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(54) Title: ADENOSINE THIOPHOSPHATE DERIVATIVES AND USES THEREOF

(57) **Abstract:** Novel adenosine 5'-thio-phosphate derivatives, represented by Formulae (I) or (I*), and salts thereof, represented by Formulae (Ia) and (I*a), as defined in the specification, and uses thereof as antioxidants and/or P2Y₁-R agonists, and/or in the treatment of medical conditions associated with oxidative stress and/or neurodegeneration, and in the treatment of Alzheimer's Disease in particular, are disclosed.

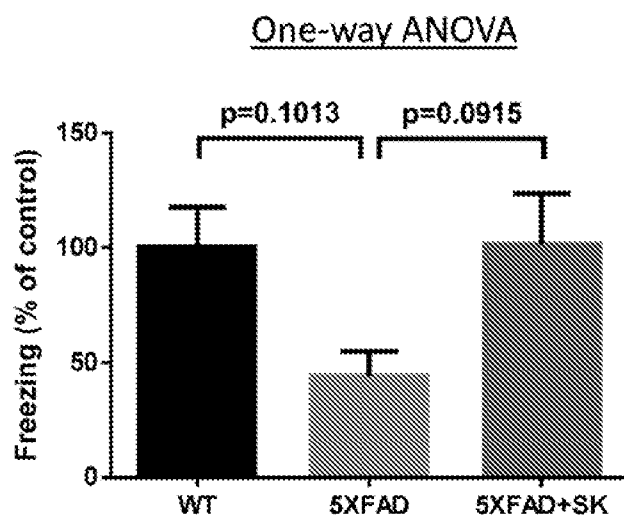


FIG. 16A



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ADENOSINE THIOPHOSPHATE DERIVATIVES AND USES THEREOF

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy and, more particularly, but not exclusively, to novel adenosine phosphate derivatives and to uses thereof in the treatment of medical conditions associated with oxidative stress and/or neurodegeneration, such as Alzheimer's Disease.

Neurodegenerative diseases and brain injury are associated with oxidative damage. In the search for treatment of neurodegenerative disorders, antioxidant agents have been mainly thought for. Activation of P2Y-receptors (P2Y-Rs), which are widely expressed in the nervous system and suggested to be involved in neuroprotection [Abbracchio et al., *Trends Neurosci.* **2009**, 32, 19-29; Volonte et al., *Curr. Drug. Targets. CNS. Neurol. Disord* **2003**, 2, 403-412], has also been suggested as an approach for treating neurodegenerative disorders.

The members of the P2 receptor (P2R) superfamily, consisting of ligand-gated ion channels (P2X-Rs) and G protein-coupled receptors (P2Y-Rs), are activated by endogenous extracellular nucleotides. Eight human P2Y-Rs subtypes are known so far - P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁-P2Y₁₄. The P2Y_{2,4,6} receptors are activated by uracil nucleotides, while the P2Y_{1,2,11} receptors are activated by adenine nucleotides (ATP, **1**, or ADP, **2**).

Neurons and astrocytes express both P2Y and P2X receptors. While ionotropic P2X receptors are mainly involved in fast synaptic neurotransmission, P2Y receptors mediate slow neuromodulatory effects [Abbracchio et al. *Pharmacol. Rev.* **2006**, 58, 281-341]. In the brain, P2Y-R mediated signaling is involved in nervous tissue remodeling following trauma, stroke, ischemia or neurodegenerative disorders. P2Y₁-R and P2Y₁₁-R mRNA are present in the human brain in large quantities as compared to mRNAs in other tissues. Neurons and astrocytes express both sub-types of P2-Rs, while P2Y₁-R is the predominant receptor in neurons.

