damage (Bjelle and Sundstrom, 1975; Nalbant *et al.*, 2003). PPi not only initiates but also regulates mineralization by suppressing hydroxyapatite crystal deposition from amorphous calcium phosphate (Ali, 1992; Anderson, 1988).

[0008] There is currently a lack of specific NPP inhibitors, and therefore, the therapeutic potential of NPP inhibition for the treatment of health disorders such as chondrocalcinosis (Johnson and Terkeltaub, 2005) remains virtually unexplored. Indeed, NPP inhibitors have scarcely been reported. Thus, suramin was reported to reduce the hydrolysis of *p*-Nph-5'-TMP by NPP by ~36% at 250 μM (Ruecker *et al.*, 2007). It is noteworthy that suramin and its derivatives antagonize most P2 receptors and also efficiently inhibit NTPDases and cannot therefore be considered as specific NPP inhibitors (Munkonda *et al.*, 2007). Recently, [3-(*t*-butyldimethylsilyloxy)-phenyl]-1,3,3-oxadiazole-2 (3*H*)-thione was reported as an NPP1 inhibitor (K_i=100 μM) (Khan *et al.*, 2009). Likewise, biscoumarin derivatives were identified as pure non-competitive inhibitors of snake venom and human NPP1 enzymes, with K_i and IC_{50} values as low as 50 and 164 μM, respectively, for human NPP1 (Choudhary *et al.*, 2006).

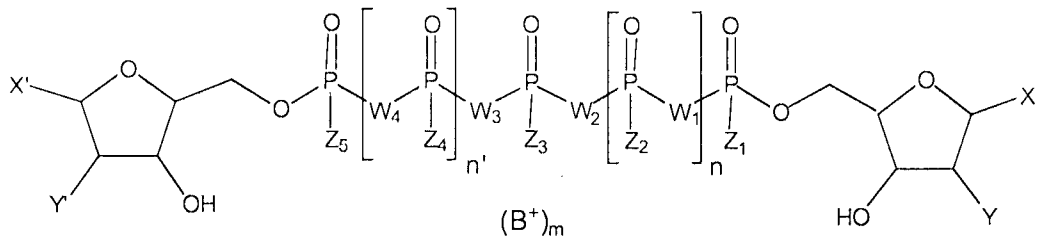
[0009] US 7,368,439 discloses diribo-, di-2'-deoxyribo, and ribo-2'-deoxyribo-nucleoside boranophosphate derivatives that can be useful for prevention or treatment of diseases or

disorders modulated by P2Y receptors such as type 2 diabetes, cystic fibrosis and cancer. WO 2009/066298 discloses non-hydrolyzable adenosine and uridine polyphosphate derivatives, said to be useful for prevention or treatment of diseases modulated by P2Y-receptors such as type 2 diabetes. Both publications, based on studies conducted in the laboratories of the present inventors, are herewith incorporated by reference in their entirety as if fully described herein.

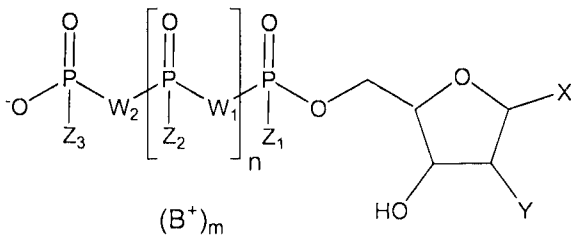
SUMMARY OF INVENTION

[0010] In one aspect, the present invention provides a pharmaceutical composition for treatment of osteoarthritis comprising a pharmaceutically acceptable carrier and either a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II:

I



II

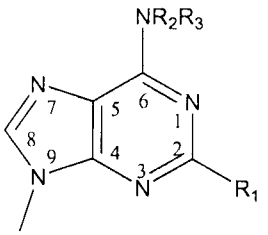


or a diastereomer or mixture of diastereoisomers thereof,

wherein

X and X' each independently is an adenine residue of the formula Ia, linked through the 9-position:

Ia



wherein

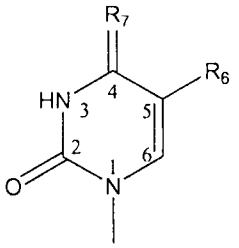
R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected

from halogen, -CN, -SCN, -NO_2, -OR_4, -SR_4, -NR_4R_5 or heteroaryl, wherein R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_2 and R_3 each independently is H or hydrocarbyl;

or X and X' each independently is an uracil residue of the formula Ib, linked through the 1-position:

Ib



wherein

R_6 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, -NR_8R_9, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected from halogen, -CN, -SCN, -NO_2, -OR_8, -SR_8, -NR_8R_9 or heteroaryl, wherein R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_7 is O or S;

Y and Y' each independently is H, -OH or -NH_2;

Z_1, Z_2, Z_3, Z_4 and Z_5 each independently is -O^-, -S^- or -BH_3^-, provided that at least one of Z_1 to Z_5 in the general formula I is -BH_3^-, and at least one of Z_1 to Z_3 in the general formula II is -BH_3^-;

W_1, W_2, W_3 and W_4 each independently is -O-, -NH- or -$C(R_{10}R_{11})$-, wherein R_{10} and R_{11} each independently is H or halogen, provided that at least one of W_1 to W_4 in the general formula I is not -O-, and at least one of W_1 to W_2 in the general formula II is not -O-;

n and n' each independently is 0 or 1;

m is 3, 4 or 5; and

B^+ represents a pharmaceutically acceptable cation.

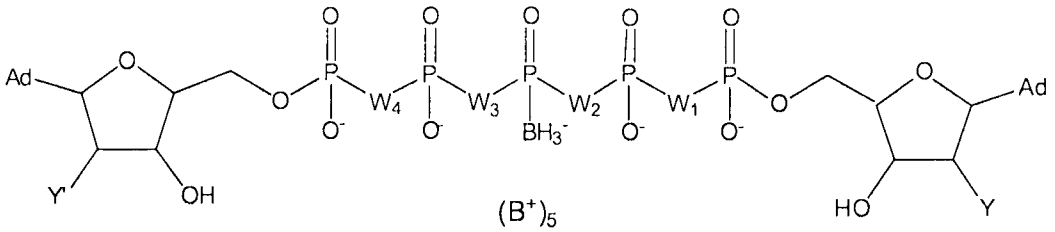
[0011] In another aspect, the present invention provides a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, for use in treatment of osteoarthritis.

[0012] In a further aspect, the present invention relates to use of a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, for the preparation of a pharmaceutical composition for treatment of osteoarthritis.

[0013] In still a further aspect, the present invention relates to a method for treatment of osteoarthritis in an individual in need thereof, comprising administering to said individual a therapeutically effective amount of a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof.

[0014] In yet another aspect, the present invention relates to a diadenosine boranophosphate derivative of the general formula III:

III

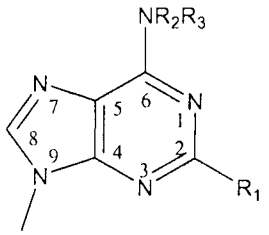


or a diastereomer or mixture of diastereoisomers thereof,

wherein

Ad is an adenine residue of the formula Ia, linked through the 9-position:

Ia



wherein

R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected from halogen, -CN, -SCN, $-NO_2$, $-OR_4$, $-SR_4$, $-NR_4R_5$ or heteroaryl, wherein R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to

which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_2 and R_3 each independently is H or hydrocarbyl;

Y and Y' each independently is H, -OH or $-NH_2$;

W_1, W_2, W_3 and W_4 each independently is -O-, -NH- or $-C(R_{10}R_{11})-$, wherein R_{10} and R_{11} each independently is H or halogen, provided that two of W_1 to W_4 are not -O-; and

B^+ represents a pharmaceutically acceptable cation.

[0015] In still another aspect, the present invention provides a pharmaceutical composition comprising a diadenosine boranophosphate derivative of the general formula III as defined above, or a diastereomer or mixture of diastereoisomers thereof, and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF DRAWINGS

[0016] **Fig. 1** shows proposed structures for nucleotide-BP_i Mg^{2+} complexes leading to products **1** and **2** (upper left side) and products **3** and **4** (upper right side), wherein Im represents imidazolyl; and Nuc represents 2'-deoxy-adenosyl.

[0017] **Fig. 2** shows the effects of analogues **1-4** on NTPDase and ecto-5'-nucleotidase activity. Either ATP (for NTPDases) or AMP (for ecto-5'-nucleotidase) was used as a substrate in the presence of compound **1** (panel A), **2** (panel B), **3** (panel C), or **4** (panel D). Both substrate and analogues **1-4** were used at 100 μM. The 100% activity was set with the nucleotide substrate alone: 1270±35, 928±55, 202±37, 129±11, and 357±10 nmol of P_i min^{-1} (mg $protein^{-1}$) for NTPDase1, -2, -3 and -8, and ecto-5'-nucleotidase, respectively. Data are presented as the mean ± SD of 3 experiments carried out in triplicate.

[0018] **Figs. 3A-3D** show that analogues **1-4** inhibit NPP activities. The activity of human NPP1 and NPP3 with either pnp-TMP (**3A**), Ap_5A (**3C**) or ATP (**3D**) as the substrate, as well as the activity of human NPP2 (membrane-bound form) with pnp-TMP (**3B**) as the substrate, is shown. Substrates and analogues **1-4** were studied at a concentration of 100 μM. In the control (ctrl), the substrate only was tested and was set to 100% of activity. The percentage of residual activity is presented at the top of each bar. Data are presented as the mean ± SD of 3-6 experiments carried out in triplicate.

[0019] **Fig. 4** shows that analogues **1-4** inhibit NPP activity at the surface of HTB-85 and HT29 cells. Substrate, pnp-TMP, and analogues **1-4** were used at the concentration of 100 μM. In the control, the substrate only was tested and was set to 100% activity. The percentage of residual activity is presented at the top of each bar. Data are presented as the mean ± SD of 3 experiments performed in triplicate.

[0020] **Figs. 5A-5B** show relative concentration-response plots for analogues **1-4** via the $P2Y_{11}$ receptor (**5A**) and the $P2Y_1$ receptor (**5B**). Data were obtained from 1321N1 cells stably expressing the $P2Y_{11}$GFP receptor (**5A**) or $P2Y_1$GFP receptor (**5B**), triggering the ligand-induced change in $[Ca^{2+}]_i$. Cells were pre-incubated with 2 μM fura-2 AM for 30 min, and the change in fluorescence ($\Delta F_{340}/F_{380}$) was monitored.

[0021] **Fig. 6** shows that analogues **22-24** are poor substrates of human NTPDases. Bars represent the mean of one experiment performed in triplicate. The relative activity was calculated using ATP hydrolysis as 100% (white bar), which was, in nmoles $Pi \cdot min^{-1} \cdot mg\ protein^{-1}$, 467 for NTPDase1; 512 for NTPDase2; 496 for NTPDase3; and 192 for NTPDase8.

[0022] **Fig. 7** shows hydrolysis of pnp-TMP and analogues **22-24** by human NPPs. Bars represent the mean of one experiment performed in triplicate. The relative activity was calculated using pnp-TMP hydrolysis as 100% (white bar), which was, in nmoles $pnp\text{-}TMP \cdot min^{-1} \cdot mg\ protein^{-1}$, 24 for NPP1; and 53 for NPP3.

[0023] **Fig. 8** shows the effect of analogues **22-24** on human NTPDase activity. Bars represent the mean of one experiment performed in triplicate. ATP (substrate) and analogues **22-24** were all used at the concentration of 100 μM. The ATPase activity of each NTPDase is indicated in **Fig. 6**.

[0024] **Fig. 9** shows that analogues **22-24** are potent inhibitors of human NPP1. Bars represent the mean of one experiment performed in triplicate. Pnp-TMP (substrate) and analogues **22-24** were all used at the concentration of 100 μM. The activity with pnp-TMP of both NPPs is indicated in **Fig. 7**.

DETAILED DESCRIPTION OF THE INVENTION

[0025] Nucleotide pyrophosphatase phosphodiesterase 1-3 (NPP1-3) have a nucleotide pyrophosphatase activity and metabolize nucleotide triphosphate (NTP) directly to nucleotide monophosphate (NMP) and pyrophosphate (PPi) (Stefan *et al.*, 2006). These enzymes can be discriminated from other ectonucleotidases, such as NTPDases, by their

ability to hydrolyze the diadenosine-5',5''-polyphosphate analogues, Ap_nA, to AMP and adenosine nucleoside 5'-(*n*-1) phosphate (Rotlian *et al.*, 2002). The regulation of the dinucleotide levels by NPPs may, in fact, be one of the dominant functions exerted by these enzymes (Stefan *et al.*, 2006).

[0026] The field of NPP enzymology is still in its infancy. Therefore, NPP specific inhibitors, which do not affect other ectonucleotidases such as NTPDases and 5'-ectonucleotidase and do not trigger nor interfere with P2 receptor activation, would be extremely valuable. Furthermore, potent and selective NPP inhibitors could be used as therapeutic agents for the treatment of osteoarthritis (Tenenbaum *et al.*, 1981) and chondrocalcinosis (Johnson and Terkeltaub, 2005).

[0027] Nucleotide scaffolds suffer from inherent limitations as therapeutic agents as they interact with numerous proteins (Nahum *et al.*, 2006) and are metabolically unstable (Sellers *et al.*, 2001). Therefore, in the study described herein, a dinucleoside polyphosphate scaffold, which offers better stability and selectivity than nucleotides (Nahum *et al.*, 2006), was selected for the development of NPP inhibitors, and four diadenosine polyphosphate derivatives herein identified by the Arabic numbers **1-4** in bold, more particularly, two diadenosine pentaphosphate derivatives identified as analogues **1** and **2**, and two diadenosine tetraphosphate derivatives identified as analogues **3** and **4**, were synthesized, taking into consideration the following points: (i) In order to prevent any activity of those derivatives toward the $P2Y_1$ receptor, the adenine ring was conserved without a methylthio substitution at the C-2 position, known to enhance potency toward the $P2Y_1$ receptor (Eliahu *et al.*, 2009); (ii) Since NPP1 hydrolyzes the P_α-P_β or P_δ-P_ϵ phosphodiester bond in Ap_nAs (Nahum *et al.*, 2006), the oxygen atoms bridging P_α-P_β and P_δ-P_ϵ in analogue **1**, and the oxygen atoms bridging P_α-P_β and P_γ-P_δ in analogue **3**, were substituted with methylene groups to obtain a hydrolysis-resistant scaffold; (iii) Since substitution of the non-bridging oxygen atom on the central phosphate of Ap_3A with a BH_3 group conferred resistance to hydrolysis by NPP1 and NPP3 (Nahum *et al.*, 2006), a central boranophosphate moiety was introduced in analogue **1**; and (iv) Since 2'-deoxyadenosine-5'-(α-thio)triphosphate was shown to be a potent inhibitor of NPPs (Wojcik *et al.*, 2007), the 2'-deoxy analogues of both analogues **1** and **3**, herein identified analogues **2** and **4**, respectively, were synthesized as well.

[0028] The full chemical structures of analogues **1-4** are depicted in Appendix A and in **Schemes 1-2** hereinafter. Analogue **1** is also identified by the name diadenosine 5',5''-

P^1,P^5,α,β-methylene-δ,ϵ-methylene-pentaphosphate-γ-borano; analogue **2** is also identified by the name di-2'-deoxyadenosine 5',5"-P^1,P^5,α,β-methylene-δ,ϵ-methylene penta-phosphate-γ-borano; analogue **3** is also identified by the name diadenosine 5',5"-P^1,P^5,α,β-methylene-γ,δ-methylene-tetraphosphate; and analogue **4** is also identified by the name di-2'-deoxyadenosine 5',5"-P^1,P^5,α,β-methylene-γ,δ-methylene-tetraphosphate.

[0029] Analogues **1-4** were evaluated for their protein selectivity as either agonists of $P2Y_{1,2,11}$ receptors or substrates for the major ectonucleotidases; and their inhibitory activity and NPP subtype selectivity were evaluated by comparison of their effects on the other main ectonucleotidases, in the presence of pnp-TMP, Ap_5A or ATP as substrates. In addition, these analogues were evaluated as inhibitors of cell surface NPP activity in two cancer cell lines. As described hereinafter, based on the various experiments conducted, a most selective NPP inhibitor was identified, and important structure-activity relationships for such inhibitors was established.

[0030] As shown in the Examples section hereinafter, analogues **1-4** strongly inhibited the metabolism of both synthetic (pnp-TMP) and natural substrates (Ap_5A and ATP) by NPP1. Additionally, analogues **1** and **4** inhibited the hydrolysis of pnp-TMP, Ap_5A, and ATP by NPP3, by 30, 50, and >90%, respectively. The kinetic parameters of the inhibition with pnp-TMP as substrate indicated that these analogues are NPP1 inhibitors. Interestingly, analogues **1-4** were not hydrolyzed by NTPDases and did not affect hydrolysis of ATP by NTPDase1 and -8. Likewise, NTPDase2 and -3 activities were reduced by $\leq$30% by these analogues. In addition, analogues **1** and **2** exhibited no inhibitory effect toward ecto-5'-nucleotidase.

[0031] In view of these findings it seems that dinucleotides having either a penta- or tetraphosphate linker, such as analogues **1** and **2**, and **3** and **4**, respectively, do not, or barely, affect NTPDase activity, and indeed, such dinucleotides are recognized neither as substrates nor as inhibitors by these enzymes. Furthermore, the fact that analogues **1** and **2** were not recognized by ecto-5'-nucleotidase may indicate that an Ap_5A scaffold is especially suitable for designing NPP-selective inhibitors.

[0032] Analogues **1** and **2** having a pentaphosphate linker inhibited Ap_5A hydrolysis by NPP1 better than analogues **3** and **4** bearing a tetraphosphate chain. Yet, analogue **1** inhibited the hydrolysis of ATP by NPP1 better than analogue **2**, implying that **1** competes with ATP because it has a 2'-OH group, i.e., recognition of ATP by NPP1 probably involves the 2'-OH group. This requirement is not important for a NPP1 inhibitor directed

against Ap_5A hydrolysis, possibly since recognition of Ap_5A does not involve a 2'-OH group.

[0033] NPP3-mediated hydrolysis of ATP was sensitive to inhibition by the dinucleotide analogues **1-4**. Apparently, the patterns of recognition of Ap_5A and ATP by NPP3 are different than those for NPP1, and therefore, NPP3 was not affected by analogues **1-4** as much as NPP1.

[0034] Finally, NPP2 nucleotidase activity for both the membrane-bound forms and the secreted forms (data not shown) was highly affected by analogues **1-4**. It is noteworthy that in addition to its nucleotidase activity, NPP2 prefers lysophospholipids as substrates. Since the hydrolysis of lysophospholipids and nucleotides is performed by the same catalytic site (Gijsbers *et al.*, 2003; Koh *et al.*, 2003), it may be speculated that analogues **1-4** might also inhibit the hydrolysis of lysophospholipids by NPP2, and potentially also by NPP4-7.

[0035] As for α,β-methylene-ADP, a known ecto-5'-nucleotidase inhibitor (Bar and Simonson, 1975), the methylene groups between α,β and γ,δ phosphates conferred strong inhibitory activity to analogues **3** and **4** toward ecto-5'-nucleotidase. In contrast, compounds **1** and **2** had no effect on ecto-5'-nucleotidase activity, further emphasizing the specificity of the latter analogues as NPP inhibitors.

[0036] Adenine nucleotide analogues with a methylene group substituting for a bridging oxygen atom and with the replacement of a nonbridging oxygen in P_α by a BH_3 group, were shown to be weak agonists of $P2Y_{1,4,6}$ receptors (Eliahu *et al.*, 2009). Likewise, replacing the P_α-P_β bridging-oxygen in the potent $P2Y_1$ receptor agonist 2-MeS-ADP with a dihalomethylene group such as CCl_2 and CF_2 resulted in reductions in potency of 390- and 1200-fold (Eliahu *et al.*, 2010). In view of these findings, in the present study, both P_α-P_β and P_δ-P_ϵ bridging oxygen atoms were replaced with methylene groups to prevent activity of the dinucleotide analogues toward the P2Y receptors.

[0037] As previously shown, P^3-borano P^1,P^5-5'-diadenosine-pentaphosphate is a highly potent $P2Y_1$ receptor agonist with EC_{50}=63 nM *vs.* 100 nM for 2-MeS-ADP (Nahum *et al.*, 2006). Upon replacing both P_α-P_β and P_δ-P_ϵ bridging oxygen atoms in the aforesaid compound with methylene groups, yielding analogue **1**, a decreased activity toward the $P2Y_1$ receptor was observed. The boranophosphate modification in analogue **1**, which in case of Ap_nA resulted in significant activity via the $P2Y_1$ receptor, increased the potency of analogue **1** toward the $P2Y_1$ receptor as compared to analogue **3**, possibly indicating an improved binding to $P2Y_1$ receptor due to the presence of the relatively lipophilic borane

moiety (Shaw *et al.*, 2000). Analogue **3** was ~60-fold less potent than ATP, while Ap_4A itself had a potency similar to that of ATP (Shaver *et al.*, 2005), indicating that replacing the bridging oxygen atom with a methylene group reduces $P2Y_1$ agonist potency. Analogue **2** was >200-fold less potent than ATP, indicating the importance of the 2'-hydroxyl group for molecular recognition by the $P2Y_1$ receptor.

[0038] Among natural diadenosine polyphosphates, only Ap_4A may be considered as an agonist of $P2Y_{11}$, which is normally activated by ATP derivatives (Communi *et al.*, 2001; Patel *et al.*, 2001). As found in the present study, Ap_4A derivatives **3** and **4** were poor $P2Y_{11}$ receptor agonists or completely inactive, probably due to the replacement of the bridging oxygen atoms in the polyphosphate chain with methylene groups, as observed for the $P2Y_1$ receptor. The most potent $P2Y_{11}$ agonist among analogues **1-4** was analogue **1**. Although a boranophosphate modification increased the potency of analogue **1** as a $P2Y_{11}$ agonist as compared to the other analogues tested, it had a lower potency than ATP, with EC_{50}=13 μM *vs.* 3.3 μM for ATP. The 2'-deoxy-related Ap_5A scaffold, **2**, was inactive up to 50 μM, indicating the essential role of the 2'-hydroxyl group for activity at the $P2Y_{11}$ receptor, as noted above for the $P2Y_1$ receptor.

[0039] In a further set of experiments, various ATP analogues described in WO 2009/066298, herein identified by the Arabic numbers **21-24** in bold, more particularly, two ATP analogues having methylthio substitution at the C-2 position of the adenine, in which either the non-bridging oxygen atom at Pα is replaced by a borano group or the Pβ-Pγ bridging oxygen atom is replaced by methylene group (analogues **21** and **22**, respectively), and two ATP analogues without or with methylthio substitution at the C-2 position of the adenine, having both borano group at the Pα and methylene group at position Pβ-Pγ (analogues **23** and **24**, respectively) were synthesized and characterized as potential NPP inhibitors. The synthesis of these analogues was carried out following the exact procedure described in WO 2009/066298. Analogues **23** and **24** were obtained as diastereomeric pairs that were separated on an HPLC column as described in the Examples.

[0040] The full chemical structures of analogues **21-24** are depicted in Appendix A. Analogue **21** is also identified by the name 2-MeS-adenosine-5'-O-(α-borano-triphosphate); analogue **22** is also identified by the name β,γ-CH_2-2-MeS-adenosine-5'-triphosphate; analogue **23** is also identified by the name adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate); and analogue **24** is also identified by the name 2-MeS-adenosine-

β,γ-CH_2-5'-O-(α-borano-triphosphate). The synthesis of those analogues was carried out following the exact procedure described in WO 2009/066298, and their chemical stability, enzymatic stability to alkaline phosphatase, and stability at human blood serum were evaluated as further described in the aforesaid publication.