Neuronal activity-dependent release of ATP is one of the principal mechanisms underlying neuron-to-astrocyte intercellular communications. In addition to the rapid neurotransmitter-like action of ATP in normal brain function, it has become evident that some of the responses to ATP released during brain injury are neuroprotective. Some

reports attributed this neuroprotective effect of ATP to its interaction with P2X-Rs and P2Y-Rs by which the P2Y₁-R appears to be the main receptor involved in the process. For instance, neuroprotection from methylmercury insult was reported to be induced by extracellular ATP through P2Y₁-R. P2Y₁-R stimulation by 2-SMe-ADP, **4**, reduced
5 ischemic neuronal lesions in mouse. In addition to 2-SMe-ADP, **4**, ATP- γ -S, **3**, and ADP- β -S, **5**, have also shown to be activators of P2Y₁-R, which enhance neuroprotection. See, Fujita et al., *Glia* **2009**, 57, 244-257; Fields et al., *Nature. Rev. Neuro.* **2006**, 7, 423-436; Tonazzini et al., *Eur. J. Neurosci.* **2007**, 26, 890-902; Noguchi et al., *Plos One* **2013**, 8, e57898; Zheng et al., *J. Cereb. Blood Flow Metabol.* **2013**, 33,
10 600-611; and Jacobson et al., *Purinerg. Signal.* **2009**, 5, 75-89.

Iron plays a vital role in various physiological functions and normal brain function. However, high level of iron can lead to significant oxidative damage via radical production within the brain, as in neurodegenerative diseases and ischemia. Specifically, OH radicals are generated from the less damaging reactive oxygen species,
15 superoxide radical anion and hydrogen peroxide, in Fenton or Haber-Weiss reactions catalyzed by Fe(II)/Cu(I), or Fe(III)/Cu(II), respectively.

Therapeutic agents, acting as Fenton reaction inhibitors, and targeting the elimination of toxic OH radicals, either by radical scavenging or by metal ion chelation mechanisms, have therefore been considered.

20 Known antioxidants include substances such as clioquinol, verapamil, desferroxamine, and tridentate triazolyl, or naturally occurring antioxidants, such as vitamin C and E26 and caffeic acid. However, many of these chelators are poorly water-soluble, and some demonstrate pro-oxidant activities at high doses.

Some of the present inventors have considered nucleotide analogues as
25 biocompatible and water soluble Fe (II)/Cu(I) chelators. Purine nucleotide analogues are natural metal-ion chelators binding metal-ions by the purine ring N7 nitrogen atom, and/or the 5'-phosphate chain. Natural nucleotides and phosphorothioate analogues thereof, as well as inorganic phosphates, have been studied as inhibitors of Fenton reaction. It was found that certain natural and synthetic adenine nucleotides at
30 submillimolar concentrations prevented OH radical production from H₂O₂ in the presence of Cu(I)/Fe(II) ions better than standard antioxidants. Specifically, ATP- γ -S proved a 100- and 20-times more active antioxidant at Fe(II)/H₂O₂ system than ATP

and the potent antioxidant Trolox, respectively. See, Richter and Fischer, *J. Biol. Inorg. Chem.* **2006**, *11*, 1063-1074; Baruch-Suchodolsky and Fischer, *J. Inorg. Biochem.* **2008**, *102*, 862-881. ATP- γ -S has been reported as a highly promising antioxidant and neuroprotective agent in PC12 cells and primary neurons exposed to oxidative stress, which was significantly more potent than ADP- β -S and GDP β -S [Danino et al., *Biochem. Pharmacol.* **2014**, *88*, 384-392]. Yet, a major limitation of ATP- γ -S is its enzymatic instability.

Arzan et al., *J. Med. Chem.* **2013**, *56*, 4938-4952, describe the design and evaluation of nucleotide and dinucleotide analogs in which a boranophosphate moiety replaces a phosphate group.

Additional background art includes U.S. Patent Application Publication No. 2011/0257109 and WO 2007/020018.

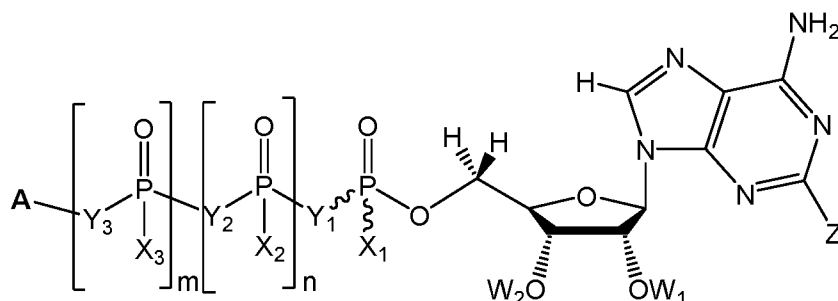
SUMMARY OF THE INVENTION

With a view to identify novel and biocompatible neuroprotectants, the present inventors have designed a series of adenosine 5'-phosphorothioate analogues (variously thiolated adenosine 5'-phosphate and derivatives thereof) capable of dual activity as both antioxidants and P2Y₁-R agonists. These analogues were evaluated as P2Y_{1,11}-R agonists, Fenton reaction inhibitors, radical scavengers, and inhibitors of ROS formation in PC12 cells and primary neurons under oxidative stress conditions. These analogues further exhibited metabolic stability.