[0041] As shown in the Examples hereinafter, while analogues **22** and **24** (B isomer) proved potent $P2Y_1$ receptor agonists, probably due to improved interactions of 2-MeS-adenine moiety (*vs.* adenine) with the $P2Y_1$ receptor binding-pocket (Mohamady and Jakeman, 2005), analogues **23** (A and B isomers) and **24** (A isomer) were practically inactive at this receptor. As further shown, analogues **22-24** were hardly degraded by all known sub-types of NTPDase, and are not inhibitors of NTPDase. This is a significantly beneficial feature of these analogues, as the local concentration of P2YR agonists in the vicinity of P2YRs is not reduced by binding to neighboring proteins of the NTPDase family. Previously we found that A- and B-isomers of analogue **21** were hydrolyzed by NTPDase1 at 14% and 59% the rate of ATP, respectively (Padyukova *et al.*, 1999). Here, the addition of a β,γ-bridging methylene to the scaffold of **21** significantly improved the resistance to enzymatic hydrolysis with negligible hydrolysis (0.9% of ATP) for both **24** (A and B isomers). Most importantly, as shown in this study, analogues **22-24**, more specifically analogue **23**, were specific inhibitors of NPP1 and were not hydrolyzed by NTPDases or NPPs, indicating that analogue **23** could be useful as a specific inhibitor of NPP1.

[0042] In summary, the study described herein shows that analogues **1-4** are moderate but effective inhibitors of NPP1 activity in either cell extracts or intact cells. Analogues **1** and **4** strongly blocked the activity of both NPP1 and -3. Yet, analogue **1**, found here to be an effective NPP1 inhibitor, exhibited a low activity toward the $P2Y_1$ and $P2Y_{11}$ receptors. On the other hand, analogue **2** did not significantly block NPP3 activity, had no activity on NTPDase1, -2, -3, and -8, as well as ecto-5'-nucleotidase, and virtually no activity toward the $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ receptors, and is therefore the most specific inhibitor of NPP1 among the analogues tested, and can be useful in treatment or management of osteoarthritis.

[0043] As further shown, analogues **23A/23B** were practically inactive at $P2Y_1$-R and $P2Y_{4/6}$-Rs; were chemically stable being hydrolyzed under conditions mimicking gastric juice pH (pH 1.4 and 37°C) with half-lives of 14.1 and 47.1 h, respectively, as compared to ATP which was hydrolyzed with half-life of 3.6 h; and completely resisted hydrolysis by

alkaline phosphatase for 30 min at 37°C. In addition, analogues **23A/23B** were hardly degraded by all plasma-bounded NTPDase sub-types, and were not inhibitors and only weakly bound to NTPDase. Analogue **23A** was a specific and potent inhibitor (K_i 500 nM) of NPP1 and was not hydrolyzed by NTPDases or NPPs. Therefore, analogue **23A** could be useful as a specific inhibitor of NPP1.

[0044] The present invention thus provides, in one aspect, a pharmaceutical composition for treatment of osteoarthritis comprising either a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, in which at least one but preferably two of the bridging-oxygens in the dinucleoside boranophosphate derivative, more preferably the α,β- and δ,ϵ-bridging-oxygens, and at least one of the bridging-oxygens in the nucleoside boranophosphate derivative, each is replaced with a group selected from -NH- or -C($R_{10}R_{11}$)-, wherein R_{10} and R_{11} each independently is H or halogen.

[0045] As used herein, the term "halogen" includes fluoro, chloro, bromo, and iodo, and is preferably fluoro or chloro.

[0046] The term "hydrocarbyl" in any of the definitions of the different radicals R_1 to R_6, R_8 and R_9 refers to a radical containing only carbon and hydrogen atoms that may be saturated or unsaturated, linear or branched, cyclic or acyclic, or aromatic, and includes alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and aryl.

[0047] The term "alkyl" as used herein typically means a straight or branched hydrocarbon radical having 1-8 carbon atoms and includes, e.g., methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, 2,2-dimethylpropyl, n-hexyl, n-heptyl, n-octyl, and the like. Preferred are (C_1-C_6)alkyl groups, more preferably (C_1-C_4)alkyl groups, most preferably methyl and ethyl. The terms "alkenyl" and "alkynyl" typically mean straight or branched hydrocarbon radicals having 2-8 carbon atoms and 1 double or triple bond, respectively, and include ethenyl, propenyl, 3-buten-1-yl, 2-ethenylbutyl, 3-octen-1-yl, and the like, and propynyl, 2-butyn-1-yl, 3-pentyn-1-yl, and the like. Preferred are (C_2-C_6)alkenyl and (C_2-C_6)alkynyl, more preferably (C_2-C_4)alkenyl and (C_2-C_4)alkynyl. Each one of the alkyl, alkenyl and alkynyl may optionally be substituted by one or more groups each independently selected from halogen, e.g., F, Cl or Br, -OH, -NO_2, -CN, -SCN, aryl, or heteroaryl, and/or interrupted by one or more heteroatoms selected from nitrogen, oxygen or sulfur.

[0048] The term "cycloalkyl" as used herein means a mono- or bicyclic saturated hydrocarbyl group having 3-10 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, bicyclo[3.2.1]octyl, bicyclo[2.2.1]heptyl, and the like, which may be substituted, e.g., with one or more groups each independently selected from halogen, e.g., F, Cl or Br, -OH, $-NO_2$, -CN, -SCN, (C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -S-(C_1-C_8)alkyl, $-NH_2$, -NH-(C_1-C_8)alkyl, or -N-$((C_1-C_8)\text{alkyl})_2$. The term "cycloalkenyl" as used herein means a mono- or bicyclic unsaturated hydrocarbyl group having 3-10 carbon atoms and 1 double bond, and include cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, cyclodecenyl, hexahydropentalenyl, octahydronaphtalenyl, bicycle[4.2.0]oct-2-enyl, and the like.

[0049] The term "aryl" as used herein denotes an aromatic carbocyclic group having 6-14 carbon atoms consisting of a single ring or multiple rings either condensed or linked by a covalent bond such as, but not limited to, phenyl, naphthyl, phenanthryl, and biphenyl. Preferred are (C_6-C_{10})aryl, more preferably phenyl. The aryl radical may optionally be substituted by one or more groups each independently selected from halogen, e.g., F, Cl or Br, -OH, $-NO_2$, -CN, -SCN, (C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -S-(C_1-C_8)alkyl, $-NH_2$, -NH-(C_1-C_8)alkyl, or -N-$((C_1-C_8)\text{alkyl})_2$.

[0050] The term "heteroaryl" refers to a radical derived from a mono- or poly-cyclic heteroaromatic ring containing one to three, preferably 1 or 2, heteroatoms selected from N, O or S. When the heteroaryl is a monocyclic ring, it is preferably a radical of a 5-6-membered ring such as, but not limited to, pyrrolyl, furyl, thienyl, thiazinyl, pyrazolyl, pyrazinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyridyl, pyrimidinyl, 1,2,3-triazinyl, 1,3,4-triazinyl, and 1,3,5-triazinyl. Polycyclic heteroaryl radicals are preferably composed of two rings such as, but not limited to, benzofuryl, isobenzofuryl, benzothienyl, indolyl, quinolinyl, isoquinolinyl, imidazo[1,2-*a*]pyridyl, benzimidazolyl, benzthiazolyl, benzoxazolyl, pyrido[1,2-a]pyrimidinyl and 1,3-benzodioxinyl. The heteroaryl may be substituted. It is to be understood that when a polycyclic heteroaryl is substituted, the substitution may be in any of the carbocyclic and/or heterocyclic rings.

[0051] In the groups $-NR_4R_5$ and $-NR_8R_9$, R_4 and R_5, and R_8 and R_9, respectively, each independently is H or hydrocarbyl as defined above, or form together with the nitrogen atom to which they are attached a saturated or unsaturated heterocyclic ring optionally containing 1 or 2 further heteroatoms selected from N, O or S. The term "heterocyclic ring" denotes a mono- or poly-cyclic non-aromatic ring of 4-12 atoms containing at least

one carbon atom and one to three, preferably 1-2 heteroatoms selected from N, O or S, which may be saturated or unsaturated, i.e., containing at least one unsaturated bond. Preferred are 5- or 6-membered heterocyclic rings. The heterocyclic ring may optionally be substituted at any carbon atom as well as at a second nitrogen atom of the ring, if present, with one or more groups each independently selected from halogen, e.g., F, Cl or Br, -OH, $-NO_2$, -CN, -SCN, (C_1-C_8)alkyl, -O-(C_1-C_8)alkyl, -S-(C_1-C_8)alkyl, $-NH_2$, -NH-(C_1-C_8)alkyl, or -N-$((C_1-C_8)alkyl)_2$. Non-limiting examples of radicals $-NR_4R_5$ and $-NR_8R_9$ include amino, dimethylamino, diethylamino, ethylmethylamino, phenylmethyl-amino, pyrrolidino, piperidino, tetrahydropyridino, piperazino, ethylpiperazino, hydroxyethyl piperazino, morpholino, thiomorpholino, thiazolino, and the like.

[0052] In certain embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula I as defined above, or a diastereomer or mixture of diastereoisomers thereof.

[0053] In particular embodiments, the active agent is a compound of the general formula I, or a diastereomer or mixture of diastereoisomers thereof, wherein (i) both n and n' are 1, two of W_1 to W_4 are -O-, and the other two of W_1 to W_4 each independently is $-C(R_{10}R_{11})-$; (ii) n is 0 and n' is 1, one of W_2 to W_4 is -O-, and the other two of W_2 to W_4 each independently is $-C(R_{10}R_{11})-$; or (iii) both n and n' are 0, and W_2 and W_3 each independently is $-C(R_{10}R_{11})-$.

[0054] In certain particular embodiments, the compound of the general formula I is a dinucleoside penta(borano)phosphate derivative wherein n and n' are 1. These derivatives may have (i) a sole borano group at position α (or α'), namely, Z_1 (or Z_5) is $-BH_3^-$, and Z_2, Z_3, Z_4 and Z_5 (or Z_1, Z_2, Z_3 and Z_4) are $-O^-$; at position β (or β'), namely, Z_2 (or Z_4) is $-BH_3^-$, and Z_1, Z_3, Z_5 and Z_4 (or Z_1, Z_2, Z_3 and Z_4) are $-O^-$; or at position γ, namely, Z_3 is $-BH_3^-$, and Z_1, Z_2, Z_4 and Z_5 are $-O^-$; (ii) two borano groups at positions α,β (or α',β'), namely, Z_1 and Z_2 (or Z_4 and Z_5) are $-BH_3^-$, and Z_3, Z_4 and Z_5 (or Z_1, Z_2 and Z_3) are $-O^-$; at positions α,γ (or α',γ), namely, Z_1 and Z_3 (or Z_3 and Z_5) are $-BH_3^-$, and Z_2, Z_4 and Z_5 (or Z_1, Z_2 and Z_4) are $-O^-$; at positions α,δ (or α',δ'), namely, Z_1 and Z_4 (or Z_2 and Z_5) are $-BH_3^-$, and Z_2, Z_3 and Z_5 (or Z_1, Z_3 and Z_4) are $-O^-$; at positions α,ϵ (or α',ϵ'), namely, Z_1 and Z_5 are $-BH_3^-$, and Z_2, Z_3 and Z_4 are $-O^-$; at positions β,γ (or β',γ), namely, Z_2 and Z_3 (or Z_3 and Z_4) are $-BH_3^-$, and Z_1, Z_4 and Z_5 (or Z_1, Z_2 and Z_5) are $-O^-$; or at positions β,δ (or β',δ'), namely, Z_2 and Z_4 are $-BH_3^-$, and Z_1, Z_3 and Z_5 are $-O^-$; (iii) three borano groups at positions α,β,γ (or α',β',γ), namely, Z_1, Z_2, and Z_3 (or Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_4 and Z_5 (or Z_1 and Z_2) are

$-O^-$; at positions α,β,δ (or α',β',δ'), namely, Z_1, Z_2 and Z_4 (or Z_2, Z_4 and Z_5) are $-BH_3^-$, and Z_5 and Z_3 (or Z_1 and Z_3) are $-O^-$; at positions α,β,ϵ (or α',β',ϵ'), namely, Z_1, Z_2 and Z_5 (or Z_1, Z_4 and Z_5) are $-BH_3^-$, and Z_3 and Z_4 (or Z_2 and Z_3) are $-O^-$; at positions α,γ,δ (or α',γ,δ'), namely, Z_1, Z_3 and Z_4 (or Z_2, Z_3 and Z_5) are $-BH_3^-$, and Z_2 and Z_5 (or Z_1 and Z_4) are $-O^-$; at positions α,γ,ϵ (or α',γ,ϵ'), namely, Z_1, Z_3 and Z_5 are $-BH_3^-$, and Z_2 and Z_4 are $-O^-$; at positions β,γ,ϵ (or β',γ,ϵ'), namely, Z_2, Z_3 and Z_5 (or Z_1, Z_3 and Z_4) are $-BH_3^-$, and Z_1 and Z_4 (or Z_2 and Z_5) are $-O^-$; or at positions β,γ,δ (or β',γ,δ'), namely, Z_2, Z_3 and Z_4 are $-BH_3^-$, and Z_1 and Z_5 are $-O^-$; (iv) four borano groups at positions $\alpha,\beta,\gamma,\delta$ (or $\alpha',\beta',\gamma,\delta'$), namely, Z_1, Z_2, Z_3 and Z_4 (or Z_2, Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_5 (or Z_1) is $-O^-$; at positions $\alpha,\beta,\gamma,\epsilon$ (or $\alpha',\beta',\gamma,\epsilon'$), namely, Z_1, Z_2, Z_3 and Z_5 (or Z_1, Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_4 (or Z_2) is $-O^-$; or at positions $\alpha,\beta,\delta,\epsilon$ (or $\alpha',\beta',\delta',\epsilon'$), namely, Z_1, Z_2, Z_4 and Z_5 are $-BH_3^-$, and Z_3 is $-O^-$; or (v) five borano groups at positions $\alpha,\beta,\gamma,\delta,\epsilon$, namely, Z_1, Z_2, Z_3, Z_4 and Z_5 are $-BH_3^-$. In specific such embodiments, the active agent is a compound of the general formula I, or a diastereomer or mixture of diastereoisomers thereof, wherein Z_3 is $-BH_3^-$, Z_1, Z_2, Z_4 and Z_5 are $-O^-$, W_2 and W_3 are -O-, and W_1 and W_4 each independently is $-C(R_{10}R_{11})-$.

[0055] In certain particular embodiments, the compound of the general formula I is a dinucleoside tetra(borano)phosphate derivative wherein n is 0 and n' is 1. These derivatives may have (i) a sole borano group at position α (or α'), namely, Z_1 (or Z_5) is $-BH_3^-$, and Z_3, Z_4 and Z_5 (or Z_1, Z_3 and Z_4) are $-O^-$; or at position β (or β'), namely, Z_3 (or Z_4) is $-BH_3^-$, and Z_1, Z_4 and Z_5 (or Z_1, Z_3 and Z_5) are $-O^-$; (ii) two borano groups at positions α,β (or α',β'), namely, Z_1 and Z_3 (or Z_4 and Z_5) are $-BH_3^-$, and Z_4 and Z_5 (or Z_1 and Z_3) are O^-; at positions α,γ (or α',γ), namely, Z_1 and Z_4 (or Z_3 and Z_5) are $-BH_3^-$, and Z_3 and Z_5 (or Z_1 and Z_4) are O^-; at positions α,δ (or α',δ'), namely, Z_1 and Z_5 are $-BH_3^-$, and Z_3 and Z_4 are O^-; or at positions β,γ (or β',γ), namely, Z_3 and Z_4 are BH_3^-, and Z_1 and Z_5 are O^-; (iii) three borano groups at positions α,β,γ (or α',β',γ), namely, Z_1, Z_3 and Z_4 (or Z_3, Z_4 and Z_5) are BH_3^-, and Z_5 (or Z_1) is O^-; or at positions α,β,δ (or α',β',δ'), namely, Z_1, Z_3 and Z_5 (or Z_1, Z_4 and Z_5) are BH_3^-, and Z_4 (or Z_3) is O^-; or (iv) four borano groups at positions $\alpha,\beta,\gamma,\delta$, namely, Z_1, Z_3, Z_4 and Z_5 are BH_3^-.

[0056] In certain particular embodiments, the compound of the general formula I is a dinucleoside tri(borano)phosphate derivative wherein n and n' are 0. These derivatives may have (i) a sole borano group at position α (or α'), namely, Z_1 (or Z_5) is BH_3^-, and Z_3 and Z_5 (or Z_1 and Z_3) are O^-; or at position β, namely, Z_3 is BH_3^-, and Z_1 and Z_5 are O^-; (ii) two borano groups at positions α,β (or α',β), namely, Z_1 and Z_3 (or Z_3 and Z_5) are BH_3^-, and

Z_5 (or Z_1) is O^-; or at positions α,γ (or α',γ), namely, Z_1 and Z_5 are BH_3^-, and Z_3 is O^-; or (iii) three borano groups at positions α,β,γ (or α',β',γ), namely, Z_1, Z_3 and Z_5 are BH_3^-.

[0057] In particular embodiments, the active agent is a compound of the general formula I, or a diastereomer or mixture of diastereoisomers thereof, wherein Y and Y' each independently is H or -OH.

[0058] In certain embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula I as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X and X' each is an adenine residue of the formula Ia. In particular such embodiments, X and X' are an adenine residue, wherein R_1 each independently is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is $(C_1\text{-}C_8)$alkyl, preferably $(C_1\text{-}C_4)$alkyl, more preferably methyl or ethyl, $(C_2\text{-}C_8)$alkenyl, preferably $(C_2\text{-}C_4)$alkenyl, $(C_2\text{-}C_8)$alkynyl, preferably $(C_2\text{-}C_4)$alkynyl, or $(C_6\text{-}C_{14})$aryl, preferably $(C_6\text{-}C_{10})$aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S. In more particular such embodiments, R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is $(C_1\text{-}C_4)$alkyl, preferably methyl or ethyl, $(C_2\text{-}C_4)$alkenyl, $(C_2\text{-}C_4)$alkynyl, or $(C_6\text{-}C_{10})$aryl, preferably phenyl. In most particular such embodiments, R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

[0059] In specific such embodiments, the active agent comprised within the pharmaceutical composition of the invention is a compound of the general formula I as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X and X' each is an adenine residue of the formula Ia, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; n and n' are 1; m is 5; Z_3 is $-BH_3^-$; Z_1, Z_2, Z_4 and Z_5 are $-O^-$; W_2 and W_3 are -O-; and W_1 and W_4 each

independently is -C($R_{10}R_{11}$)-, wherein said hydrocarbyl each independently is methyl or ethyl. More specific such embodiments are those wherein R_1 each independently is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; n and n' are 1; m is 5; Z_3 is $-BH_3^-$; Z_1, Z_2, Z_4 and Z_5 are $-O^-$; W_2 and W_3 are -O-; and W_1 and W_4 each independently is $-CH_2-$, $-CCl_2-$ or $-CF_2-$, preferably $-CH_2-$. In particular specific such embodiments, the active agent comprised within the pharmaceutical composition of the invention is a compound of the general formula I as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X and X' each is an adenine residue of the formula Ia, wherein R_1 is H; R_2 and R_3 are H; Y and Y' each independently is -OH or H; n and n' are 1; m is 5; Z_3 is $-BH_3^-$; Z_1, Z_2, Z_4 and Z_5 are $-O^-$; W_2 and W_3 are -O-; and W_1 and W_4 each independently is $-CH_2-$ (analogues **1** and **2**, respectively).

[0060] In certain embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula I as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X and X' each is an uracil residue of the formula Ib. In particular such embodiments, X and X' are an uracil residue, wherein R_6 each independently is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, -NR_8R_9, heteroaryl, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_7 is O, wherein said hydrocarbyl each independently is (C_1-C_8)alkyl, preferably (C_1-C_4)alkyl, more preferably methyl or ethyl, (C_2-C_8)alkenyl, preferably (C_2-C_4)alkenyl, (C_2-C_8)alkynyl, preferably (C_2-C_4)alkynyl, or (C_6-C_{14})aryl, preferably (C_6-C_{10})aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S. In more particular such embodiments, R_6 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_8R_9, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl. In most particular such embodiments, R_6 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_8R_9, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is methyl or ethyl.

[0061] In other embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof.

[0062] In particular embodiments, the active agent is a compound of the general formula II, or a diastereomer or mixture of diastereoisomers thereof, wherein (i) n is 1, one of W_1 and W_2 is -O-, and another one of W_1 and W_2 is $-C(R_{10}R_{11})-$; or (ii) n is 0, and W_2 is $-C(R_{10}R_{11})-$.

[0063] In certain particular embodiments, the compound of the general formula II is a nucleoside tri(borano)phosphate derivative wherein n is 1. These derivatives may have (i) a sole borano group at position α, namely, Z_1 is $-BH_3^-$, and Z_2 and Z_3 are $-O^-$; at position β, namely, Z_2 is $-BH_3^-$, and Z_1 and Z_3 are $-O^-$; or at position γ, namely, Z_3 is $-BH_3^-$, and Z_1 and Z_2 are $-O^-$; (ii) two borano groups at positions α,β, namely, Z_1 and Z_2 are $-BH_3^-$, and Z_3 is $-O^-$; at positions α,γ, namely, Z_1 and Z_3 are $-BH_3^-$, and Z_2 is $-O^-$; or at positions β,γ, namely, Z_2 and Z_3 are $-BH_3^-$, and Z_1 is $-O^-$; or (iii) three borano groups at positions α,β,γ, namely, Z_1, Z_2 and Z_3 are $-BH_3^-$.

[0064] In specific such embodiments, the active agent is a compound of the general formula II, or a diastereomer or mixture of diastereoisomers thereof, wherein Z_1 is $-BH_3^-$, Z_2 and Z_3 are $-O^-$, W_1 is -O-, and W_2 is $-C(R_{10}R_{11})-$.

[0065] In certain particular embodiments, the compound of the general formula II is a nucleoside di(borano)phosphate derivative wherein n is 0. These derivatives may have (i) a sole borano group at position α, namely, Z_1 is $-BH_3^-$, and Z_3 is $-O^-$; or at position β, namely, Z_3 is $-BH_3^-$, and Z_1 is $-O^-$; or (ii) two borano groups at positions α,β, namely, Z_1 and Z_3 are $-BH_3^-$.

[0066] In particular embodiments, the active agent is a compound of the general formula II, or a diastereomer or mixture of diastereoisomers thereof, wherein Y is H or -OH.

[0067] In certain embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X is an adenine residue of the formula Ia. In particular such embodiments, X is an adenine residue, wherein R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S;

and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is $(C_1\text{-}C_8)$alkyl, preferably $(C_1\text{-}C_4)$alkyl, more preferably methyl or ethyl, $(C_2\text{-}C_8)$alkenyl, preferably $(C_2\text{-}C_4)$alkenyl, $(C_2\text{-}C_8)$alkynyl, preferably $(C_2\text{-}C_4)$alkynyl, or $(C_6\text{-}C_{14})$aryl, preferably $(C_6\text{-}C_{10})$aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S. In more particular such embodiments, R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is $(C_1\text{-}C_4)$alkyl, preferably methyl or ethyl, $(C_2\text{-}C_4)$alkenyl, $(C_2\text{-}C_4)$alkynyl, or $(C_6\text{-}C_{10})$aryl, preferably phenyl. In most particular such embodiments, R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

[0068] In specific such embodiments, the active agent comprised within the pharmaceutical composition of the invention is a compound of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X is an adenine residue of the formula Ia, wherein R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H; Y is H or -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-C(R_{10}R_{11})-$, wherein said hydrocarbyl each independently is methyl or ethyl. More specific such embodiments are those wherein R_1 is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y is H or -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-CH_2-$, $-CCl_2-$ or $-CF_2-$, preferably $-CH_2-$. In one particular such embodiments, the active agent comprised within the pharmaceutical composition of the invention is a compound of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X is an adenine residue of the formula Ia, wherein R_1 is H; R_2 and R_3 are H; Y is -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-CH_2-$ (analogues **23**). Both isomers **23A** and **23B** can be used, i.e., the isomers having a retention time (Rt) of 7.64 min or 9.67 min, respectively, when separated from a mixture of diastereoisomers using a semi-preparative reverse-phase Gemini 5u column (C-18 110A, 250×10 mm, 5 micron), and isocratic elution [100 mM triethylammonium acetate, pH 7: MeOH, 89:11] with flow rate of 5 ml/min.