Adenosine 5'-phosphorothioate derivatives, featuring a substituent such as thioalkoxy at position 2 of the adenosine, were found to be highly potent antioxidants and P2Y₁-R agonists, and improved cognitive and behavioural functions in Alzheimer's Disease (AD) mice model.

According to an aspect of some embodiments of the present invention there is provided a compound represented by Formula I:

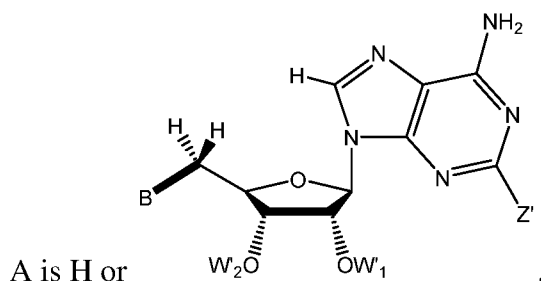
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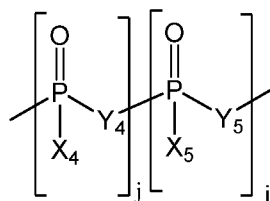
Formula I

wherein:

- 5 the curled lines denote an Rp or Sp configuration, when relevant;
 n and m are each independently 0 or 1;
 Z is selected from $-\text{SR}_1$, $\text{S}(=\text{O})\text{R}_1$, $\text{S}(=\text{O})\text{NHR}_1$, OR_1 , NHR_1 , R_1 and halo;
 R_1 is alkyl, cycloalkyl, aryl or acyl;
 W_1 and W_2 are each independently selected from H, alkyl and acyl;
 10 Y_1 , Y_2 and Y_3 are each independently O, S, CH_2 , $\text{C}(\text{L})_2$ or NH, wherein L is halo;
 X_1 , X_2 and X_3 are each independently $-\text{OH}$ or $-\text{SH}$, provided that at least one of X_1 , X_2 and X_3 is SH; and



- 15 wherein Z' is selected from $-\text{SR}'_1$, $\text{S}(=\text{O})\text{R}'_1$, OR'_1 , NHR'_1 , R'_1 and halo; R'_1 is alkyl, cycloalkyl, aryl or acyl; W'_1 and W'_2 are each independently selected from H,



- alkyl and acyl; and B is absent or is
 independently O, S, CH_2 , $\text{C}(\text{L}')_2$ or NH, wherein L' is halo; X_4 and X_5 are each
 independently $-\text{OH}$ or $-\text{SH}$; and j and i are each independently an integer of 0, 1 or 2, or
 20 a pharmaceutically acceptable salt thereof.

5

According to some of any of the embodiments described herein, A is H.

According to some of any of the embodiments described herein, Y₁, Y₂ and Y₃ are each O.

According to some of any of the embodiments described herein, m is 0.

5 According to some of any of the embodiments described herein, X₁ is -SH.

According to some of any of the embodiments described herein, X₂ is -OH.

According to some of any of the embodiments described herein, Z is -SR₁.

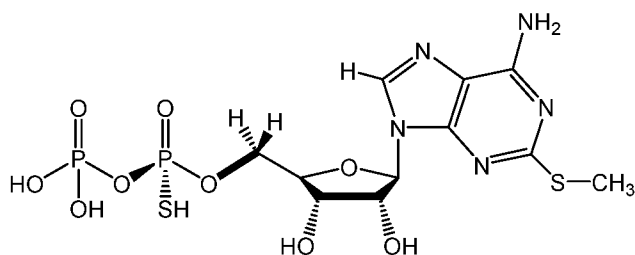
According to some of any of the embodiments described herein, Z is -SR₁ and R₁ is alkyl.

10 According to some of any of the embodiments described herein, R₁ is methyl.

According to some of any of the embodiments described herein, the curled lines denote an Rp configuration.