[0069] In another particular such embodiments, the active agent comprised within the pharmaceutical composition of the invention is a compound of the general formula II as

defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X is an adenine residue of the formula Ia, wherein R_1 is S-methyl; R_2 and R_3 are H; Y is -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-CH_2-$ (analogues **24**). The preferred isomer in this case is 24A, i.e., the isomer having a retention time (Rt) of 5.29 min when separated from a mixture of diastereoisomers using a semi-preparative reverse-phase Gemini 5u column (C-18 110A, 250×10 mm, 5 micron), and isocratic elution [100 mM triethylammonium acetate, pH 7: MeOH, 75:25] with flow rate of 5 ml/min.

[0070] In certain embodiments, the active agent comprised within the pharmaceutical composition of the present invention is a compound of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein X is an uracil residue of the formula Ib. In particular such embodiments, X is an uracil residue, wherein R_6 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, heteroaryl, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_7 is O, wherein said hydrocarbyl each independently is (C_1-C_8)alkyl, preferably (C_1-C_4)alkyl, more preferably methyl or ethyl, (C_2-C_8)alkenyl, preferably (C_2-C_4)alkenyl, (C_2-C_8)alkynyl, preferably (C_2-C_4)alkynyl, or (C_6-C_{14})aryl, preferably (C_6-C_{10})aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S. In more particular such embodiments, R_6 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl. In most particular such embodiments, R_6 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is methyl or ethyl.

[0071] The compounds of the general formula I or II may be synthesized according to any technology or procedure known in the art. Procedures for the synthesis of compounds of the general formula I are described in detail, e.g., in US 7,368,439 and in the Examples section hereinafter. Procedures for the preparation of compounds of the general formula II may are described, inter alia, in WO 2009/066298.

[0072] Both the compounds of the general formula I and the compounds of the general formula II may have one or more asymmetric centers, e.g., in the Pα, and may accordingly exist as pairs of diastereoisomers. In cases a pair of diastereoisomers exists, the separation and characterization of the different diastereoisomers may be accomplished using any technology known in the art, e.g., using HPLC. According to the present invention, treatment of osteoarthritis could be carried out by administration of all such isomers and mixtures thereof.

[0073] The compounds of the general formula I are in the form of pharmaceutically acceptable salts.

[0074] In certain embodiments, the cation B is an inorganic cation of an alkali metal, e.g., lithium, sodium or potassium, or an alkaline earth metal, e.g., calcium or magnesium. In other embodiments, the cation B is ammonium (NH_4^+) or is an organic cation derived from an amine of the formula R_4N^+, wherein each one of the Rs independently is selected from H, C_1-C_{22}, preferably C_1-C_6 alkyl, such as methyl, ethyl, propyl, isopropyl, butyl, and the like, phenyl, or heteroaryl such as pyridyl, imidazolyl, pyrimidinyl, and the like, or two of the Rs together with the nitrogen atom to which they are attached form a 3-7 membered ring optionally containing a further heteroatom selected from N, S and O, such as pyrrolydine, piperidine and morpholine.

[0075] In further embodiments, the cation B is a cationic lipid or a mixture of cationic lipids. Cationic lipids are often mixed with neutral lipids prior to use as delivery agents. Neutral lipids include, but are not limited to, lecithins; phosphatidylethanolamine; diacyl phosphatidylethanolamines such as dioleoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine and distearoyl phosphatidylethanolamine; phosphatidylcholine; diacyl phosphatidylcholines such as dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, palmitoyloleoyl phosphatidylcholine and distearoyl phosphatidylcholine; phosphatidylglycerol; diacyl phosphatidylglycerols such as dioleoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol and distearoyl phosphatidylglycerol; phosphatidylserine; diacyl phosphatidylserines such as dioleoyl- or dipalmitoyl phosphatidylserine; and diphosphatidylglycerols; fatty acid esters; glycerol esters; sphingolipids; cardiolipin; cerebrosides; ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3β hydroxy-sterols.

[0076] Examples of cationic lipid compounds include, without being limited to, Lipofectin® (Life Technologies, Burlington, Ontario) (1:1 (*w/w*) formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoylphosphatidyl-ethanolamine); Lipofectamine™ (Life Technologies, Burlington, Ontario) (3:1 (*w/w*) formulation of polycationic lipid 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanamin-iumtrifluoroacetate and dioleoylphosphatidyl-ethanolamine), Lipofectamine Plus (Life Technologies, Burlington, Ontario) (Lipofectamine and Plus reagent), Lipofectamine 2000 (Life Technologies, Burlington, Ontario) (Cationic lipid), Effectene (Qiagen, Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, San Luis Obispo, Calif.) (ethanolic cationic lipids numbers 1 through 12: $C_{52}H_{106}N_6O_4{\cdot}4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2{\cdot}4CF_3CO_2H$, $C_{40}H_{84}NO_3P{\cdot}CF_3CO_2H$, $C_{50}H_{103}N_7O_3{\cdot}4CF_3CO_2H$, $C_{55}H_{116}N_8O_2{\cdot}6CF_3CO_2H$, $C_{49}H_{102}N_6O_3{\cdot}4CF_3CO_2H$, $C_{44}H_{89}N_5O_3{\cdot}2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2{\cdot}8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9{\cdot}13CF_3CO_2H$, $C_{43}H_{88}N_4O_2{\cdot}2CF_3CO_2H$, $C_{43}H_{88}N_4O_3{\cdot}2CF_3CO_2H$, $C_{41}H_{78}NO_8P$); Cytofectene (Bio-Rad, Hercules, Calif.) (mixture of a cationic lipid and a neutral lipid), GenePORTER® (Gene Therapy Systems, San Diego, Calif.) (formulation of a neutral lipid (Dope) and a cationic lipid) and FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Ind.) (Multi-component lipid based non-liposomal reagent).

[0077] The pharmaceutical compositions provided by the present invention may be prepared by conventional techniques, e.g., as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995. The compositions can be prepared, e.g., by uniformly and intimately bringing the active agent, i.e., the compound of the general formula I or II, into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulation. The compositions may be in liquid, solid or semisolid form and may further include pharmaceutically acceptable fillers, carriers, diluents or adjuvants, and other inert ingredients and excipients. In one embodiment, the pharmaceutical composition of the present invention is formulated as nanoparticles.

[0078] The compositions can be formulated for any suitable route of administration, but they are preferably formulated for parenteral, e.g., intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, subcutaneous, transdermal or topical, or for oral administration.

The dosage will depend on the state of the patient, and will be determined as deemed appropriate by the practitioner.

[0079] The pharmaceutical composition of the invention may be in the form of a sterile injectable aqueous or oleagenous suspension, which may be formulated according to the known art using suitable dispersing, wetting or suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Acceptable vehicles and solvents that may be employed include, without limiting, water, Ringer's solution and isotonic sodium chloride solution.

[0080] The pharmaceutical compositions of the invention, when formulated for administration route other than parenteral administration, may be in a form suitable for oral use, e.g., as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and may further comprise one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active agent in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients may be, e.g., inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, or sodium phosphate; granulating and disintegrating agents, e.g., corn starch or alginic acid; binding agents, e.g., starch, gelatin or acacia; and lubricating agents, e.g., magnesium stearate, stearic acid, or talc. The tablets may be either uncoated or coated utilizing known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated using the techniques described in the US Patent Nos. 4,256,108, 4,166,452 and 4,265,874 to form osmotic therapeutic tablets for control release. The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsion.

[0081] Pharmaceutical compositions according to the invention, when formulated for inhalation, may be administered utilizing any suitable device known in the art, such as metered dose inhalers, liquid nebulizers, dry powder inhalers, sprayers, thermal vaporizers, electrohydrodynamic aerosolizers, and the like.

[0082] The pharmaceutical compositions of the invention may be formulated for controlled release of the active agent. Such compositions may be formulated as controlled-release matrix, e.g., as controlled-release matrix tablets in which the release of a soluble active agent is controlled by having the active diffuse through a gel formed after the swelling of a hydrophilic polymer brought into contact with dissolving liquid (*in vitro*) or gastro-intestinal fluid (*in vivo*). Many polymers have been described as capable of forming such gel, e.g., derivatives of cellulose, in particular the cellulose ethers such as hydroxypropyl cellulose, hydroxymethyl cellulose, methylcellulose or methyl hydroxypropyl cellulose, and among the different commercial grades of these ethers are those showing fairly high viscosity. In other configurations, the compositions comprise the active agent formulated for controlled release in microencapsulated dosage form, in which small droplets of the active agent are surrounded by a coating or a membrane to form particles in the range of a few micrometers to a few millimeters.

[0083] Another contemplated formulation is depot systems, based on biodegradable polymers, wherein as the polymer degrades, the active agent is slowly released. The most common class of biodegradable polymers is the hydrolytically labile polyesters prepared from lactic acid, glycolic acid, or combinations of these two molecules. Polymers prepared from these individual monomers include poly (D,L-lactide) (PLA), poly (glycolide) (PGA), and the copolymer poly (D,L-lactide-co-glycolide) (PLG).

[0084] In another aspect, the present invention provides a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, for use in treatment of osteoarthritis.

[0085] In a further aspect, the present invention relates to use of a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof, for the preparation of a pharmaceutical composition for treatment of osteoarthritis.

[0086] In still a further aspect, the present invention relates to a method for treatment of osteoarthritis in an individual in need thereof, comprising administering to said individual a therapeutically effective amount of a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined above, or a diastereomer or mixture of diastereoisomers thereof.

[0087] Osteoarthritis, also known as degenerative arthritis or degenerative joint disease, is the most common form of arthritis and refers to a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone. Symptoms may include joint pain, tenderness, stiffness, locking, and sometimes an effusion, i.e., an accumulation of excess fluid in or around the knee joint. A variety of causes including hereditary, developmental, metabolic and mechanical may initiate processes leading to loss of cartilage. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged. As a result of decreased movement secondary to pain, regional muscles may atrophy, and ligaments may become more lax.

[0088] Osteoarthritis can be either primary or secondary in case there is an identifiable underlying cause, although the resulting pathology is the same. Primary osteoarthritis is a chronic degenerative disorder related to but not caused by aging. As a person ages, the water content of the cartilage decreases as a result of a reduced proteoglycan content, thus causing the cartilage to be less resilient. Without the protective effects of the proteoglycans, the collagen fibers of the cartilage can become susceptible to degradation and thus exacerbate the degeneration. Inflammation of the surrounding joint capsule can also occur, though often mild compared to that which occurs in rheumatoid arthritis. This can happen as breakdown products from the cartilage are released into the synovial space, and the cells lining the joint attempt to remove them. New bone outgrowths, called "spurs" or osteophytes, can form on the margins of the joints, possibly in an attempt to improve the congruence of the articular cartilage surfaces. These bone changes, together with the inflammation, can be both painful and debilitating. Secondary osteoarthritis is caused by other factors such as congenital disorders of joints; diabetes; inflammatory diseases, e.g., Perthes' disease and Lyme disease, and all chronic forms of arthritis, e.g., costochondritis, gout and rheumatoid arthritis; injury to joints as a result of an accident or orthodontic operations; septic arthritis, i.e., infection of a joint; ligamentous deterioration; Marfan syndrom, obesity; alkaptonuria; and hemochromatosis and Wilson's disease.

[0089] One of the conditions frequently coexisting and associated with osteoarthritis is known as the calcium pyrophosphate dehydrate (CPPD) disease, i.e., the deposition of calcium pyrophosphate dehydrate crystals in the synovial fluid, which causes stiffness and severe pain, and eventually leads to cartilage damage. The CPPD crystals deposition is lead by excess of extracellular PPi resulting from over expression of NPP1. Controlled blocking of NPP1 could thus control the concentration of extracellular PPi and

consequently decrease symptoms of CPPD. Crystal formation in both articular cartilage and synovial fluid, as well as low-grade inflammation, a consequence of crystal deposition, should be monitored during therapy.

[0090] The only available treatment for osteoarthritis is symptomatic and does not deal with the causes underlying the disease. The compounds of the general formulas I and II are useful in treatment or management of osteoarthritis. The term "treatment" as used herein with respect to osteoarthritis refers to blocking of NPP1 over expression and consequently extracellular PPi concentration, thus reducing CPPD crystals deposition and attenuating, i.e., limiting or reducing, the various symptoms of the disease as defined above. The term "management" as used herein with respect to osteoarthritis refers to a continuous treatment of the disease during which NPP1 over expression is constantly controlled so as to maintain balanced levels of extracellular PPi thus constantly reducing CPPD crystals deposition. The term "therapeutically effective amount" as used herein refers to the quantity of the compound of the general formula I or II as defined above, or a diastereomer or mixture of diastereomers thereof, that is useful to treat or manage osteoarthritis.

[0091] In yet another aspect, the present invention relates to a diadenosine penta(γ-borano)phosphate derivative of the general formula III as defined above, i.e., a particular embodiment of the compound defined by the general formula I above, in which a borano group replaces a non-bridging oxygen atom at position Pγ and two of the bridging-oxygens, preferably the α,β- and δ,ϵ-bridging-oxygens, each is replaced with a group selected from -NH- or -C($R_{10}R_{11}$)-, wherein R_{10} and R_{11} each independently is H or halogen.

[0092] In certain embodiments, the diadenosine penta(borano)phosphate derivative of the invention is a compound of the general formula III, wherein W_2 and W_3 are -O-, and W_1 and W_4 each independently is -NH- or -C($R_{10}R_{11}$)-, preferably -CH_2-, -CCl_2- or -CF_2-.

[0093] In certain embodiments, the diadenosine penta(borano)phosphate derivative of the invention is a compound of the general formula III, wherein Y and Y' each independently is H or -OH.

[0094] In certain embodiments, the diadenosine penta(borano)phosphate derivative of the invention is a compound of the general formula III as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein Ad each independently is an adenine residue of the formula Ia. In particular such embodiments, Ad each independently is an adenine residue, wherein R_1 each independently is H, halogen, -O-hydrocarbyl, -S-

hydrocarbyl, -NR_4R_5, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is (C_1-C_8)alkyl, preferably (C_1-C_4)alkyl, more preferably methyl or ethyl, (C_2-C_8)alkenyl, preferably (C_2-C_4)alkenyl, (C_2-C_8)alkynyl, preferably (C_2-C_4)alkynyl, or (C_6-C_{14})aryl, preferably (C_6-C_{10})aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S. In more particular such embodiments, R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl. In most particular such embodiments, R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

[0095] In specific such embodiments, the diadenosine penta(borano)phosphate derivative of the invention is a compound of the general formula III as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein Ad each is an adenine residue of the formula Ia, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; W_2 and W_3 are -O-; and W_1 and W_4 each independently is -$C(R_{10}R_{11})$-, wherein said hydrocarbyl each independently is methyl or ethyl. More specific such embodiments are those wherein R_1 each independently is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; W_2 and W_3 are -O-; and W_1 and W_4 each independently is -CH_2-, -CCl_2- or -CF_2-, preferably -CH_2-. In particular specific such embodiments, the diadenosine penta(borano)phosphate derivative of the invention is a compound of the general formula III as defined above, or a diastereomer or mixture of diastereoisomers thereof, wherein Ad each is an adenine residue of the formula Ia, wherein R_1 is H; R_2 and R_3 are H; Y and Y' each independently is -OH or H; W_2 and W_3 are -O-; and W_1 and W_4 are -CH_2- (analogues **1** and **2**, respectively).

[0096] In still another aspect, the present invention provides a pharmaceutical composition comprising a diadenosine boranophosphate derivative of the general formula III as defined above, or a diastereomer or mixture of diastereoisomers thereof, and a pharmaceutically acceptable carrier.

[0097] The invention will now be illustrated by the following non-limiting Examples.

EXAMPLES

Abbreviations

[0098] **BP_i**, boranophosphate; **$[Ca^{2+}]_i$**, intracellular Ca^{2+} concentration; **CDI**, carbodiimidazole; **DMF**, N,N-dimethylformamide; **E-NPP**, ecto-nucleotide pyrophosphatase/phosphodiesterase; **E-NTPDase**, ecto-nucleoside triphosphate diphosphohydrolase; **ESI**, electron spray ionization; **FBS**, fetal bovine serum; **HRMS-MALDI**, high resolution mass spectrometry matrix-assisted laser desorption/ionization; **MPLC**, medium pressure liquid chromatography; **pnp-TMP**, thymidine 5'-monophosphate *p*-nitrophenyl ester; **P2R**, P2 receptor; **RT**, room temperature; **TEAA**, triethylammonium acetate.

Experimental

General

[0099] All commercial reagents were used without further purification, unless otherwise noted. All air- and moisture-sensitive reactions were carried out in flame-dried, argon-flushed, two-neck flasks sealed with rubber septa, and the reagents were introduced with a syringe. Progress of reactions was monitored by TLC using pre-coated Merck silica gel plates (60F-253). Reactants and products were visualized using UV light (Isco, UA-5). Compounds were characterized by NMR using Bruker AC-200, DPX-300 or DMX-600 spectrometers. ^1H NMR spectra were measured at 200, 300 or 600 MHz. Nucleotides were characterized also by ^{31}P NMR in D_2O, using 85% H_3PO_4 as an external reference on Bruker AC-200 and DMX-600 spectrometers. High resolution mass spectra were recorded on an AutoSpec-E FISION VG mass spectrometer by chemical ionization. Nucleotides were analyzed using electron spray ionization (ESI) on a Q-TOF micro-instrument (Waters, UK). Primary purification of the nucleotides was achieved on a LC (Isco UA-6) system using a column of Sephadex DEAE-A25, swollen in 1 M $NaHCO_3$ at 3°C for 24 h. The resin was washed with deionized water before use. LC separation was monitored by

UV detection at 280 nm. Final purification of the nucleotides and separation of the diastereomeric pairs were achieved on an HPLC (Merck-Hitachi) system using a semi-preparative reverse-phase column (Gemini 5u C-18 110A 250x10 mm; 5 micron; Phenomenex, Torrance, USA). The purity of the dinucleotides was evaluated on an analytical reverse-phase HPLC column system (Gemini 5u C-18 110A, 150 × 3.60 mm; 5 micron; Phenomenex) in two-solvent systems with either solvent systems I and II or solvent system III. Solvent system I was: (A) 100 mM TEAA, pH 7, (B) MeOH; solvent system II: (A) 100 mM TEAA, pH 7, (B) CH_3CN; solvent system III: (A) 0.01 M KH_2PO_3, pH = 3.5, (B) CH_3CN. The details of the solvent system gradients used for the separation of each product are provided below. The products, obtained as triethylammonium salts, were generally ≥95% pure. All reactants in moisture-sensitive reactions were dried overnight in a vacuum oven. 2',3'-*O*-Methoxymethylidene adenosine, **6** (Nahum *et al.*, 2002), α,β-methylene-ADP, **9**, and 2'-deoxy-α,β-methyleme-ADP, **13** (Davisson *et al.*, 1987) were prepared as previously described. α,β-methylene-ADP and 2'-deoxy-α,β-methylene-ADP were separated using a MPLC system (Biotage; Kungsgatan, Uppsala, Sweden) using a RP-C18 (12+M) column and the following gradient scheme: 3 column volumes of 100:0 (A:B ratio) (A: 100 mM TEAA; B: MeOH), 9 column volumes of a gradient from 100:0 to 60:30 A:B, followed by 5 column volumes of 60:30 A:B, at a flow rate of 12 ml/min.

Typical procedure for the preparation of diadenosine-5',5''-(boranated) poly-phosphonate derivatives 1-4

[00100] As depicted in **Schemes 1** and **2**, respectively, α,β-methylene-ADP$(Bu_3NH)^+_2$, **9**, and 2'-deoxy-α,β-methylene-ADP $(Bu_3NH)^+_2$, **13**, were prepared by applying the corresponding ADP analogues through a column of activated Dowex 50WX-8 (200 mesh, H^+ form). The eluate was collected in an ice-cooled flask containing tributylamine (2 eq) and EtOH. The resulting solution was freeze-dried to yield **9** and **13** as viscous oil. Bis(tributylammonium) α,β-methylene-ADP salt (100 mg, 0.16 mmol) was dissolved in dry DMF (2 ml), and CDI (180 mg, 1.11 mmol, 5 eq) was added. The resulting solution was stirred at RT for 12 h. $BP_i(Bu_3NH^+)_2$, **15**, (130 mg, 0.27 mmol, 1.7 eq) in dry DMF (1.5 ml), and $MgCl_2$ (120 mg, 1.28 mmol, 8 equiv) were added. The resulting solution was stirred at RT for 23 h. The semisolid obtained after evaporation of the solvent was chromatographed at RT on a Sephadex DEAE-A25 column, which was swelled in 1 M

$NaHCO_3$ prior to column preparation. The separation was monitored by UV detection (λ= 280 nm). A buffer gradient of water (1 l) to 0.7 M NH_4HCO_3 (1 l) was applied. The relevant fractions were pooled and freeze-dried to yield a white solid. Final purification was achieved on a semipreparative C18 HPLC column. Compound **1** was obtained in 10% yield (16 mg). Compound **3** was obtained in 20% yield (29 mg) (Pankiewicz *et al.*, 1997). The spectral data for **3** are consistent with literature (Pankiewicz *et al.*, 1997). Compound **2** was obtained in 21% yield (15 mg). Compound **4** was obtained in 28% yield (20 mg) after LC separation.

Diadenosine 5', 5''-P^1, P^5, α,β-methylene-δ,ϵ-methylene-pentaphosphate-γ-borano, 1, - purification and characterization

[00101] Compound **1** was purified by HPLC on a semi-preparative reverse-phase column, using solvent system I, with a gradient from 95:5 to 75:25 A:B over 15 min at a flow rate of 3 ml/min. Retention time: 12.88 min. ^{1}H NMR (D_2O; 300 MHz): δ 8.31 (s; H-8; 2H), 8.08 (s; H-2; 2H), 5.99 (d; J = 6.00 Hz; H-1'; 2H), (H2' signals are hidden by the water signal at 3.78 ppm), 3.53 (m; H-3'; 2H), 3.29 (m; H-3', 2H), 3.13 (m; H-5'; 3H), 2.31 (t; J = 20.71 Hz; CH_2; 3H), 0.50 (m; BH_3; 3H) ppm. ^{31}P NMR (D_2O; 81 MHz): 78.38 (m; P_γ-BH_3; 1P), 20.55 (d, J = 7.93 Hz; P_α; 2P), 10.77 (m; P_β; 2P) ppm. HRMS MALDI (negative) *m/z* $C_{22}H_{27}D_5N_{10}O_{20}P_5$: calculated 916.0796 found 916.0791 [MD_5]. TLC (NH_4OH:H_2O: isopropanol 2:8:11), R_f = 0.17. Purity data obtained on an analytical column: retention time: 1.81 min (99.97% purity) using solvent system II with a gradient from 80:20 to 70:30 A:B over 10 min at a flow rate of 1 ml/min. Retention time: 8.00 min (98.31% purity) using solvent system III with a gradient from 100:0 to 70:30 A:B over 10 min at a flow rate of 1 ml/min.

Di-2'-deoxyadenosine 5', 5''-P^1, P^5, α,β-methylene-δ,ϵ-methylene pentaphosphate-γ-borano, 2, - purification and characterization

[00102] Compound **2** was purified by HPLC on a semi-preparative reverse-phase column, using solvent system I, with a gradient from 95:5 to 70:30 A:B over 20 min at a flow rate of 5 ml/min. Retention time: 15.35 min. ^{1}H NMR (D_2O; 300 MHz): δ 8.33 (s; H-8; 1H), 8.33 (s; H-8; 1H), 8.06 (s; H-2; 1H), 8.05 (s; H-2; 1H), 6.32 (t; J = 6.90 Hz; H-1'; 2H), (H2' and H3' signals are hidden by the water signal at 3.78 ppm), 3.19 (m; H-3', 2H), 3.07 (m; H-5'; 3H), 2.38 (t; J = 20.70 Hz; CH_2; 3H), 0.50 (m; BH_3; 3H) ppm. ^{31}P NMR (D_2O; 81 MHz): 76.00 (m; P_γ-BH_3, 1P), 17.82 (s; P_α; 2P), 9.13 (d; J= 32.08 Hz; P_β; 2P) ppm. MS

ESI *m/z*: 899 ($M^- Na^+$). TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.12. Purity data obtained on an analytical column: retention time: 2.18 min (93.1% purity) using solvent system I with a gradient from 70:30 to 50:50 A:B over 10 min at a flow rate of 1 ml/min. Retention time: 1.37 min (99.5% purity) using solvent system III with a gradient from 85:15 to 50:50 A:B over 10 min at a flow rate of 1 ml/min.

Di-2'-deoxyadenosine 5', 5''-P^1, P^5, α,β-methylene-γ,δ-methylene-tetraphosphate, 4, - purification and characterization

[00103] Compound **4** was purified by HPLC on a semi-preparative reverse-phase column, using solvent system I, with a gradient from 95:5 to 75:25 A:B over 20 min at a flow rate of 5 ml/min. Retention time: 16.26 min. ^1H NMR (D_2O; 300 MHz): δ 8.33 (s; H-8; 2H), 8.06 (s; H-2; 2H), 6.32 (t; J = 6.30 Hz; H-1'; 2H), (H2' and H3' signals are hidden by the water signal at 3.78 ppm), 3.18 (m; H-3', 2H), 3.06 (m; H-5'; 3H), 2.31 (t; J = 2.31 Hz; CH_2; 3H) ppm. ^{31}P NMR (D_2O; 81 MHz): 18.02 (s; P_α; 2P), 8.15 (s; P_β; 2P) ppm. HRMS MALDI (negative) *m/z* $C_{22}H_{31}N_{10}O_{15}P_3$: calculated 799.0920 found 799.0915 [M^{3-}]. TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.22. Purity data obtained on an analytical column: retention time: 3.39 min (100% purity) using solvent system I with a gradient from 80:20 to 60:30 A:B over 10 min at a flow rate of 1 ml/min. Retention time: 1.51 min (98.5% purity) using solvent system III with a gradient from 85:15 to 50:50 A:B over 10 min at a flow rate of 1 ml/min.

Plasmids

[00104] The plasmids used in this study, except for human NPP2, have all been described in published reports: human NTPDase1 (GenBank accession No. U87967) (Kaczmarek *et al.*, 1996); human NTPDase2 (NM_203368) (Knowles and Chiang, 2003); human NTPDase3 (AF033830) (Smith and Kirley, 1998); human NTPDase8 (AY330313) (Fausther *et al.*, 2007); human NPP1 (NM_006208) (Belli and Goding, 1994); and human NPP3 (NM_005021) (Jin-Hua *et al.*, 1997). The unpublished human NPP2 (XXU13871, submitted to NCBI by JA Malone) was subcloned here in the expression vector pcDNA3.1.

Cell culture and transfection

[00105] HTB-85 and HT29 cell lines were grown in 10 cm-plates and were then transferred into 1 cm-plates and incubated at 37°C in α-MEM medium and Dulbecco's modified Eagle's medium/F-12 nutrient mixture (DMEM/F-12), respectively, in the

presence of 10% FBS. After reaching full confluence, cells were used in intact cell assays (see below).

[00106] Ectonucleotidases were produced by transiently transfecting COS-7 cells in 10-cm plates using Lipofectamine (Invitrogen, Burlington, ON, Canada), as previously described (Kukulski *et al.*, 2005). Briefly, 80-90% confluent cells were incubated for 5 h at 37°C in Dulbecco's modified Eagle's medium, nutriment mix F-12 (DMEM/F-12) in the absence of FBS with 6 μg of plasmid DNA and 23 μl of Lipofectamine reagent. The reaction was stopped by the addition of an equal volume of DMEM/F-12 containing 20% FBS and the cells were harvested 33-72 h later. The conditioned medium of NPP2-transfected cells was also collected.

[00107] Green fluorescent protein (GFP) constructs of human $P2Y_1$ and $P2Y_{11}$ receptors were expressed in 1321N1 astrocytoma cells, which lack endogenous expression of both P2X and P2Y receptors. The respective cDNA of the given receptor gene was cloned into the pEGFPN1 vector, and after transfection using the FuGENE 6 Transfection Reagent (Roche Molecular Biochemicals, Mannheim, Germany), cells were selected with 0.5 mg/ml G318 (Merck Chemicals, Darmstadt, Germany) and grown in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 U/ml streptomycin at 37°C and 5% CO_2. The functional expression of the receptor was confirmed by GFP fluorescence and the change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) upon incubation with the respective standard receptor agonists.

Preparation of protein fractions

[00108] For the preparation of protein extracts enriched in membrane proteins, transfected cells were washed three times with Tris-saline buffer at 4°C, collected by scraping in harvesting buffer (95 mM NaCl, 0.1 mM phenylmethylsulphonyl fluoride (PMSF) and 35 mM Tris at pH 7.5), and washed twice again by 300 g centrifugation for 10 min at 3°C. Cells were re-suspended in the harvesting buffer supplemented with 10 mg/ml of aprotinin, and sonicated. Nucleus and cellular debris were discarded by centrifugation at 300 g for 10 min at 3°C and the supernatant (crude protein extract) was aliquoted and stored at -80°C until used for activity assays. The secreted form of NPP2 was prepared from the conditioned media of transfected cells, which were frozen and stored at -80°C until tested for activity. Protein concentration was estimated by the Bradford microplate assay using bovine serum albumin (BSA) as standard (Bradford, 1976).

Enzymatic activity assays

[00109] ***NTPDases and eto-5'-nucleotidase***. Activity was measured as previously described (Kukulski *et al.*, 2005) in 0.2 ml of incubation medium Tris-Ringer buffer (in mM, 120 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_3$, 25 $NaHCO_3$, 5 glucose, 80 Tris, pH 7.3) at 37°C with or without analogues **1-4** (final concentration 100 µM), and with or without 100 µM ATP (for NTPDases) or 100 µM AMP (for ecto-5'-nucleotidase) as a substrate. The analogues were added without ATP or AMP when tested as potential substrate, and with ATP or AMP when tested for their effect on nucleotide hydrolysis. Either NTPDase or ecto-5'-nucleotidase protein extracts were added to the incubation mixture and pre-incubated at 37°C for 3 min. The reaction was initiated by the addition of a substrate (ATP, AMP or one of analogues **1-4**) and stopped after 15 min with 50 µl of malachite green reagent. The released inorganic phosphate (P_i) was measured at 630 nm as previously described (Baykov *et al.*, 1988).

[00110] ***NPPs***. Evaluation of the effect of compounds **1-4** on human NPP1, -2 and -3 activity was carried out either with pnp-TMP, ATP or Ap_5A as a substrate (Belli and Goding, 1994). The reactions were carried out at 37°C in 0.2 ml of the following incubation mixture: in mM, 1 $CaCl_2$, 130 NaCl, 5 KCl and 50 Tris, pH 8.5, with or without compounds **1-4** and/or substrates. Substrates and compounds **1-4** were all used at a final concentration of 100 µM. Recombinant human NPP1, -2 or -3 cell lysates, as well as soluble proteins containing the secreted form of NPP2, were added to the incubation mixture and preincubated at 37°C for 3 min. The reaction was initiated by addition of the substrate. For pnp-TMP hydrolysis, the production of *p*-nitrophenol was measured at 405 nm, 15 min after the initiation of the reaction. For Ap_5A and ATP, the reaction was stopped after 30 min by transferring an aliquot of 0.1 ml from the reaction mixture to 0.125 ml ice-cold 1 M perchloric acid. The samples were centrifuged for 5 min at 13,000 x *g*. Supernatants were neutralized with 1 M KOH (3°C) and centrifuged for 5 min at 13,000 x *g*. An aliquot of 20 µl was separated by reverse-phase HPLC to evaluate the nucleotide content of each reaction sample (see below). The type of inhibition, IC_{50} and K_i, were calculated by plotting the data of three independent experiments using pnp-TMP as a substrate according to Dixon and Cornish-Bowden kinetics.

[00111] ***Separation and quantification of nucleotides and dinucleotides by HPLC***. An aliquot of 20 µl of the reaction products (described above) was used for nucleotide analysis by HPLC using a 15 cm x 3.6 mm, 3 µm SUPELCOSIL™ LC-18-T column (Supelco,

Bellefonte, PA). ATP, Ap_5A, analogues **1-4** and their hydrolysis products were separated with a mobile phase made of 25 mM TBA, 5 mM EDTA, 100 mM KH_2PO_3/K_2HPO_3, pH 7.0 and 2% methanol (v/v), at a flow rate of 1 ml/min for 30 min. Separated nucleotides were detected by UV absorption at 260 and 253 nm, identified and quantified by the comparison of the retention time with the appropriate standards.

[00112] ***Activity assays with intact HTB-85 and HT29 cell lines.*** For intact cells, activity assays at the cell surface were carried out in 0.25 ml of the incubation mixture containing 135 mM NaCl in 24-well plates. Reaction was initiated by the addition of pnp-TMP to obtain a final concentration of 100 μM. After 20 min, 0.2 ml of the reaction mixture was transferred to a 96-well plate and the production of *p*-nitrophenol was measured at 310 nm.

Calcium measurements

[00113] 1321N1 astrocytoma cells were transfected with the respective plasmid for P2YR-GFP expression, i.e., pEGFPN1 expression vector plasmids encoding the cDNA for human $P2Y_1$ or $P2Y_{11}$ receptors (Ecke *et al.*, 2006), and the $P2Y_2$ receptor (Ginsburg-Shmuel *et al.*, 2010; Tulapurkar *et al.*, 2004), respectively. Cells plated on cover slips (22-mm diameter) and grown to approximately 80% density were incubated with 2 μM fura 2/AM and 0.02% pluronic acid in HEPES-buffered saline (in mM, 135 NaCl, 5.3 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 25 glucose, 20 HEPES/Tris, pH 7.3) for 30 min at 37°C. Cells were superfused (1 ml/min, 37°C) with different concentrations of compounds **1-4** and the change in $[Ca^{2+}]_i$ was monitored from the respective emission intensity at 510 nm after alternating between 330 nm and 380 nm as excitation wavelengths (Ubl *et al.*, 1998). Concentration-response data were analyzed with the SigmaPlot software (SPSS Inc., Chicago, IL, USA) using the ratio of the fluorescence intensities with 330 nm and 380 nm excitation wavelengths (Ecke *et al.*, 2006; Ecke *et al.*, 2008).

Example 1. Synthesis of dinucleoside polyphosphonate analogues 1-4

[00114] Dinucleoside polyphosphates are conventionally prepared via the activation of the 5'-terminal phosphate of a nucleotide, thus forming a phosphoryl donor (P-donor), followed by reaction with a non-activated nucleoside 5'-phosphate, or phosphonate analogue (phosphoryl acceptor; P-acceptor). A common method for activation of phosphates/phosphonates uses CDI to form phosphoroimidazolides. The latter may be generated *in situ* or isolated prior to the reaction with the corresponding nucleotides (Zatorski *et al.*, 1995). We had previously synthesized diadenosine (γ-borano)penta-

phosphate, **5**, using an inorganic boranophosphate salt (BP_i) (Nahum and Fischer, 2004) as a P-acceptor and two nucleoside phosphoroimidazolides (NDP-Im) as P-donors (Nahum *et al.*, 2006). Here, we used the same synthetic method to prepare the corresponding phosphonate analogues, **1** and **2**.

[00115] As depicted in **Schemes 1** and **2**, respectively, diadenosine α,β-δ,ϵ-dimethylene-pentaphosphonate, **1**, and di-2'-deoxyadenosine α,β-δ,ϵ-dimethylene-pentaphosphonate, **2**, were prepared as described in the Experimental from the α,β-methylene-ADP building blocks, **9**, and α,β-methylene-2'-deoxy-ADP, **13**, respectively. α,β-Methylene-ADP derivatives were synthesized as previously described (Davisson *et al.*, 1987). As specifically shown in **Scheme 1**, adenosine analogue **6**, which is 2' and 3'-OH protected, was activated with tosyl chloride to form the activated nucleoside **7**, which was then coupled with a tris(tetra-*n*-butylammonium)methylene diphosphonate salt to form analogue **8**, followed by removal of the protecting group, which provided product **9**. As shown in **Scheme 2**, the related scaffold **13** was prepared from 2'-deoxyadenosine. A selective tosylation at the 5'-OH position of **11** was carried out at 0°C to form product **12**, which was then coupled with a tris(tetra-n-butylammonium) methylenediphosphonate salt to yield product **13** (Liang *et al.*, 2008).

[00116] Nucleotides **9** and **13** were activated with CDI *in situ* to form P-donors **10** and **14**, respectively, which were then treated with BP_i, **15**, as a P-acceptor. $MgCl_2$ was added as an activator to overcome the low nucleophilicity of BP_i as a P-acceptor (Hoard and Ott, 1965). Compounds **1** and **2** were obtained in 10% and 21% overall yields, respectively, after LC separation.

[00117] Dinucleoside poly(borano)phosphonate **1** and **2** are probably formed due to a preorganization of a P-acceptor (BP_i) and two P-donors (nucleoside phosphorimidazolides) coordinated with one Mg^{2+} ion, as shown in **Fig. 1**. Specifically, the Mg^{2+} ion probably stabilized the folded structure, which involved two molecules of α,β-methylene-ADP-imidazolide (Im) or α,β-methylene-2'-deoxy-ADP-Im and one BPi ion. This structure provided the correct orientation and proximity for a nucleophilic attack of both nucleoside phosphoroimidazolide reactants by BP_i in an octahedral complex (**Fig. 1**, upper left side, paths a and b). Although we expected to obtain analogues **1** and **2** as exclusive products, by-products **3** and **4** were also isolated with 20% and 28% yields, respectively. The formation of **3** and **4** products is driven by α,β-methylene-ADP, **9**, and α,β-methylene-2'-deoxy-ADP, **13**, which remained in the reaction mixture due to incomplete reaction with

CDI. Thus, the activated forms α,β-methylene-ADP-Im, **10**, and α,β-methylene-2'-deoxy-ADP-Im, **14**, become P-donors, whereas α,β-methylene-ADP, **9**, and α,β-methylene-2'-deoxy-ADP, **13**, rather than BP_i, function as P-acceptors (**Fig 1**, upper right side, path c). Furthermore, since the phosphonate is assumed to be a better nucleophile than BP_i, **15**, byproducts **3/4** are obtained and not adenosine-α,β-CH_2-γ-borano-triphosphate, **16**.

[00118] The identity and purity of compounds **1-4** were established by ^1H and ^{31}P NMR, ESI or MALDI negative mass spectrometry, and HPLC in two solvent systems. ^{31}P NMR spectra of compounds **1** and **2** showed a typical P_γ signal as a multiplet at about 80 ppm in addition to two phosphonate signals at 20 and 10 ppm. ^1H NMR spectra showed borane hydrogen atoms as a very broad signal at ~0.3 ppm, and a typical triplet at 2.3 ppm of the bridging methylene group.

Example 2. The effect of analogues 1-4 on ectonucleotidase activity and on recombinant ectonucleotidases

[00119] The experiments were carried out with protein extracts of COS-7 cells transfected with an expression vector encoding one of each ectonucleotidase or, alternatively, with intact cell lines exhibiting NPP activity. In addition, medium from NPP2-transfected cells was assayed for activity to test the secreted form of this enzyme. The protein extracts and media of non-transfected COS-7 cells exhibited a negligible level of NTPDase and NPP activity, thus allowing the analysis of each ectonucleotidase in its native membrane-bound form (Lévesque *et al.*, 2007).

[00120] As shown in **Table 1**, none of the analogues **1-4** was metabolized by human NTPDases or ecto-5'-nucleotidase, and all of them were modestly hydrolyzed by NPPs. While analogues **1** and **3** were hydrolyzed by NPP1 and -3 at ~7.5-13% of the level observed for ATP hydrolysis, analogues **2** and **4** were more resistant to hydrolysis. As shown in **Fig. 2**, the hydrolysis of ATP by NTPDase1 and -8 was not affected by any of the analogues **1-4** when used at the same concentration as the substrate (100 μM). NTPDase2 and -3 were modestly inhibited (10-30%) by these analogues. While analogues **3** and **4** inhibited ecto-5'-nucleotidase activity by 90 and 80%, respectively, analogues **1** and **2** did not affect the latter enzymatic activity.

[00121] The effect of analogues **1-4** on NPP activities was tested using synthetic (pnp-TMP) and natural substrates (Ap_5A and ATP). The level of hydrolysis of pnp-TMP by NPP1 was decreased by over 90% by all compounds tested, as shown in **Fig. 3A**. In the

presence of analogues **1-4**, the hydrolysis of pnp-TMP by NPP2 was similarly blocked at ~95%, as shown in **Fig. 3B**, and at 60-70% by the secreted form of NPP2 (data not shown). The activity of NPP1 with Ap_5A as the substrate was reduced by 60-80% by analogues **1**, **2** and **4**, and by 20% by analogue **3**. When using ATP as the substrate, NPP1 was inhibited by ~70-80% in the presence of analogues **2** and **3**, and by more than 90% by analogues **1** and **4** (**Figs. 3C-3D**). The presence of analogues **1-4** reduced the hydrolysis of pnp-TMP by NPP3 by ~30% (**Fig. 3A**), and the hydrolysis of Ap_5A by ~10-60% (**Fig. 3C**). The inhibition of NPP3 activity using ATP as the substrate was more pronounced (~90%) in the presence of analogues **1** and **4**, and around 65% with analogues **2** and **3** (**Fig. 3D**).

Table 1: Hydrolysis of analogues **1-4** by human ectonucleotidases

Human ectonucleotidases (substrate used)	**Relative activity (%±SD of ATP, AMP or Ap_5A hydrolysis)**			
	analogues			
	1	**2**	**3**	**4**
NTPDase1 (ATP)	0.5 ± 0.02	ND	1.5 ± 0.1	1.1 ± 0.04
NTPDase2 (ATP)	ND	0.2 ± 0.01	0.1 ± 0.01	ND
NTPDase3 (ATP)	ND	ND	5.3 ± 0.2	1.8 ± 0.1
NTPDase8 (ATP)	ND	ND	ND	ND
ecto-5'-nucleotidase (AMP)	ND	ND	ND	ND
NPP1 (Ap_5A)	7.5 ± 0.3	ND	13 ± 0.6	2.3 ± 0.1
NPP3 (Ap_5A)	12 ± 0.6	5.5 ± 0.2	11 ± 0.6	5.5 ± 0.3

Dinucleotide analogues **1-4** (each at 100 μM) were incubated with the indicated ectonucleotidases. The activity with 100 μM ATP (for NTPDases) or AMP (for the ecto-5'-nucleotidase) was set as 100%: 1270±35; 928±55; 202±37; 129±11; and 357±10 nmol Pi min^{-1} (mg $protein^{-1}$) for NTPDase1, -2, -3, and -8, and ecto-5'-nucleotidase, respectively. 100% of the activity with 100 μM Ap_5A as a substrate was 71±5 and 98±9 nmol of nucleotide min^{-1} (mg $protein^{-1}$) for NPP1 and NPP3, respectively (n=3). ND = no hydrolysis detected.

[00122] As analogues **1-4** significantly reduced the activity of human NPP1, we have tested IC_{50} values as well as some kinetic parameters (K_i and K_i') of the inhibition with pnp-TMP as the substrate. IC_{50} values were similar for all tested analogues, on the order of 10-60 μM, while inhibition constants (K_i) were in the range of 10-50 μM, always smaller than (enzyme-substrate)-inhibitor dissociation constants (K_i) that were in the range of 30-150 μM. The lowest K_i value was observed for analogue **2**, as shown in **Table 2**. Kinetic analysis of K_i and K_i' using the Dixon and Cornish-Bowden methods showed a mixed-type, predominantly competitive, inhibition of NPP1 by all tested analogues (data not shown).

Table 2: IC_{50}, K_i and K_i' analysis of the inhibition of NPP1 by analogues **1-4**

Analouge	IC_{50}±SD [μM]	K_i±SD [μM]	K_i'±SD [μM]
1	63±4.4	20±1.4	31±1.5
2	13±0.7	9±0.5	145±6.9
3	33±1.7	13±0.7	147±7.2
4	50±2.0	51±2.5	80±3.8

For the determination of K_i and K_i', pnp-TMP substrate and analogues **1-4** were used in the concentration range of $2.5x10^{-5}$ to $1x10^{-4}$ M. For the determination of IC_{50}, the pnp-TMP concentration was $5x10^{-5}$ M and the concentrations of the inhibitors were in the range of $5x10^{-7}$ to $1x10^{-3}$ M. All experiments were performed three times in triplicate.

Example 3. The effect of analogues 1-4 on NPP activity at the surface of two cell lines

[00123] NPP1 is critical in regulating mineralization by generating inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal growth. On the other hand, NPP3 is associated with carcinogenesis. Human osteoblastic SaOS-2 cells (HTB-85) are used to investigate the activity of NPP1 (Vaingankar *et al.*, 2004), as well as HT29, a human colon cancer cell line (Baricault *et al.*, 1995). HTB-85 and HT29 catabolized pnp-TMP, indicating the presence of NPPs at their surface. As for the enzymes obtained from cell extract, NPP activity exhibited by both cell lines was blocked by ~90% by analogues **1** and **2**, and by about 80% by analogues **3** and **4**, as shown in **Fig. 4**.

Example 4. The activity of analogues 1-4 on the $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ receptors

[00124] GFP constructs of human $P2Y_1$ and $P2Y_{11}$ receptors were expressed in 1321N1 astrocytoma cells, which lack endogenous expression of both P2X and P2Y receptors. The cells were then incubated with various concentrations of analogues **1-4**, and the Ca^{2+} response to each one of the analogues was compared with that due to ATP, as shown in **Figs. 5A-5B**.

[00125] As shown in **Table 3**, analogues **2-4** were weak agonists of the $P2Y_1$ receptor. Analogue **1** was 2-fold less potent than the standard agonist ATP (EC_{50}=0.15 μM) and 30-fold more potent than the Ap_4A derivative **3** (EC_{50}=9 μM). The 2'-deoxy analogues **2** and **4** exhibited comparably weak activities with EC_{50} values of ≳30 μM for the $P2Y_1$ receptor. No clear plateau was reached up to 100 μM for analogue **2**.