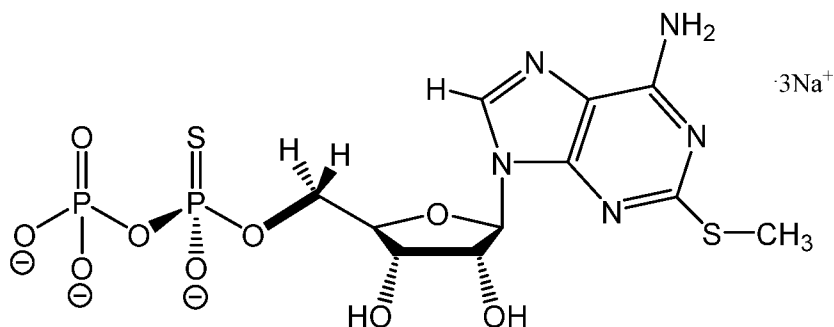
According to some of any of the embodiments described herein, W₁ and W₂ are each H.

15 According to some of any of the embodiments described herein, the compound is



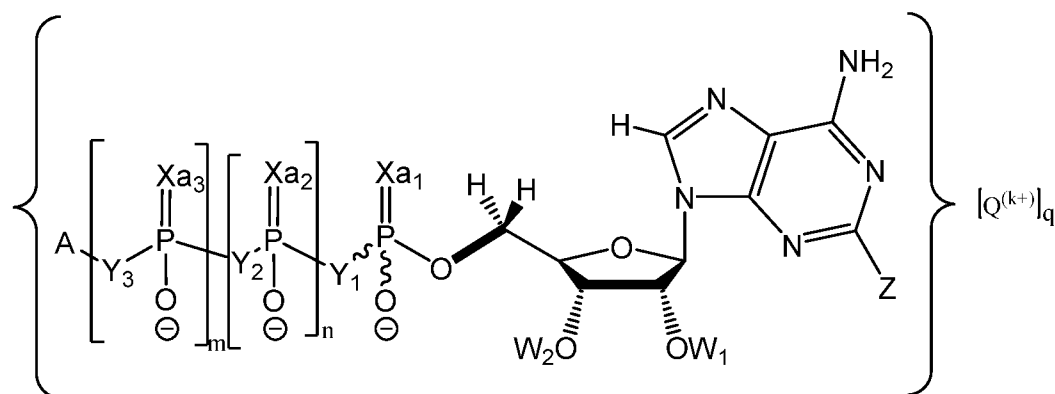
or a pharmaceutically acceptable salt thereof, for example,

20



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According to some of any of the embodiments described herein, a compound as described herein is a pharmaceutically acceptable salt of a compound represented by Formula I, and is represented by Formula I*a:



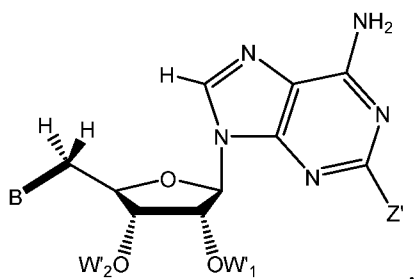
5

Formula I*a

wherein:

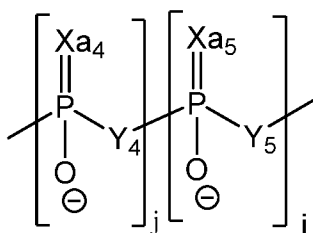
10 Y_1 , Y_2 , Y_3 , W_1 , W_2 , Z , n , m and the curled lines are as defined for Formula I;

Xa_1 , Xa_2 and Xa_3 are each independently S (sulfur) or O (oxygen), at least one of Xa_1 , Xa_2 and Xa_3 being S (sulfur);



A is absent or is

wherein Z' , W'_1 and W'_2 are as defined for Formula I; and B is absent or is



15

, wherein Y_4 and Y_5 , i and j are as defined for Formula I, and Xa_4 and Xa_5 are each independently O (oxygen) or S (sulfur);

Q is a cation, k is the cation valence, and q is the number of cations, whereby q corresponds to k and is such that the number of cations provide for a positive charge that is equal to the number of negatively charged groups in the compound.

According to some of any of the embodiments described herein, $Q^{(k+)}$ is selected
5 from Na^+ , K^+ , Li^+ , and Cs^+ .

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising the compound as described herein in any of the respective embodiments, and a pharmaceutically acceptable carrier.