[00126] Analogue **1** also exhibited the highest $P2Y_{11}$ receptor agonist potency (EC_{50}=13 μM) among the diadenosine polyphosphate analogues. The maximal response reached ~80% of that obtained with the standard agonist ATP (EC_{50}=3.3 μM), but with a 4-

fold lower potency. Analogue **3** was found to be a very weak agonist of the $P2Y_{11}$ receptor, with an $EC_{50} \geq 40$ μM, whose maximal response corresponded only to 15% of that of ATP. The 2'-deoxy analogues **2** and **4** were both inactive at concentrations ≤ 50 μM. Analogues **1-4** were completely inactive toward the $P2Y_2$ receptor at concentrations ≤ 25 μM.

Table 3: EC_{50} values for $[Ca^{2+}]_i$ elevation by analogues **1-4**, mediated by the $P2Y_{1,2,11}$ receptors

Receptor subtype	Analouge	EC_{50} (μM) $[Ca^{2+}]_i$ elevation	Reduction of affinity vs. ATP
$P2Y_1$	**4**	30	200
	2	≥30	≥200
	3	9	60
	1	0.3	2
	ATP[a]	0.15	1
$P2Y_2$	**1-4**	not active up to 25 μM	-
$P2Y_{11}$	**4**	not active up to 50 μM	-
	2	not active up to 50 μM	-
	3	≥40 (~15% of ATP)	-
	1	13	4
	ATP[a]	3.3	1

[a] ATP was selected as the common reference agonist at both $P2Y_1$ and $P2Y_{11}$ receptors, although ADP is the preferred endogenous $P2Y_1$ receptor agonist.

Example 5. Adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 23, separation and characterization

[00127] The separation of the diastereomeric pair of **23**, obtained as described in WO 2009/066298, was accomplished using a semi-preparative reverse-phase Gemini 5u column (C-18 110A, 250×10.00 mm, 5 micron) and isocratic elution using Solvent System I, by applying (A) 100 mM TEAA, pH 7 to (B) MeOH, at a flow rate of 5 ml/min, at 89:11 A:B at a flow rate of 5 ml/min, followed by a final separation of the two diastereoisomers using an analytical Gemini 5u column (C-18 110A, 150×4.60 mm) by applying Solvent System I with a gradient from 90:10 to 70:30 A:B over 20 min at a flow rate of 1 ml/min. Fractions containing the same isomer [Rt = 6.33 min (isomer A), 7.73 min (isomer B)] were collected and freeze-dried. The excess buffer was removed by repeated freeze-drying cycles with the solid residue dissolved each time in deionized water. Diastereoisomers **23A** and **23B** were obtained in 36% overall yield after LC separation.

Adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 23A, characterization

[00128] Retention time on a semi-preparative column: 7.64 min. ^{1}H NMR (D_2O, 600 MHz): δ 8.59 (s, H-8, 1H), 8.25 (s, H-2, 1H), 6.14 (d, J = 4.8 Hz, H-1', 1H), (H2' signal is hidden by the water signal at 4.78 ppm), 4.60 (m, H-3', 1H), 4.39 (m, H-4', 1H), 4.27 (m, H-5', 1H), 4.14 (m, H-5", 1H), 2.25 (t, J = 20.4 Hz, CH_2, 2H), 0.37 (m, BH_3, 3H) ppm. ^{31}P NMR (D_2O, 600 MHz): δ 82.81 (m, P_α-BH_3), 13.92 (s, P_γ), 11.22 (br s, P_β) ppm. MS-ESI *m/z*: 502 (M^-). TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.23. Purity data obtained on an analytic column: Retention time: 3.55 min (100% purity) using Solvent System I with a gradient from 90:10 to 70:30 A:B over 10 min at a flow rate of 1 ml/min). Retention time: 2.53 min (95.5% purity) using Solvent System II, a gradient from 90:10 to 80:20 of (A) 0.01 M KH_2PO_4, pH = 4.5 to (B) MeOH over 10 min at a flow rate of 1 ml/min).

Adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 23B, characterization

[00129] Retention time on a semi-preparative column: 9.67 min. ^{1}H NMR (D_2O, 300 MHz): δ 8.56 (s, H-8, 1H), 8.24 (s, H-2, 1H), 6.14 (d, J = 5.1 Hz, H-1', 1H), (H2' signal is hidden by the water signal at 4.78 ppm), 4.52 (m, H-3', 1H), 4.39 (m, H-4', 1H), 4.23 (m, H-5', 1H), 4.17 (m, H-5", 1H), 2.30 (t, J = 20.10 Hz, CH_2, 2H), 0.40 (m, BH_3, 3H) ppm. ^{31}P NMR (D_2O, 600 MHz): δ 82.50 (m, P_α-BH_3), 14.10 (s, P_γ), 11.03 (br s, P_β) ppm. MS-ESI *m/z*: 502 (M^-). TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.23. Purity data obtained on an analytic column: Retention time: 4.09 min (92.6% purity) using Solvent System I with a gradient from 90:10 to 70:30 A:B over 10 min at a flow rate of 1 ml/min). Retention time: 3.66 min (95.5% purity) using Solvent System II with a gradient from 95:10 to 80:20 A:B over 10 min at a flow rate of 1 ml/min).

Example 6. 2-MeS-adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 24, separation and characterization

[00130] The separation of the diastereomeric pair of **24**, obtained as described in WO 2009/066298, was accomplished using a semi-preparative reverse-phase Gemini 5u column (C-18 110A, 250×10.00 mm, 5 micron), and isocratic elution by applying Solvent System I (see Example 5) at 75:25 A:B at a flow rate of 5 ml/min. Final separation of the two diastereoisomers was achieved using an analytical Gemini 5u column (C-18 110A, 150×4.6 mm) and Solvent System I with a gradient from 82:18 to 74:26 A:B over 20 min at a flow rate of 1 ml/min. Fractions containing the same isomer [Rt = 9.79 min (isomer A), 11.53 min (isomer B)] were collected and freeze-dried. The excess buffer was removed

by repeated freeze-drying cycles with the solid residue dissolved each time in deionized water. Diastereoisomers **24A** and **24B** were obtained in 28% overall yield after LC separation.

2-MeS-adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 24A, characterization.

[00131] Retention time on a semi-preparative column: 5.29 min. ^{1}H NMR (D_2O, 600 MHz): δ 8.30 (s, H-8, 1H), 6.12 (d, J = 4.98 Hz, H-1', 1H), (H2' signal is hidden by the water signal at 4.78 ppm), 4.50 (m, H-3', 1H), 4.25 (m, H-4', 1H), 4.14 (m, H-5', 1H), 4.05 (m, H-5", 1H), 2.95 (s, CH_3, 3H), 2.17 (t, J = 20.10 Hz, CH_2, 2H), 0.42 (m, BH_3, 3H) ppm. ^{31}P NMR (D_2O, 600 MHz): δ 83.60 (m, P_α-BH_3), 14.61 (s, P_γ), 10.26 (br s, P_β) ppm. MS-ES *m/z*: 548 (M^-). TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.44. Purity data obtained on an analytic column: Retention time: 4.24 min (94.3% purity) using Solvent System I with a gradient from 80:20 to 60:40 A:B over 10 min at a flow rate of 1 ml/min). Retention time: 2.99 min (99.5% purity) using Solvent System II (see Example 5) with a gradient from 75:25 to 65:35 A:B over 10 min at a flow rate of 1 ml/min).

2-MeS-adenosine-β,γ-CH_2-5'-O-(α-borano-triphosphate), 24B, characterization

[00132] Retention time on a semi-preparative column: 5.57 min. ^{1}H NMR (D_2O, 600 MHz): δ 8.29 (s, H-8, 1H), 6.99 (m, H-1', 1H), (H2' signal is hidden by the water signal at 4.78 ppm), 4.47 (m, H-3', 1H), 4.27 (m, H-4', 1H), 4.15 (m, H-5', 1H), 4.08 (m, H-5", 1H), 2.49 (s, CH_3, 3H) 2.18 (t, J = 19.20 Hz, CH_2, 2H), 0.32 (m, BH_3, 3H) ppm. ^{31}P NMR (D_2O, 600 MHz): δ 84.13 (m, P_α-BH_3), 14.85 (s, P_γ), 10.04 (br s, P_β) ppm. MS-ESI *m/z*: 548 (M^-). TLC (NH_4OH:H_2O:isopropanol 2:8:11), R_f = 0.44. Retention time: 2.12 min (94% purity) using a gradient of (A) 100 mM TEAA, pH 7 to (B) CH_3CN from 70:30 to 40:60 A:B over 10 min at a flow rate of 1 ml/min). Retention time: 1.38 min (100% purity) using Solvent System II with a gradient from 50:50 to 40:60 A:B over 10 min at a flow rate of 1 ml/min).

Example 7. The effect of analogues 22-24 on human ectonucleotidase activity, NPP1 and NPP3 activity, and on the $P2Y_1$, $P2Y_2$ and $P2Y_4$ and $P2Y_6$ receptors

[00133] Ectonucleotidases were produced by transiently transfecting 293T cells using Lipofectamine (Invitrogen, Burlington, ON, Canada), and protein fractions were prepared as described in Experimental.

[00134] NTPDase activity was measured as previously described (Kukulski *et al.*, 2005) in 0.2 ml of Tris-Ringer buffer (in mM 120 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 5 glucose, 80 Tris, pH 7.4) at 37°C with or without analogues **21-24**. NTPDase protein extracts were added to the incubation mixture and pre-incubated at 37°C for 3 min. The reaction was initiated by the addition of the substrate (ATP, ADP or one of the analogues) to a final concentration of 100 μM and stopped after 20 min with 50 μl of malachite green reagent. The released inorganic phosphate (P_i) was measured at 630 nm according to Baykov *et al.* (1988). The activity obtained with protein extracts from untransfected cells was subtracted from the activity obtained with extracts from NTPDase transfected cells. The activity with this control protein extract did not exceed 5% of the activity of any NTPDase extract.

[00135] Evaluation of the inhibition of human NPP1 and NPP3 activities by each one of the analogues was carried out with pnp-TMP as the substrate, as previously described previously. Reactions were carried out at 37°C in 0.2 ml of the following incubation mixture, in mM 1 $CaCl_2$, 140 NaCl, 5 KCl, and 50 Tris, pH 8.5, with or without an analogue (100 μM). Human NPP1 or NPP3 extract was added to the incubation mixture and pre-incubated at 37°C for 3 min. Reaction was initiated by the addition of the indicated substrate (pnp-TMP or an analogue) at the final concentration of 100 μM. For pnp-TMP, the production of p-nitrophenol was measured at 410 nm, 20 min after the initiation of the reaction. When one of the analogues was used as a substrate, the reaction was stopped after 20 min by transferring a 0.1 ml aliquot from the reaction mixture to 0.125 ml ice-cold 1 M perchloric acid. These samples were centrifuged for 5 min at 13,000 g. Supernatants were neutralized with 1 M KOH (4°C) and centrifuged for 5 min at 13,000 g. An aliquot of 20 μl was separated by reverse-phase HPLC to evaluate the decrease in the analogue level. Protein extracts from non-transfected cells did not show any NPP activity. After NPP1 and NPP3 enzymatic reactions, the substrates and their products were separated on a SUPELCOSIL™ LC-18-T column (15 cm x 4.6 mm, 3 μm Supelco, Bellefonte, Pennsylvania, USA) with a mobile phase composed of 25 mM TBA, 5 mM EDTA, 100 mM KH_2PO_4/K_2HPO_4, pH 7.0 and 2% (v/v) methanol at a flow rate of 1 ml/min.

[00136] As shown in **Fig. 6**, in comparison to the physiological substrate ATP, analogues **22-24** were almost not hydrolyzed by NTPDases. Analogue **22** was the most efficiently hydrolyzed analogue by NTPDases, and was hydrolyzed by human NTPDase1, 3 and 8 at

about 7-8% of the rate of ATP and by human NTPDase2, at about 2% of the rate of ATP. As shown in **Fig. 7**, NPP1 hydrolyzed **22** at 20% the rate of pnp-TMP hydrolysis, while human NPP3 hydrolyzed **22** and **24A**, at 10% of the rate of pnp-TMP hydrolysis. The other analogues were hydrolyzed by these ectonucleotidases at less than 5% of the rate of pnp-TMP hydrolysis.

[00137] In general, analogues **22-24** did not affect significantly the activity of NTPDases. A weak inhibition of ATP hydrolysis by human NTPDase1 (17%) was observed when equal concentrations of the substrate ATP and **23** were used, and similar levels of inhibition of human NTPDase3 activity were obtained with **22** (17%), **24A** (16%) and **24B** (18%), as shown in **Fig. 8**. Similar inhibition profiles were obtained for analogues **22-24** with ADP as a substrate (data not shown).

[00138] **Fig. 9** shows that all analogues **22-24** inhibited the hydrolysis of pnp-TMP by human NPP1. The molecule with the weakest inhibitory properties was **22** that blocked 66% of NPP1 activity and the most potent inhibitor was **23** that inhibited 93% of the hydrolysis of pnp-TMP. The activity of human NPP3 was more modestly inhibited (between 15-23%) by the analogues **22-24**.

[00139] The activities of analogues **22-24** were further examined at the G protein-coupled P2YRs, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$, expressed in human 1321N1 astrocytoma cells. As shown in **Table 4**, analogues **22** and **24B** were agonists of the $P2Y_1R$ with EC_{50}'s of 0.08 and 17.2 μM, respectively, as compared to 0.004 μM for 2-MeSADP, and were virtually ineffective agonists of $P2Y_2R$, $P2Y_4R$ and $P2Y_6R$. Analogues **23A, 23B** and **24A** had insignificant activities at all the P2YRs tested.

Table 4: Activity of analogues **22**, **23** and **24** at $P2Y_{1/2/4/6}R$

Analogue	Compound-induced activation of $[Ca^{2+}]$ EC_{50} (μM) values			
	$P2Y_1$	$P2Y_2$	$P2Y_4$	$P2Y_6$
22	0.08±0.03	nr	nr	sr
23A	nr	nr	nr	nr
23B	nr	nr	nr	sr
24A	nr	nr	nr	nr
24B	17.2±5.3	nr	nr	sr
2-MeS-ADP	0.004±0.002			
UTP		0.64±0.25	0.48±0.31	
UDP				0.20±0.06

* sr = slight response at 100 μM; nr = no response

Example 8. *In vivo* models for evaluating NPP1 inhibitors activity

[00140] Several animal models to study crystal deposition diseases have been reported. Unfortunately, there are no such models that are totally satisfactory for mimicking osteoarthritis symptoms in humans, but may be useful for studying osteoarthritis aspects like CPPD decrease in deposition.

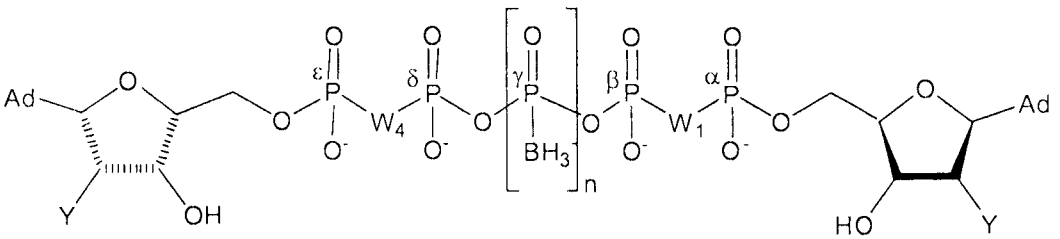
[00141] First promising animal model comes from the use of guinea pigs (Bendele *et al.*, 1989). These animals spontaneously develop extensive calcification of the meniscus. The animals start calcification from birth under gene control, but it is speculated that direct intervention with the drug will slow the progression of the chronic crippling arthritic condition. The guinea pig model is currently being used to ascertain changes in calcifying meniscus and articular cartilage degeneration in the presence of both salt forms of phosphocitrate (Sallis and Cheung, 2003).

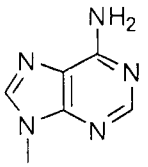
[00142] Mouse phenotypes express pathologic calcification in soft tissues and articular cartilage, allowing insights into the extra- and intracellular events associated with osteoarthritis (Masuda and Hirose, 2002). Using a murine progressive ankylosis model of ank/ank mice, Krug et al. (1993) have reported a therapeutic effect of phosphocitrate to decrease calcification before the disease is fully established. Histology and clinical function tests (e.g., ability to cling to cage wire) all confirmed an impressive improvement in the disease progression. This model itself has limitations for interpreting the role of crystals in osteoarthritis because it more closely resembles reactive arthritis rather than the degenerative disease.

[00143] Using a different experimental model (the rat air pouch model), new studies conclude that increasing levels of low density lipoprotein might be beneficial in CPPD crystal-induced inflammation (Kumagai *et al.*, 2001). In associated calcific studies in the rat with phosphocitrate, the response to a new and even more potent formulation of this compound recently was reported (Demadis et al., 2001). This model allowed long-term studies as a calcium-sodium salt of phosphocitrate (as opposed to the neutralized tetrasodium form) is several times stronger in blocking calcification of a chemically induced calcific plaque in rats.

[00144] All publications mentioned in this Example are herewith incorporated by reference in their entirety as if fully described herein.

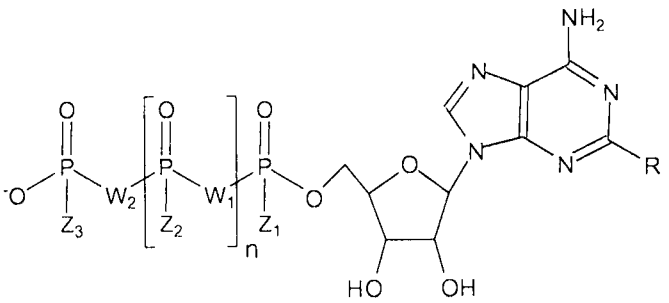
APPENDIX A





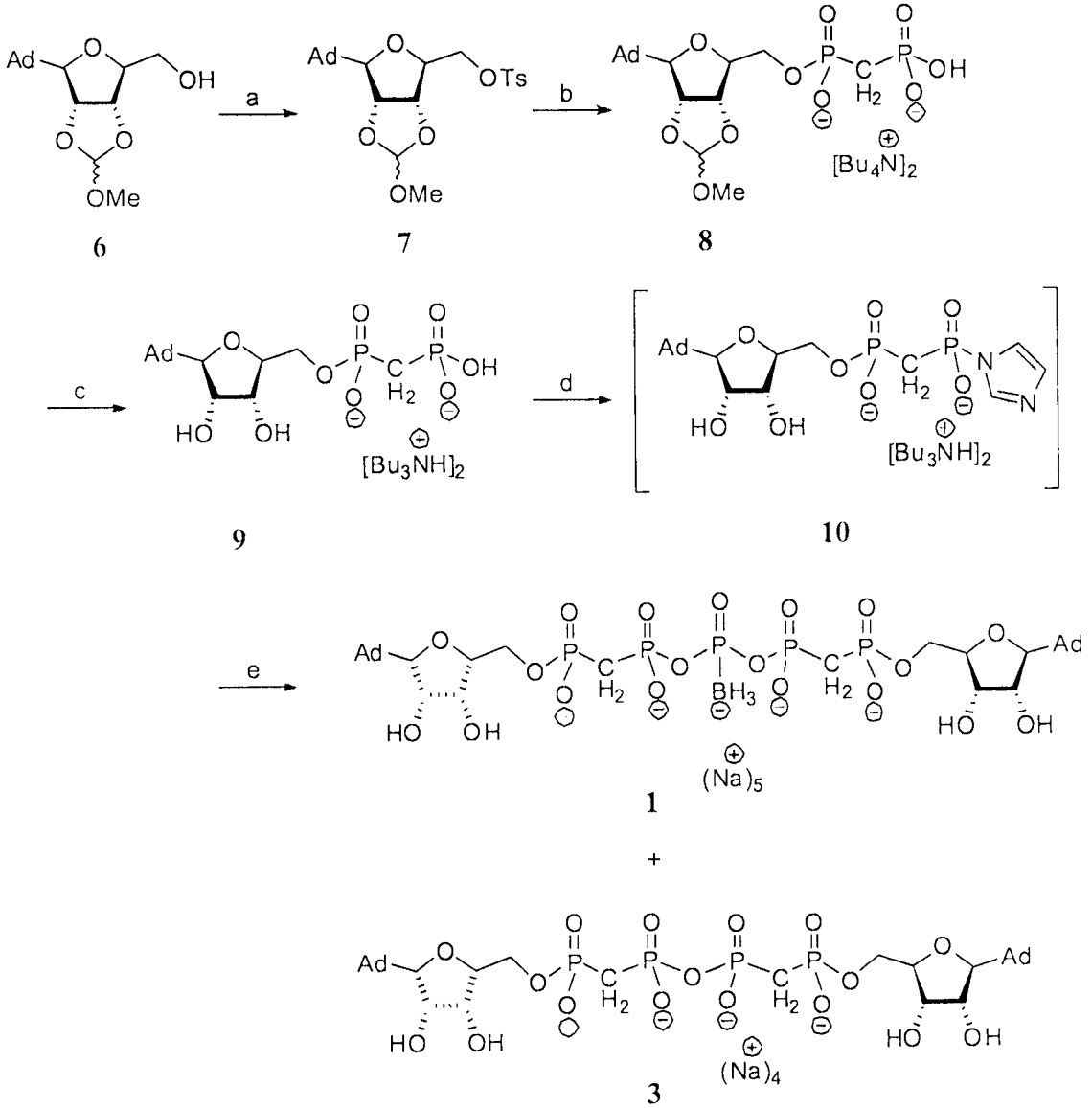
Ad

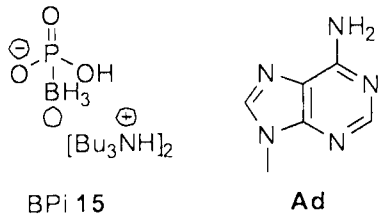
Compound	Y	W_1	W_4	n
1	OH	CH_2	CH_2	1
2	H	CH_2	CH_2	1
3	OH	CH_2	CH_2	0
4	H	CH_2	CH_2	0



Compound	R	Z_1	Z_2,Z_3	W_1	W_2	n
21	SMe	BH^{3-}	O^-	O	O	1
22	SMe	O^-	O^-	O	CH_2	1
23	H	BH^{3-}	O^-	O	CH_2	1
24	SMe	BH^{3-}	O^-	O	CH_2	1

Scheme 1: Synthesis of compounds **1** and **3**



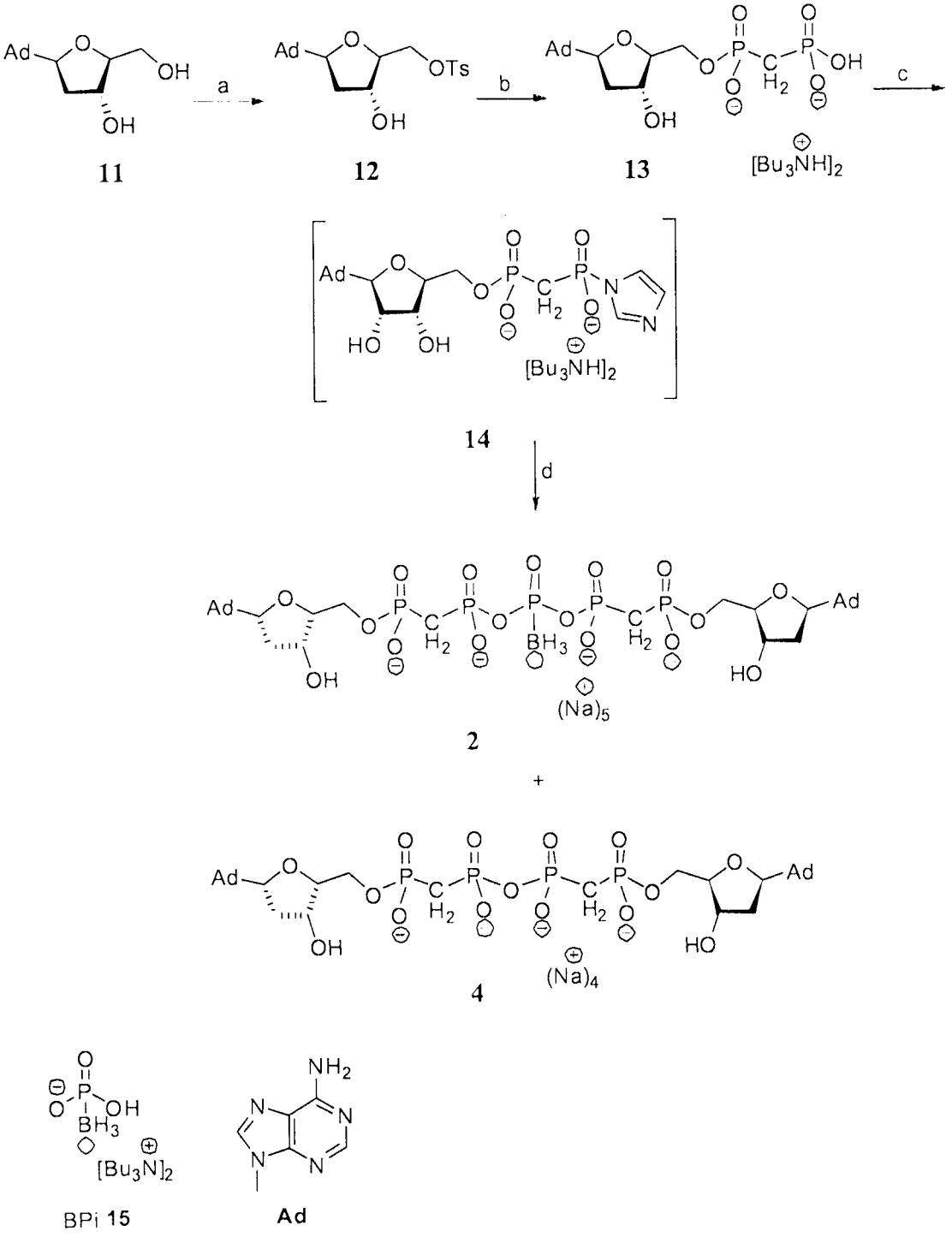


BPi **15** **Ad**

Reaction conditions

a) CH_2Cl_2, DMAP, TsCl, RT, 12 h, 67%; **b)** Tetra-(n-butylammonium)methyl enediphosphonate, dry DMF, RT , 38 h, 63%; **c)** 1) 18% HCl, pH 2.3, RT, 3 h; and 2) 23% NH_4OH, pH 9, RT, 35 min, 63%; **d)** CDI, DMF, RT, 12 h; **e)** BP_i, **15**, $MgCl_2$, RT, 23 h, **1** and **3** were obtained in 10 and 20% yields, respectively.

Scheme 2: Synthesis of compounds **2** and **4**



Reaction conditions

a) Pyridine, DMAP, TsCl, 0°C, 3 h, 62%; **b**) Tetra-(n-butylammonium)methyl enediphosphonate, dry DMF, RT , 38 h, 62%; **c**) CDI, DMF, RT, 12 h; **d**) BP_i, **15**, $MgCl_2$, RT, 23 h, **2** and **4** were obtained in 21 and 28% overall yield of step c and d, respectively.

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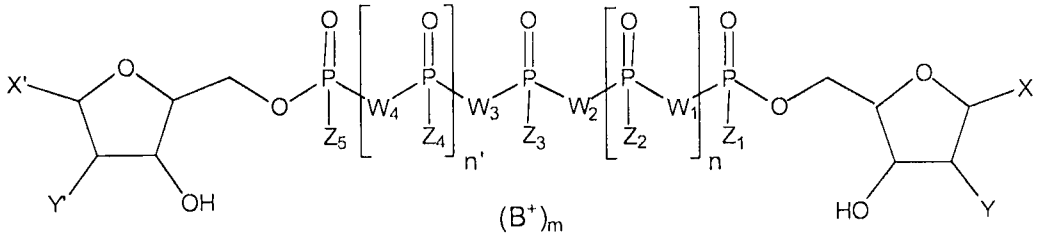
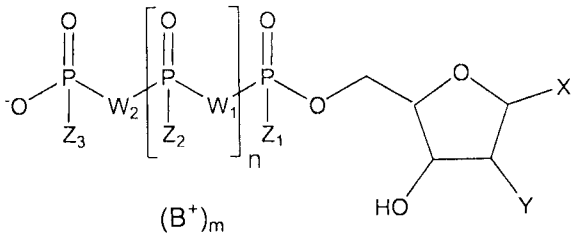
CLAIMS

1. A pharmaceutical composition for treatment of osteoarthritis comprising a pharmaceutically acceptable carrier and either a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II:

I



II

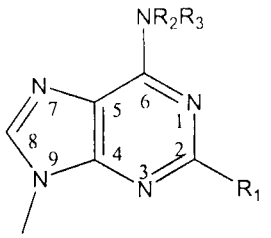


or a diastereomer or mixture of diastereoisomers thereof,

wherein

X and X' each independently is an adenine residue of the formula Ia, linked through the 9-position:

Ia

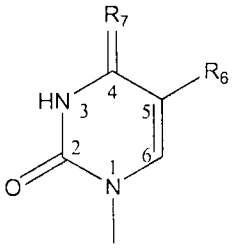


wherein

R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected from halogen, -CN, -SCN, $-NO_2$, $-OR_4$, $-SR_4$, $-NR_4R_5$ or heteroaryl, wherein R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_2 and R_3 each independently is H or hydrocarbyl;

or X and X' each independently is an uracil residue of the formula Ib, linked through the 1-position:

Ib 

wherein

R_6 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected from halogen, -CN, -SCN, $-NO_2$, $-OR_8$, $-SR_8$, $-NR_8R_9$ or heteroaryl, wherein R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_7 is O or S;

Y and Y' each independently is H, -OH or $-NH_2$;

Z_1, Z_2, Z_3, Z_4 and Z_5 each independently is $-O^-$, $-S^-$ or $-BH_3^-$, provided that at least one of Z_1 to Z_5 in the general formula I is $-BH_3^-$, and at least one of Z_1 to Z_3 in the general formula II is $-BH_3^-$;

W_1, W_2, W_3 and W_4 each independently is -O-, -NH- or $-C(R_{10}R_{11})-$, wherein R_{10} and R_{11} each independently is H or halogen, provided that at least one of W_1 to W_4 in the general formula I is not -O-, and at least one of W_1 to W_2 in the general formula II is not -O-;

n and n' each independently is 0 or 1;

m is 3, 4 or 5; and

B^+ represents a pharmaceutically acceptable cation.

2. The pharmaceutical composition of claim 1, comprising a dinucleoside boranophosphate derivative of the general formula I, or a diastereomer or mixture of diastereoisomers thereof.

3. The pharmaceutical composition of claim 1, comprising a nucleoside boranophosphate derivative of the general formula II, or a diastereomer or mixture of diastereoisomers thereof.

4. The pharmaceutical composition of claim 2, wherein:

(i) n and n' are 1, two of W_1 to W_4 are -O-, and the other two of W_1 to W_4 each independently is $-C(R_{10}R_{11})-$;

(ii) n is 0 and n' is 1, one of W_2 to W_4 is -O-, and the other two of W_2 to W_4 each independently is $-C(R_{10}R_{11})-$; or

(iii) n and n' are 0, and W_2 and W_3 each independently is $-C(R_{10}R_{11})-$.

5. The pharmaceutical composition of claim 4, wherein n and n' are 1, and:

(i) Z_1 (or Z_5) is $-BH_3^-$, and Z_2, Z_3, Z_4 and Z_5 (or Z_1, Z_2, Z_3 and Z_4) are $-O^-$; Z_2 (or Z_4) is $-BH_3^-$, and Z_1, Z_3, Z_5 and Z_4 (or Z_1, Z_2, Z_3 and Z_4) are $-O^-$; or Z_3 is $-BH_3^-$, and Z_1, Z_2, Z_4 and Z_5 are $-O^-$;

(ii) Z_1 and Z_2 (or Z_4 and Z_5) are $-BH_3^-$, and Z_3, Z_4 and Z_5 (or Z_1, Z_2 and Z_3) are $-O^-$; Z_1 and Z_3 (or Z_3 and Z_5) are $-BH_3^-$, and Z_2, Z_4 and Z_5 (or Z_1, Z_2 and Z_4) are $-O^-$; Z_1 and Z_4 (or Z_2 and Z_5) are $-BH_3^-$, and Z_2, Z_3 and Z_5 (or Z_1, Z_3 and Z_4) are $-O^-$; Z_1 and Z_5 are $-BH_3^-$, and Z_2, Z_3 and Z_4 are $-O^-$; Z_2 and Z_3 (or Z_3 and Z_4) are $-BH_3^-$, and Z_1, Z_4 and Z_5 (or Z_1, Z_2 and Z_5) are $-O^-$; or Z_2 and Z_4 are $-BH_3^-$, and Z_1, Z_3 and Z_5 are $-O^-$;

(iii) Z_1, Z_2, and Z_3 (or Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_4 and Z_5 (or Z_1 and Z_2) are $-O^-$; Z_1, Z_2 and Z_4 (or Z_2, Z_4 and Z_5) are $-BH_3^-$, and Z_5 and Z_3 (or Z_1 and Z_3) are $-O^-$; Z_1, Z_2 and Z_5 (or Z_1, Z_4 and Z_5) are $-BH_3^-$, and Z_3 and Z_4 (or Z_2 and Z_3) are $-O^-$; Z_1, Z_3 and Z_4 (or Z_2, Z_3 and Z_5) are $-BH_3^-$, and Z_2 and Z_5 (or Z_1 and Z_4) are $-O^-$; Z_1, Z_3 and Z_5 are $-BH_3^-$, and Z_2 and Z_4 are $-O^-$; Z_2, Z_3 and Z_5 (or Z_1, Z_3 and Z_4) are $-BH_3^-$, and Z_1 and Z_4 (or Z_2 and Z_5) are $-O^-$; or Z_2, Z_3 and Z_4 are $-BH_3^-$, and Z_1 and Z_5 are $-O^-$;

(iv) Z_1, Z_2, Z_3 and Z_4 (or Z_2, Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_5 (or Z_1) is $-O^-$; Z_1, Z_2, Z_3 and Z_5 (or Z_1, Z_3, Z_4 and Z_5) are $-BH_3^-$, and Z_4 (or Z_2) is $-O^-$; or Z_1, Z_2, Z_4 and Z_5 are $-BH_3^-$, and Z_3 is $-O^-$; or

(v) Z_1, Z_2, Z_3, Z_4 and Z_5 are $-BH_3^-$.

6. The pharmaceutical composition of claim 4, wherein n is 0 and n' is 1, and:

(i) Z_1 (or Z_5) is $-BH_3^-$, and Z_3, Z_4 and Z_5 (or Z_1, Z_3 and Z_4) are $-O^-$; or Z_3 (or Z_4) is $-BH_3^-$, and Z_1, Z_4 and Z_5 (or Z_1, Z_3 and Z_5) are $-O^-$;

(ii) Z_1 and Z_3 (or Z_4 and Z_5) are $-BH_3^-$, and Z_4 and Z_5 (or Z_1 and Z_3) are O^-; Z_1 and Z_4 (or Z_3 and Z_5) are $-BH_3^-$, and Z_3 and Z_5 (or Z_1 and Z_4) are O^-; Z_1 and Z_5 are $-BH_3^-$, and Z_3 and Z_4 are O^-; or Z_3 and Z_4 are BH_3^-, and Z_1 and Z_5 are O^-; or

(iii) Z_1, Z_3 and Z_4 (or Z_3, Z_4 and Z_5) are BH_3^-, and Z_5 (or Z_1) is O^-; Z_1, Z_3 and Z_5 (or Z_1, Z_4 and Z_5) are BH_3^-, and Z_4 (or Z_3) is O^-; or

(iv) Z_1, Z_3, Z_4 and Z_5 are BH_3^-.

7. The pharmaceutical composition of claim 4, wherein n and n' are 0, and:

(i) Z_1 (or Z_5) is BH_3^-, and Z_3 and Z_5 (or Z_1 and Z_3) are O^-; or Z_3 is BH_3^-, and Z_1 and Z_5 are O^-;

(ii) Z_1 and Z_3 (or Z_3 and Z_5) are BH_3^-, and Z_5 (or Z_1) is O^-; or Z_1 and Z_5 are BH_3^-, and Z_3 is O^-; or

(iii) Z_1, Z_3 and Z_5 are BH_3^-.

8. The pharmaceutical composition of claim 5, wherein Z_3 is $-BH_3^-$, Z_1, Z_2, Z_4 and Z_5 are $-O^-$, W_2 and W_3 are -O-, and W_1 and W_4 each independently is $-C(R_{10}R_{11})-$.

9. The pharmaceutical composition of claim 2, wherein Y and Y' each independently is H or -OH.

10. The pharmaceutical composition of any one of claims 2 or 4 to 9, wherein X and X' are an adenine residue, wherein R_1 each independently is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_2 and R_3 each independently is H or hydrocarbyl,

wherein said hydrocarbyl each independently is $(C_1$-$C_8)$alkyl, preferably $(C_1$-$C_4)$alkyl, more preferably methyl or ethyl, $(C_2$-$C_8)$alkenyl, preferably $(C_2$-$C_4)$alkenyl, $(C_2$-$C_8)$alkynyl, preferably $(C_2$-$C_4)$alkynyl, or $(C_6$-$C_{14})$aryl, preferably $(C_6$-$C_{10})$aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S.

11. The pharmaceutical composition of claim 10, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl.

12. The pharmaceutical composition of claim 11, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

13. The pharmaceutical composition of any one of claims 2 or 4 to 9, wherein X and X' are an uracil residue, wherein R_6 each independently is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, heteroaryl, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_7 is O,

wherein said hydrocarbyl each independently is (C_1-C_8)alkyl, preferably (C_1-C_4)alkyl, more preferably methyl or ethyl, (C_2-C_8)alkenyl, preferably (C_2-C_4)alkenyl, (C_2-C_8)alkynyl, preferably (C_2-C_4)alkynyl, or (C_6-C_{14})aryl, preferably (C_6-C_{10})aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S.

14. The pharmaceutical composition of claim 13, wherein R_6 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl.

15. The pharmaceutical composition of claim 14, wherein R_6 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is methyl or ethyl.

16. The pharmaceutical composition of claim 10, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; n and n' are 1; m is 5; Z_3 is $-BH_3^-$; Z_1, Z_2, Z_4 and Z_5 are $-O^-$; W_2 and W_3 are -O-; and W_1 and W_4 each independently is $-C(R_{10}R_{11})-$, wherein said hydrocarbyl each independently is methyl or ethyl.

17. The pharmaceutical composition of claim 16, wherein R_1 each independently is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; n and n' are 1; m is 5; Z_3 is $-BH_3^-$; Z_1, Z_2, Z_4 and Z_5 are $-O^-$; W_2 and W_3 are -O-; and W_1 and W_4 each independently is $-CH_2-$, $-CCl_2-$ or $-CF_2-$, preferably $-CH_2-$.

18. The pharmaceutical composition of claim 3, wherein:

(i) n is 1, one of W_1 and W_2 is -O-, and another one of W_1 and W_2 is $-C(R_{10}R_{11})-$; or

(ii) n is 0, and W_2 is $-C(R_{10}R_{11})-$.

19. The pharmaceutical composition of claim 18, wherein n is 1, and:

(i) Z_1 is $-BH_3^-$, and Z_2 and Z_3 are $-O^-$; Z_2 is $-BH_3^-$, and Z_1 and Z_3 are $-O^-$; or Z_3 is $-BH_3^-$, and Z_1 and Z_2 are $-O^-$;

(ii) Z_1 and Z_2 are $-BH_3^-$, and Z_3 is $-O^-$; Z_1 and Z_3 are $-BH_3^-$, and Z_2 is $-O^-$; or Z_2 and Z_3 are $-BH_3^-$, and Z_1 is $-O^-$; or

(iii) Z_1, Z_2 and Z_3 are $-BH_3^-$.

20. The pharmaceutical composition of claim 18, wherein n is 0, and:

(i) Z_1 is $-BH_3^-$, and Z_3 is $-O^-$; or Z_3 is $-BH_3^-$, and Z_1 is $-O^-$; or

(ii) Z_1 and Z_3 are $-BH_3^-$.

21. The pharmaceutical composition of claim 19, wherein Z_1 is $-BH_3^-$, Z_2 and Z_3 are $-O^-$, W_1 is -O-, and W_2 is $-C(R_{10}R_{11})-$.

22. The pharmaceutical composition of claim 3, wherein Y is H or -OH.

23. The pharmaceutical composition of any one of claims 3 or 18 to 22, wherein X is an adenine residue, wherein R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5

together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_2 and R_3 each independently is H or hydrocarbyl,

wherein said hydrocarbyl each independently is $(C_1$-$C_8)$alkyl, preferably $(C_1$-$C_4)$alkyl, more preferably methyl or ethyl, $(C_2$-$C_8)$alkenyl, preferably $(C_2$-$C_4)$alkenyl, $(C_2$-$C_8)$alkynyl, preferably $(C_2$-$C_4)$alkynyl, or $(C_6$-$C_{14})$aryl, preferably $(C_6$-$C_{10})$aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S.

24. The pharmaceutical composition of claim 23, wherein R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is $(C_1$-$C_4)$alkyl, preferably methyl or ethyl, $(C_2$-$C_4)$alkenyl, $(C_2$-$C_4)$alkynyl, or $(C_6$-$C_{10})$aryl, preferably phenyl.

25. The pharmaceutical composition of claim 24, wherein R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

26. The pharmaceutical composition of any one of claims 3 or 18 to 22, wherein X is an uracil residue, wherein R_6 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, heteroaryl, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl, or R_8 and R_9 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_7 is O,

wherein said hydrocarbyl each independently is $(C_1$-$C_8)$alkyl, preferably $(C_1$-$C_4)$alkyl, more preferably methyl or ethyl, $(C_2$-$C_8)$alkenyl, preferably $(C_2$-$C_4)$alkenyl, $(C_2$-$C_8)$alkynyl, preferably $(C_2$-$C_4)$alkynyl, or $(C_6$-$C_{14})$aryl, preferably $(C_6$-$C_{10})$aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S.

27. The pharmaceutical composition of claim 26, wherein R_6 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl;

and R_7 is O, wherein said hydrocarbyl each independently is $(C_1$-$C_4)$alkyl, preferably methyl or ethyl, $(C_2$-$C_4)$alkenyl, $(C_2$-$C_4)$alkynyl, or $(C_6$-$C_{10})$aryl, preferably phenyl.

28. The pharmaceutical composition of claim 27, wherein R_6 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_8R_9$, or hydrocarbyl; R_8 and R_9 each independently is H or hydrocarbyl; and R_7 is O, wherein said hydrocarbyl each independently is methyl or ethyl.

29. The pharmaceutical composition of claim 23, wherein R_1 is H, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H; Y is H or -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-C(R_{10}R_{11})$-, wherein said hydrocarbyl each independently is methyl or ethyl.

30. The pharmaceutical composition of claim 29, wherein R_1 is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y is H or -OH; n is 1; m is 4; Z_1 is $-BH_3^-$; Z_2 and Z_3 are $-O^-$; W_1 is -O-; and W_2 is $-CH_2$-, $-CCl_2$- or $-CF_2$-, preferably $-CH_2$-.

31. The pharmaceutical composition of claim 1, wherein B is a cation of an alkali metal, NH_4^+, an organic cation of the formula R_4N^+ wherein each one of the Rs independently is H or C_1-C_{22}, preferably C_1-C_6, alkyl, a cationic lipid or a mixture of cationic lipids.

32. The pharmaceutical composition of any one of claims 1 to 31, for intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, subcutaneous, transdermal, topical or oral administration.

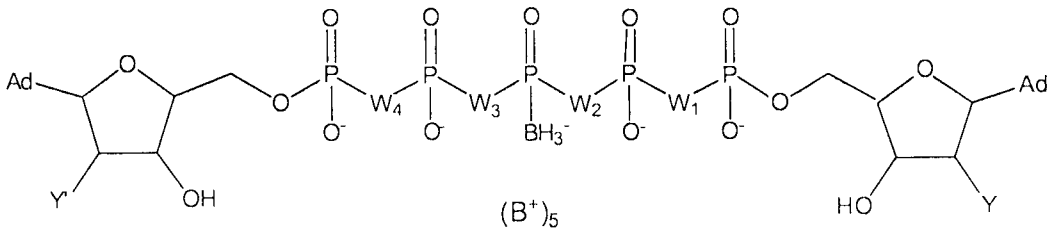
33. A dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined in claim 1, or a diastereomer or mixture of diastereoisomers thereof, for use in treatment of osteoarthritis.

34. Use of a dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined in claim 1, or a diastereomer or mixture of diastereoisomers thereof, for the preparation of a pharmaceutical composition for treatment of osteoarthritis.

35. A method for treatment of osteoarthritis in an individual in need thereof, comprising administering to said individual a therapeutically effective amount of a

dinucleoside boranophosphate derivative of the general formula I or a nucleoside boranophosphate derivative of the general formula II as defined in claim 1, or a diastereomer or mixture of diastereoisomers thereof.

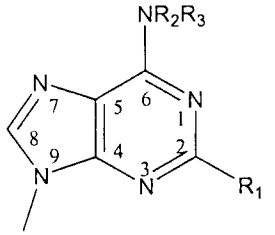
36. A diadenosine boranophosphate derivative of the general formula III:

III 

or a diastereomer or mixture of diastereoisomers thereof,

wherein

Ad is an adenine residue of the formula Ia, linked through the 9-position:

Ia 

wherein

R_1 is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, $-NR_4R_5$, heteroaryl, or hydrocarbyl optionally substituted by one or more groups each independently selected from halogen, -CN, -SCN, $-NO_2$, $-OR_4$, $-SR_4$, $-NR_4R_5$ or heteroaryl, wherein R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S, wherein the additional nitrogen is optionally substituted by alkyl; and

R_2 and R_3 each independently is H or hydrocarbyl;

Y and Y' each independently is H, -OH or $-NH_2$;

W_1, W_2, W_3 and W_4 each independently is -O-, -NH- or $-C(R_{10}R_{11})-$, wherein R_{10} and R_{11} each independently is H or halogen, provided that two of W_1 to W_4 are not -O-; and

B^+ represents a pharmaceutically acceptable cation.

37. The diadenosine boranophosphate derivative of claim 36, wherein W_2 and W_3 are -O-, and W_1 and W_4 each independently is -NH- or -C($R_{10}R_{11}$)-, preferably -CH_2-, -CCl_2- or -CF_2-.

38. The diadenosine boranophosphate derivative of claim 36, wherein Y and Y' each independently is H or -OH.

39. The diadenosine boranophosphate derivative of any one of claims 36 to 38, wherein R_1 each independently is H, halogen, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, heteroaryl, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl, or R_4 and R_5 together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated or unsaturated heterocyclic ring optionally containing 1-2 further heteroatoms selected from N, O or S; and R_2 and R_3 each independently is H or hydrocarbyl,

wherein said hydrocarbyl each independently is (C_1-C_8)alkyl, preferably (C_1-C_4)alkyl, more preferably methyl or ethyl, (C_2-C_8)alkenyl, preferably (C_2-C_4)alkenyl, (C_2-C_8)alkynyl, preferably (C_2-C_4)alkynyl, or (C_6-C_{14})aryl, preferably (C_6-C_{10})aryl, more preferably phenyl; and said heteroaryl is a 5-6-membered monocyclic heteroaromatic ring containing 1-2 heteroatoms selected from N, O or S.