According to an aspect of some embodiments of the present invention there is
10 provided a compound as described herein in any of the respective embodiments or a composition comprising same, as described herein, for use in activating hP2Y₁ receptor in a subject in need thereof.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein in any of the respective embodiments or a
15 composition comprising same, as described herein, for use in treating a medical condition treatable by activating hP2Y₁ receptor.

According to some of any of the embodiments described herein, the medical condition is treatable also by activating hP2Y₁₁ and/or hP2Y₁₂ receptor.

According to an aspect of some embodiments of the present invention there is
20 provided a compound as described herein in any of the respective embodiments or a composition comprising same, as described herein, for use in the treatment of a medical condition associated with oxidative stress.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein in any of the respective embodiments or a
25 composition comprising same, as described herein, for use in the treatment of a neurodegenerative disease or disorder.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein in any of the respective embodiments or a composition comprising same, as described herein, for use in the treatment of
30 Alzheimer's disease.

Further according to the present embodiments, there are provided process of preparing the compounds as described herein, essentially in accordance with the procedures described in the Examples section hereinbelow.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-C present concentration-response curves for agonists at hP2Y₁-R: ADP(α -S), **6A** and **6B**, and 2-SMe-ADP(α -S), **7A** and **7B** (FIG. 1A); 2-Cl-ADP(α -S), **8A** and **8B**, ADP, **2**, and 2-SMe-ADP, **4** (FIG. 1B); 2-SMe-ADP(β -S), **10**, 2-Cl-ADP(β -S), **11**, ADP, **2**, and ADP(β -S), **5** (FIG. 1C). Data was obtained by determining the agonist-induced change in $[Ca^{2+}]_i$ of 1321N1 cells stably expressing the human P2Y₁GFP receptor. Cells were pre-incubated with 2 μ M fura-2 AM for 30 min and change in fluorescence ($\Delta F_{340\text{ nm}/F_{380\text{ nm}}}$) was detected.

FIGs. 2A-C present concentration-response curves for agonists at hP2Y₁₁-R: ADP(α -S), **6A** and **6B**, and 2-SMe-ADP(α -S), **7A** and **7B** (FIG. 2A); 2-Cl-ADP(α -S), **8A** and **8B**, and ADP, **2** (FIG. 2B), 2-SMe-ADP(β -S), **10**, 2-Cl-ADP(β -S), **11**, ADP, **2**, and ADP(β -S), **5** (FIG. 2C). Data were obtained by determining the agonist-induced change in $[Ca^{2+}]_i$ of 1321N1 cells stably expressing the human P2Y₁₁GFP receptor.

Cells were pre-incubated with 2 μ M fura-2 AM for 30 min and change in fluorescence ($\Delta F_{340\text{ nm}/F_{380\text{ nm}}}$) was detected.

FIG. 3 presents comparative plots showing the effect of 2-SMe-ADP(α -S), **7A** and **7B**, vs. ADP, **2**, on the amount of DMPO-OH adduct formed under Fenton reaction conditions. The amount is given as the percentage of control, which contains only FeSO₄, H₂O₂, and DMPO.

FIG. 4 presents comparative plots showing the effect of hydrolysis of 2-SMe-ADP(α -S), **7A**, ADP, **2** and ADP- α -S, **6A**, in human blood serum and RPMI-1640 medium over 24 hours at 37 °C, as monitored by HPLC.

FIGs. 5A-B present bar graphs showing a reduction of antioxidant activity of 2-SMe-ADP(α -S), **7A**, in PC12 cells under oxidizing conditions upon blocking P2Y₁₂-R with 2-SMe-AMP (FIG. 5A) and a reduction of antioxidant activity of **7A**, in Ntera-2 cells by blocking P2Y₁-R with MRS2179 (FIG. 5B). The results shown are the mean $\pm$ SD of three independent experiments performed in triplicate (* $P < 0.05$, **7A** vs. **7A** with P2Y_{1/12}-R inhibitors).

FIG. 6 is a bar graph showing the neuroprotective activity of 2-SMe-ADP(α -S), **7A** vs. **7B**. Cortical neurons were treated with **7A** or **7B** 0.01-25 μ M and 3 μ M FeSO₄ for 24 hours. After 24 hours, cell viability was measured by a MTT assay. The results shown are the mean $\pm$ S.D. of three independent experiments in triplicate ($P < 0.05$).