40. The diadenosine boranophosphate derivative of claim 39, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 each independently is H or hydrocarbyl, wherein said hydrocarbyl each independently is (C_1-C_4)alkyl, preferably methyl or ethyl, (C_2-C_4)alkenyl, (C_2-C_4)alkynyl, or (C_6-C_{10})aryl, preferably phenyl.

41. The diadenosine boranophosphate derivative of claim 40, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; and R_2 and R_3 are H, wherein said hydrocarbyl each independently is methyl or ethyl.

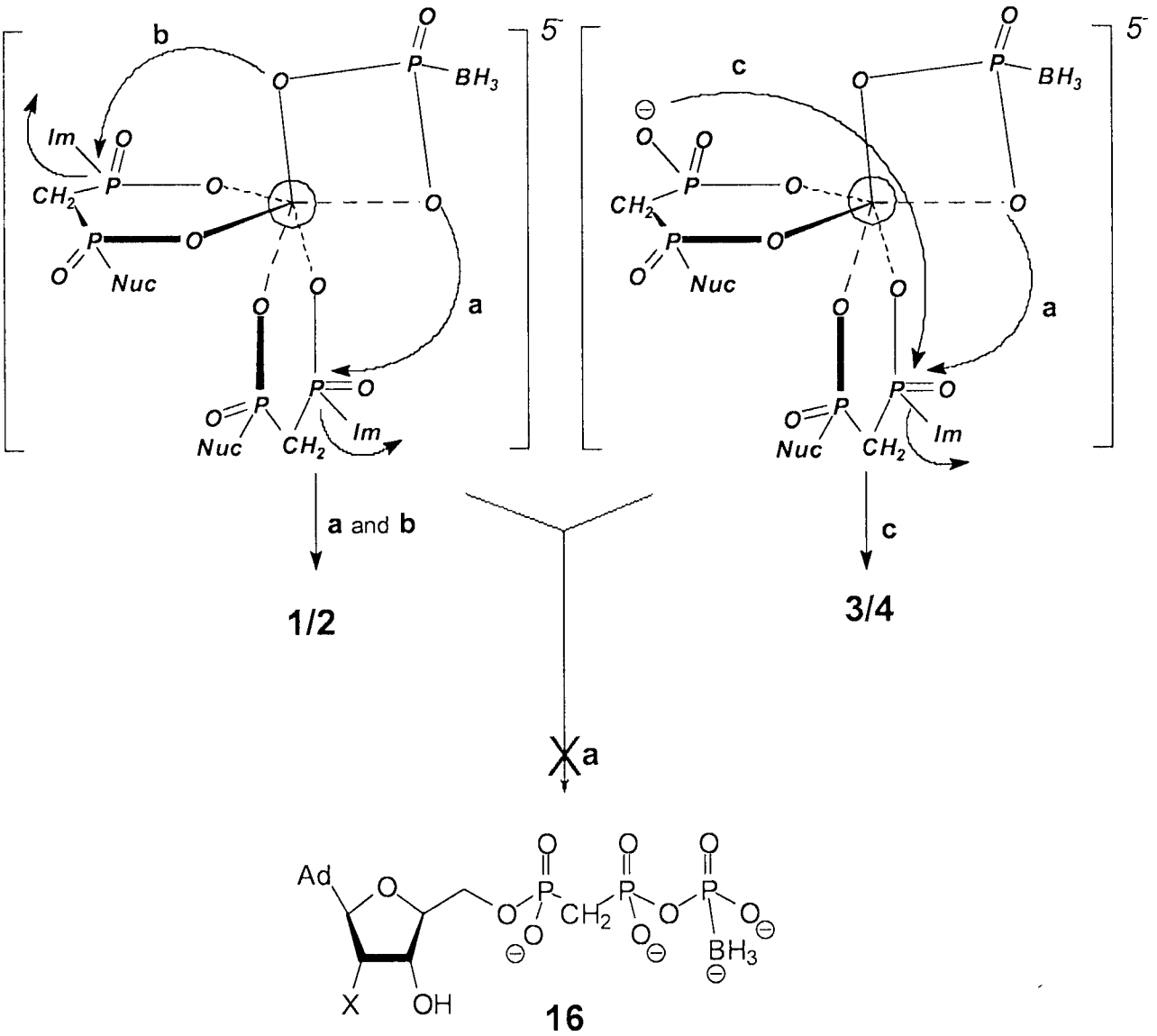
42. The diadenosine boranophosphate derivative of claim 39, wherein R_1 each independently is H, -O-hydrocarbyl, -S-hydrocarbyl, -NR_4R_5, or hydrocarbyl; R_4 and R_5 each independently is H or hydrocarbyl; R_2 and R_3 are H, Y and Y' each independently is H or -OH; W_2 and W_3 are -O-; and W_1 and W_4 each independently is -C($R_{10}R_{11}$)-, wherein said hydrocarbyl each independently is methyl or ethyl.

43. The diadenosine boranophosphate derivative of claim 42, wherein R_1 each independently is H, -O-methyl or -S-methyl; R_2 and R_3 are H; Y and Y' each independently is H or -OH; W_2 and W_3 are -O-; and W_1 and W_4 each independently is $-CH_2-$, $-CCl_2-$ or $-CF_2-$, preferably $-CH_2-$.

44. The diadenosine boranophosphate derivative of claim 36, wherein B is a cation of an alkali metal, NH_4^+, an organic cation of the formula R_4N^+ wherein each one of the Rs independently is H or C_1-C_{22}, preferably C_1-C_6, alkyl, a cationic lipid or a mixture of cationic lipids.

45. A pharmaceutical composition comprising a diadenosine boranophosphate derivative according to any one of claims 36 to 44 and a pharmaceutically acceptable carrier.

Fig. 1



(2'-deoxy)-adenosine-5'-α,β-CH_2-γ-borano-triphosphate

○ = Mg^{2+}

Im = imidazolyl

Nuc = adenosyl (2'-deoxy-adenosyl)

Fig. 2

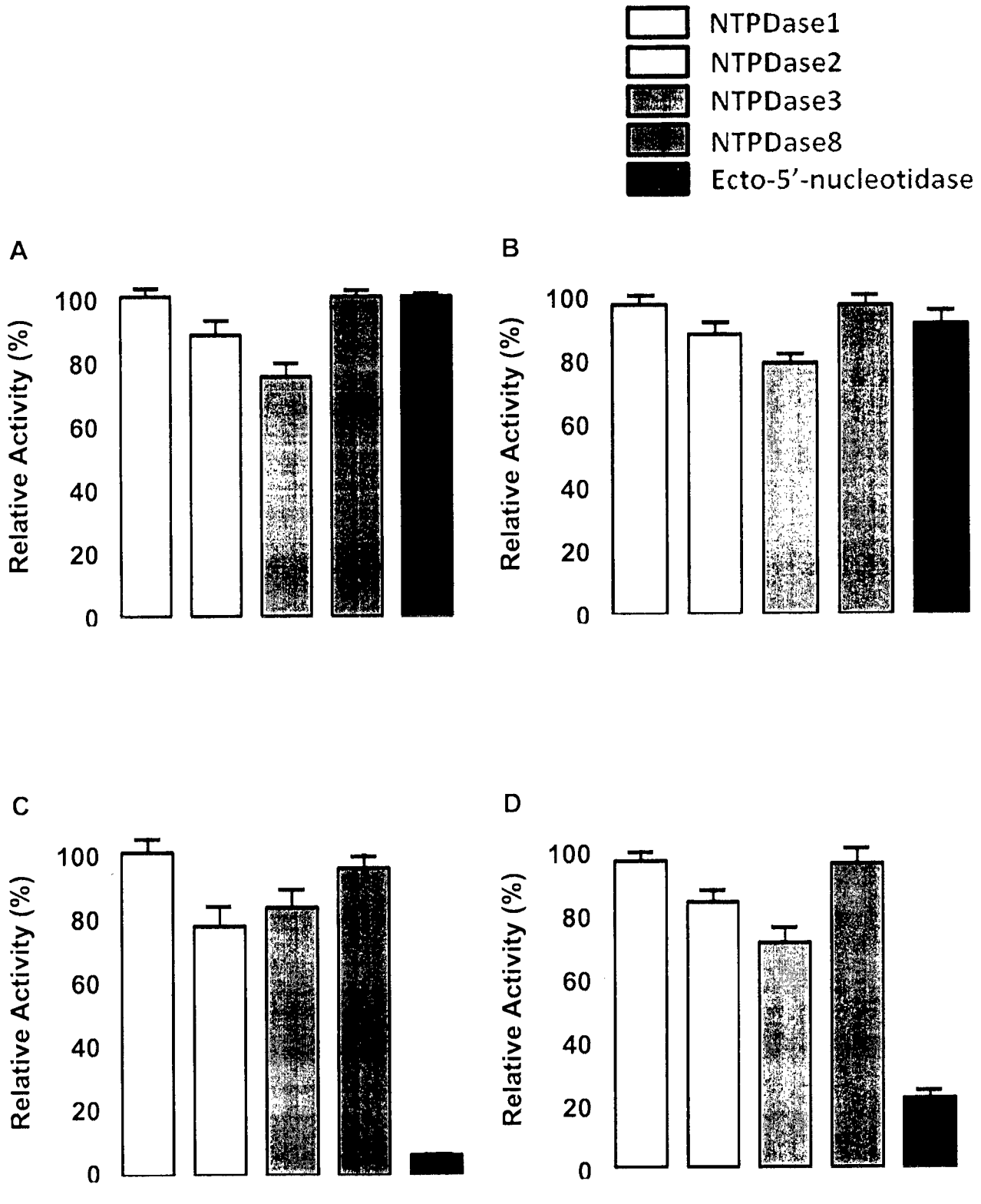

NTPDase1
NTPDase2
NTPDase3
NTPDase8
Ecto-5'-nucleotidase
A
B
C
D
Relative Activity (%)
100
80
60
40
20
0

Fig. 3A

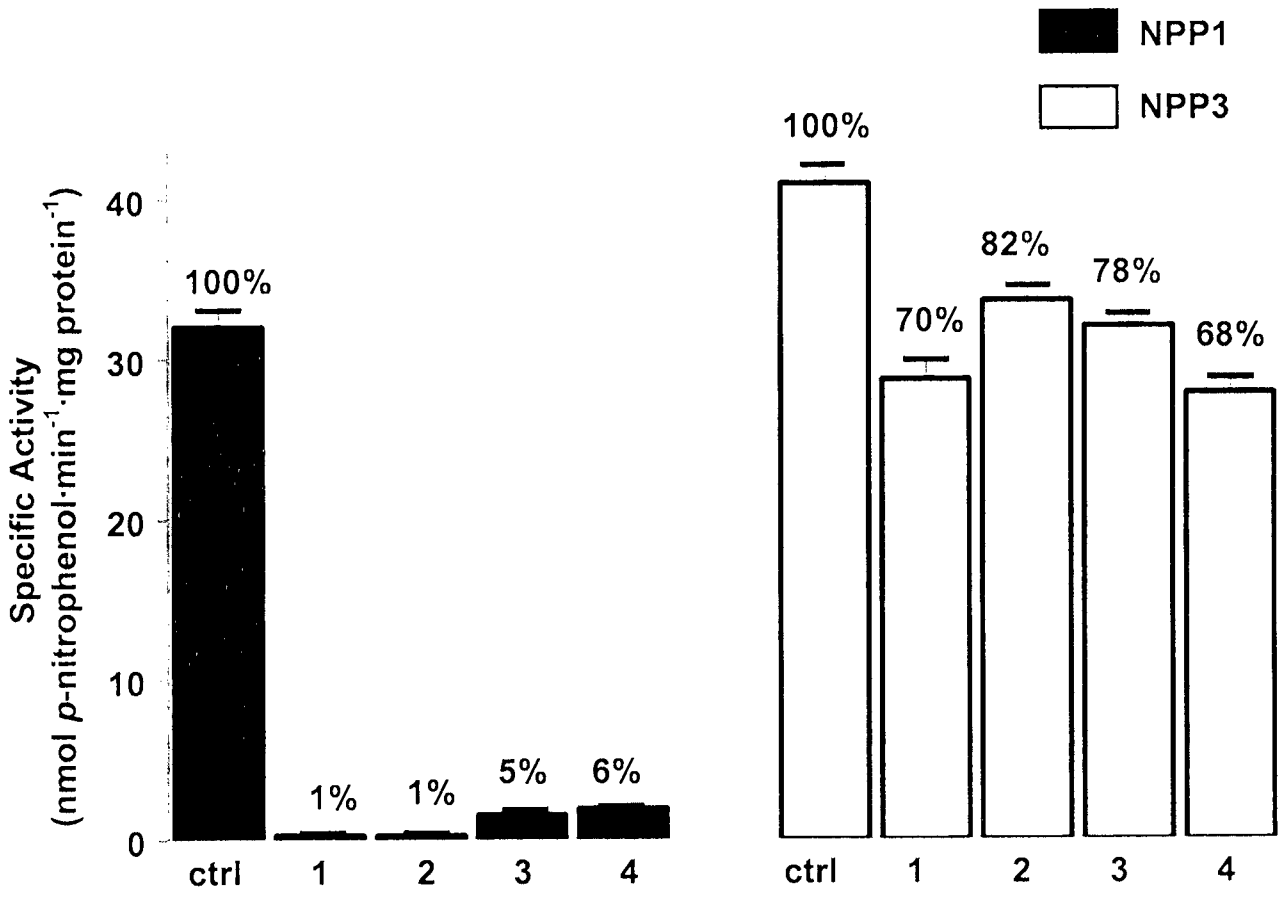

NPP1
NPP3
Specific Activity
(nmol p-nitrophenol·min-1·mg protein-1)
0
10
20
30
40
100%
1%
1%
5%
6%
ctrl
1
2
3
4
100%
70%
82%
78%
68%
ctrl
1
2
3
4

Fig. 3B

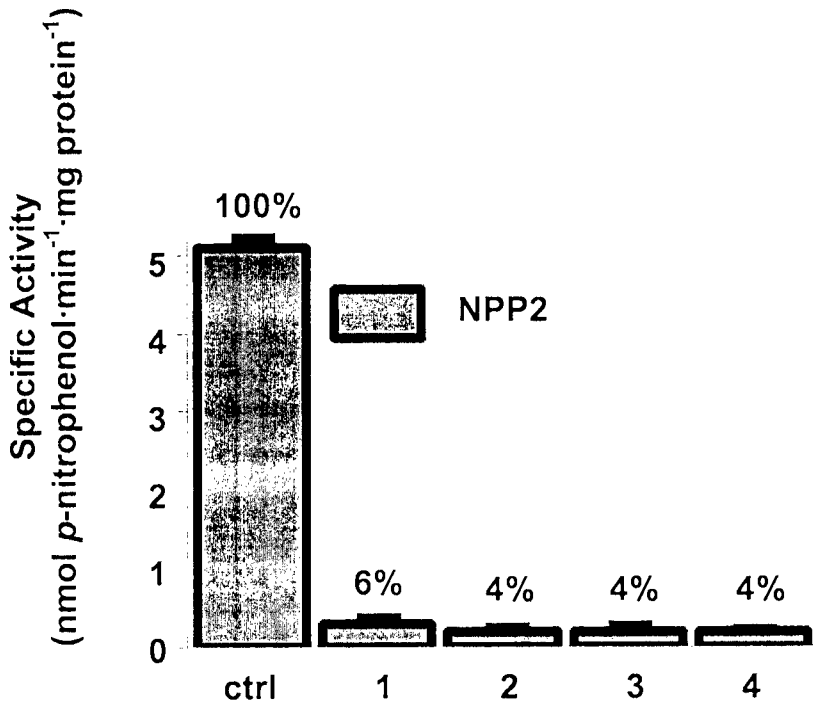

Specific Activity
(nmol p-nitrophenol·min-1·mg protein-1)
100%
NPP2
6%
4%
4%
4%
0
1
2
3
4
5
ctrl
1
2
3
4

Fig. 3C

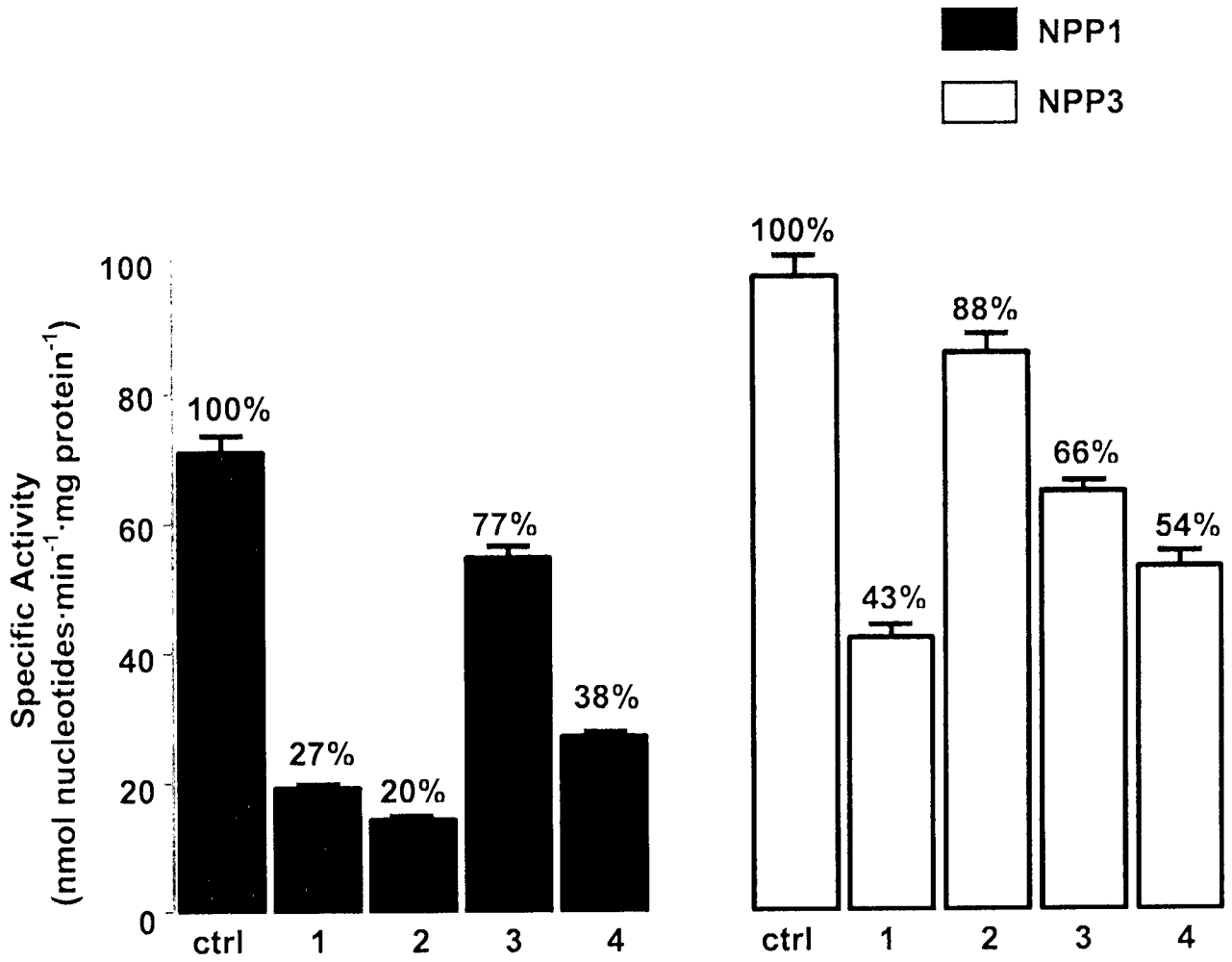

NPP1
NPP3
Specific Activity
(nmol nucleotides·min-1·mg protein-1)
100
80
60
40
20
0
100%
27%
20%
77%
38%
ctrl
1
2
3
4
100%
43%
88%
66%
54%
ctrl
1
2
3
4

Fig.3D

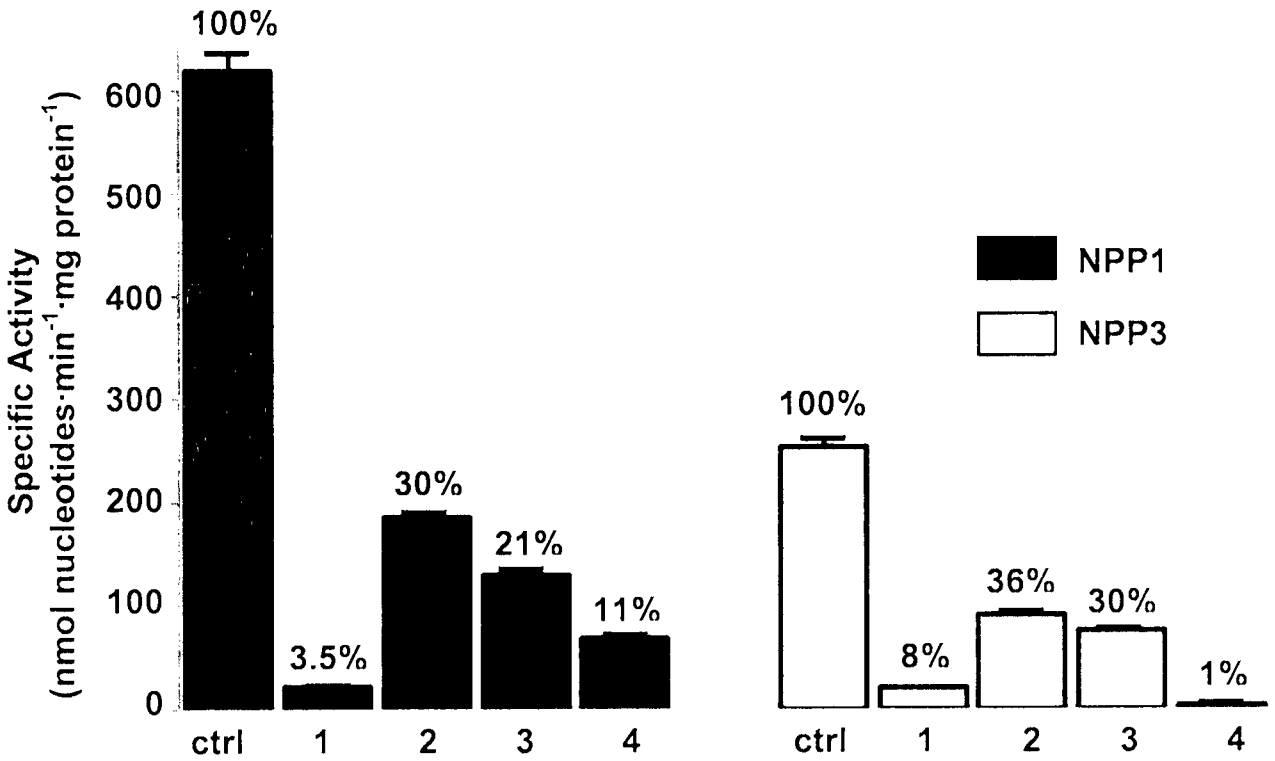

Specific Activity
(nmol nucleotides·min-1·mg protein-1)
600
500
400
300
200
100
0
100%
3.5%
30%
21%
11%
ctrl
1
2
3
4
NPP1
NPP3
100%
8%
36%
30%
1%
ctrl
1
2
3
4

Fig. 4

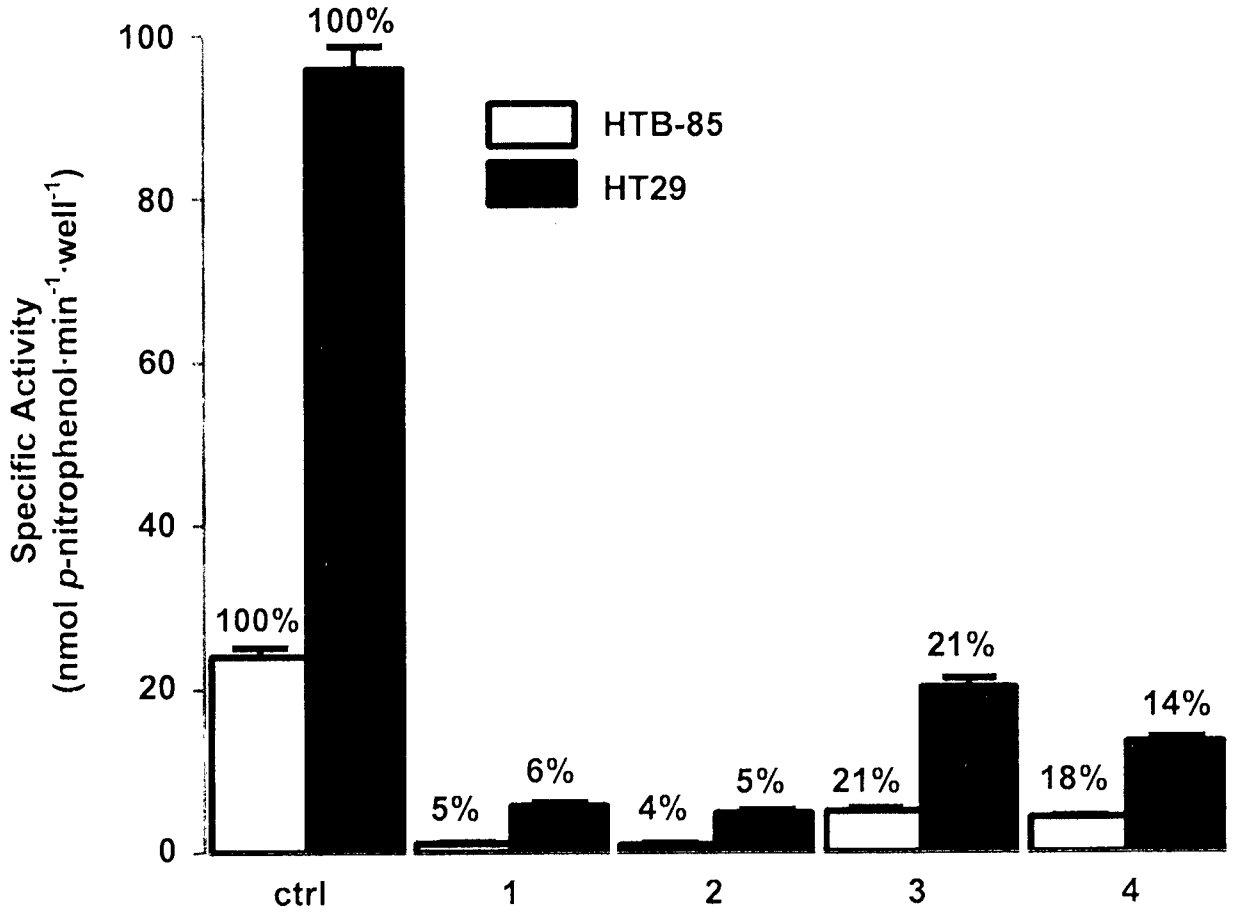

Specific Activity
(nmol p-nitrophenol·min-1·well-1)
100
80
60
40
20
0
HTB-85
HT29
100%
100%
5%
6%
4%
5%
21%
21%
18%
14%
ctrl
1
2
3
4

Fig. 5A

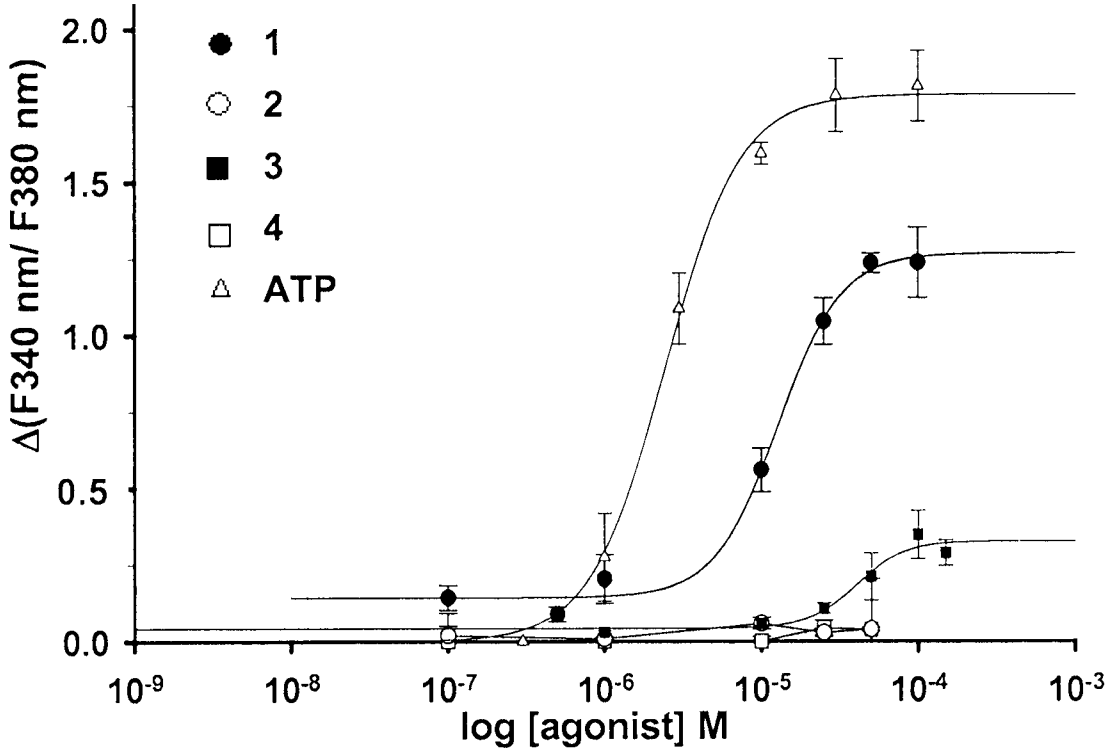

2.0
1.5
1.0
0.5
0.0
Δ(F340 nm/ F380 nm)
1
2
3
4
ATP
10-9
10-8
10-7
10-6
10-5
10-4
10-3
log [agonist] M

Fig. 5B

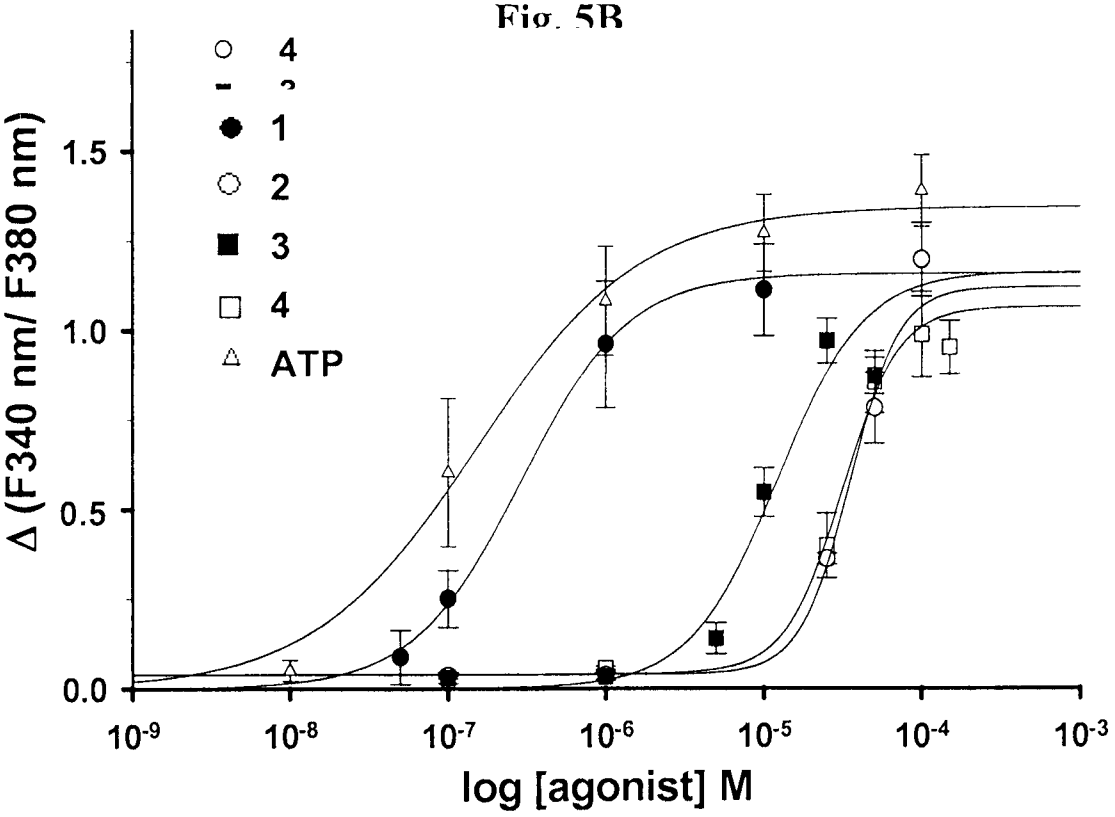

4
1
2
3
4
ATP
1.5
1.0
0.5
0.0
Δ (F340 nm/ F380 nm)
10-9
10-8
10-7
10-6
10-5
10-4
10-3
log [agonist] M

Fig. 6

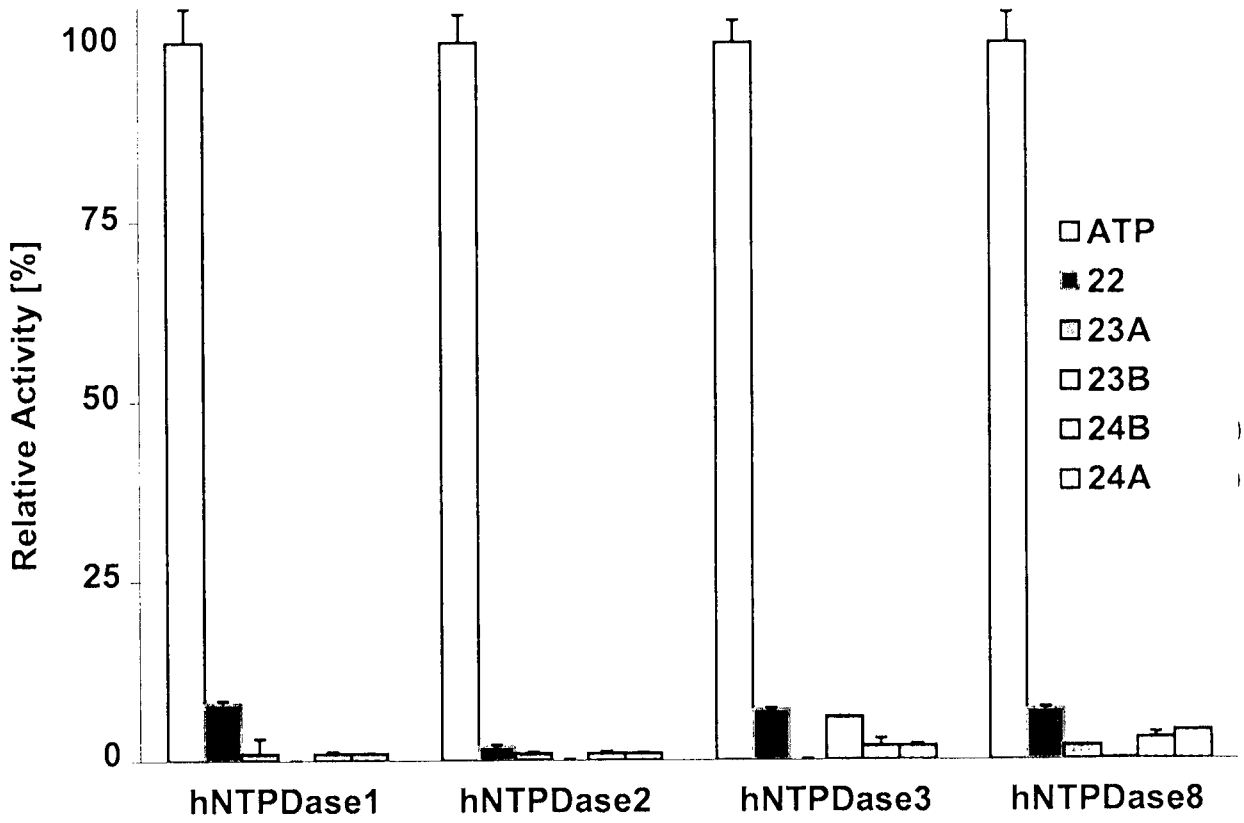

100
75
50
25
0
Relative Activity [%]
ATP
22
23A
23B
24B
24A
hNTPDase1
hNTPDase2
hNTPDase3
hNTPDase8

Fig. 7

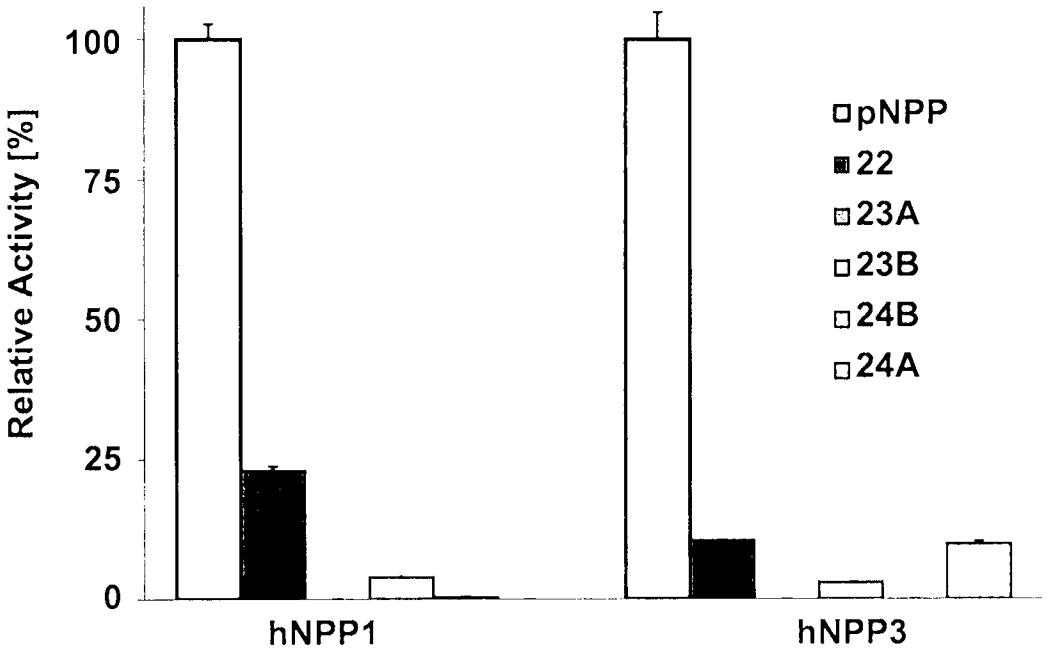

Relative Activity [%]
100
75
50
25
0
hNPP1
hNPP3
pNPP
22
23A
23B
24B
24A

Fig. 8

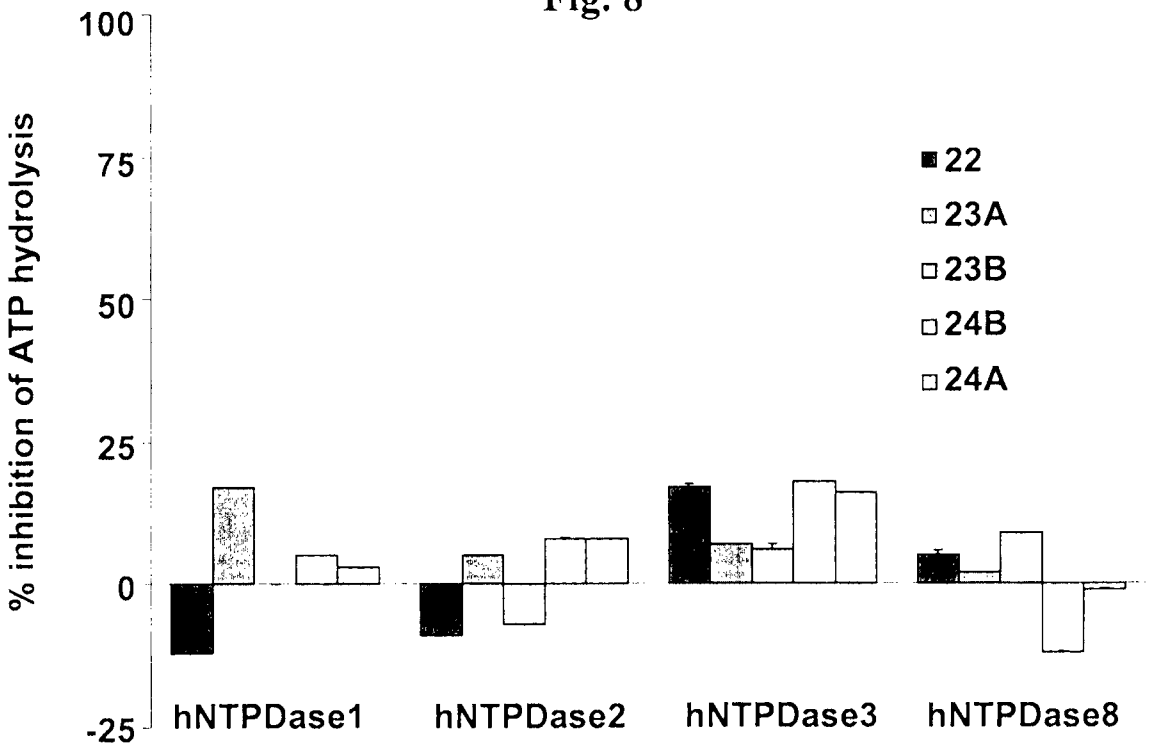
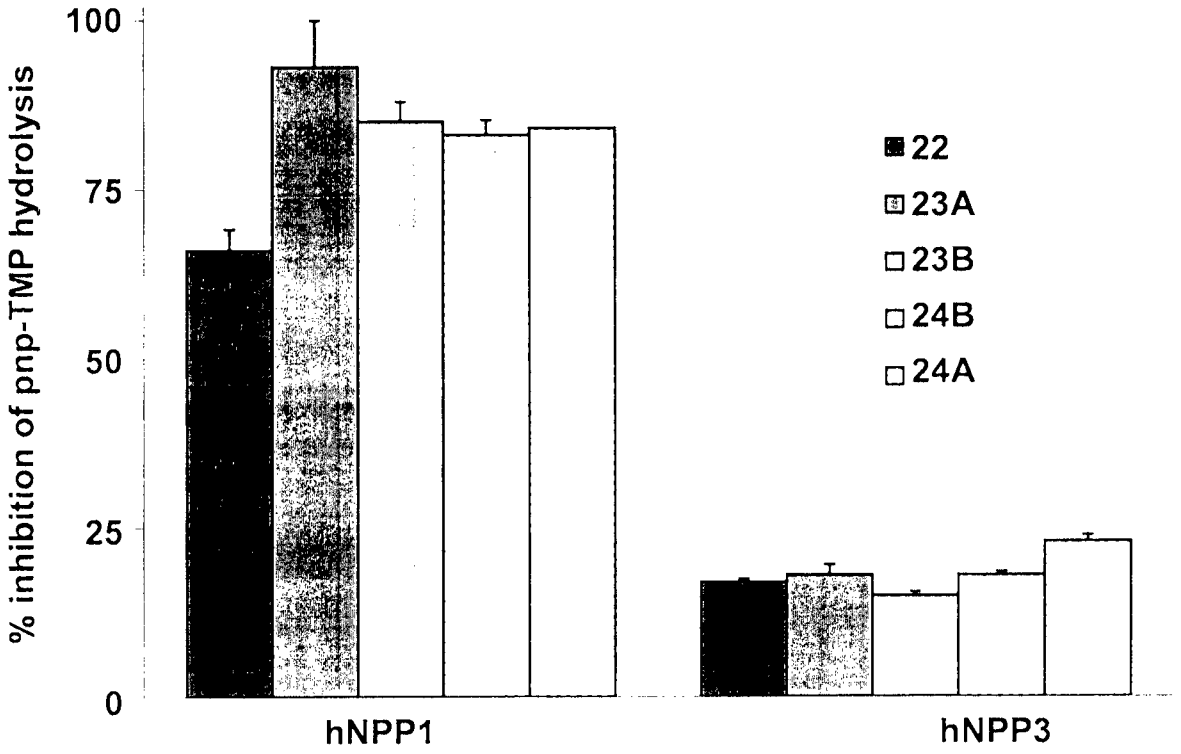

% inhibition of ATP hydrolysis
100
75
50
25
0
-25
hNTPDase1
hNTPDase2
hNTPDase3
hNTPDase8
22
23A
23B
24B
24A

Fig. 9


100
75
50
25
0
% inhibition of pnp-TMP hydrolysis
22
23A
23B
24B
24A
hNPP1
hNPP3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000713

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7072 A61K31/7076 A61K31/7084 C07H19/06 C07H19/16
C07H23/00 A61P19/02
ADD.
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)
A61K C07H

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	SHAY ELIAHU ET AL: "Diadenosine 5',5''-(Boranated)polyphosphonate Analogues as Selective Nucleotide Pyrophosphatase/Phosphodiesterase Inhibitors [bottom]", JOURNAL OF MEDICINAL CHEMISTRY, vol. 53, no. 24, 23 December 2010 (2010-12-23), pages 8485-8497, XP55015129, ISSN: 0022-2623, DOI: 10.1021/jm100597c the whole document	1-45

X	WO 03/000056 A1 (INSPIRE PHARMACEUTICALS INC [US]; COWLEN MATTHEW S [US]; YERXA BENJAMI) 3 January 2003 (2003-01-03)	1-3, 9-17, 22-35
Y	claims 2,4,7,8	1-45

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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

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"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

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 January 2012	24/01/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kollmannsberger, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2011/000713

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2005/053612 A1 (GRANSTEIN RICHARD D [US] ET AL) 10 March 2005 (2005-03-10) paragraph [0014] paragraph [0029] paragraph [0070]	1,3,22, 26-28, 31-35 1-45
Y	US 2006/287271 A1 (FISCHER BILHA [IL] ET AL) 21 December 2006 (2006-12-21) cited in the application paragraph [0015] - paragraph [0037]	1-45
Y	ELIAHU S E ET AL: "Identification of hydrolytically stable and selective P2Y1 receptor agonists", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 44, no. 4, 1 April 2009 (2009-04-01), pages 1525-1536, XP026003085, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2008.07.015 [retrieved on 2008-07-22] compounds 1-4	1-45
Y	WO 2009/066298 A1 (UNIV BAR ILAN [IL]; FISCHER BILHA [IL]; ELYAHU SHAY [IL]) 28 May 2009 (2009-05-28) claims 1,28 page 43; compounds	1-35
Y	SHAY ELIAHU ET AL: "2-MeS-beta,gamma-CCl2-ATP is a Potent Agent for Reducing Intraocular Pressure", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 53, no. 8, 1 April 2010 (2010-04-01), pages 3305-3319, XP002635428, ISSN: 0022-2623, DOI: 10.1021/JM100030U [retrieved on 2010-03-25] figures 1,2; compounds page 3309; compound 38	1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IL2011/000713

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