FIGs. 7A-E present photographs showing the effect of 2-SMe-ADP(α -S), **7A**, and FeSO₄ treatment on the morphology of primary cortical neurons. Neuronal cells were photographed before (FIG. 7A) and after treatment with 3 μ M FeSO₄ (FIG. 7B) for 24 hours. In order to reduce the oxidative stress the cells were treated with **7A** at 0.2 (FIG. 7C), 5 μ M (FIG. 7D), and 100 μ M (FIG. 7E) and photographed by light microscopy (100 $\times$ magnification). The arrows mark the areas that maintain the normal morphology in the soma and the extensions.

FIG. 8 presents comparative plots showing the chelation of ferrous ions by 2-SMe-ADP(α -S), **7A**, and ADP. Values represent mean $\pm$ S.D of three experiments.

FIGs. 9A-C present a graph showing the effect of 2-SMe-ADP(α -S), **7A**, at various concentrations, on the viability of primary mixed culture of neurons and astrocytes in the presence of A β ₄₂ (FIG. 9A), a bar graph showing the effect of 2-SMe-ADP(α -S), **7A**, at various concentrations, on reduction of LDH levels (FIG. 9B), and a

graph showing the effect of 2-SMe-ADP(α -S), **7A**, (50 μ M) on disaggregation of A β 42-Zn²⁺ (50 μ M).

FIGs. 10A-D present computational modeling structures of P2Y₁R. FIG. 10A presents experimental crystal structure, 4XNV, with BPTU antagonist; FIG. 10B presents experimental crystal structure, 4XNW, with MRS-2500 antagonist; FIG. 10C presents 2SMe-ADP(β -S), Compound **7A**, docked in the 4XNV_{MOD} receptor; and FIG. 10D presents 2-SMe-ADP(β -S), **7A**, docked in the 4XNW receptor. Green oval shapes indicate the binding pocket.

FIGs. 11A-D are bar graphs presenting experimental EC₅₀ values (FIG. 11A) and predicted EC₅₀ values (FIG. 11B) using ligand CDOCKER interaction energy with the 4XNV_{MOD} receptor, predicted EC₅₀ values using solvation energy (FIG. 11C) and predicted EC₅₀ values using both ligand CDOCKER interaction energy with the 4XNV_{MOD} receptor and solvation energy (FIG. 11D).

FIGs. 12A-B present computational modeling structures showing the binding cavity of 4XNV_{MOD} receptor complex with ADP (**2**) (FIG. 12A) and 2-SMe-ADP(α -S) (**7A**) (FIG. 12B).

FIG. 13 is schematic illustration presenting the protocol of the in vivo studies conducted for chronic treatment with Compound **7A** (**SK**) in 5XFAD mouse model of Alzheimer's Disease.

FIGs. 14A-C present the data obtained for female (FIG. 14B) and male (FIG. 14C) mice, in the elevated plus maze test shown in FIG. 14A. * = p<0.05, n.s = not significant

FIGs. 15A-C present the data obtained for female (FIG. 15B) and male (FIG. 15C) mice, in the T maze memory test shown in FIG. 15A. * = p<0.05, # = p=0.07.

FIGs. 16A-C present the data (FIGs. 16A-B) obtained in fear conditioning test for long term cognitive memory depicted in FIG. 16C. * = p<0.05.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy and, more particularly, but not exclusively, to novel adenosine phosphate derivatives and to uses thereof as antioxidants and/or P2Y₁-R agonists, and in the treatment of medical

conditions associated with oxidative stress and/or neurodegeneration, such as Alzheimer's Disease.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

With a view to identify novel and biocompatible neuroprotectants, the present inventors have designed a series of nucleoside 5'-thiophosphate analogues (thiolated adenosine 5'-phosphate derivatives), and have identified some structural features that attribute to an improved activity of such analogs, compared to, for example, ADP, ATP, SMe-ADP, and adenosine 5'-phosphorothioate.

Recently, it was reported that adenosine 5'-boranophosphate analogues act as selective P2Y₁-R agonists (EC₅₀ of 7-150 nM) [Azran et al., 2013, *supra*]. The thiophosphate analogues disclosed herein were found to be active agonists at P2Y₁-R, and also better Fe(II) chelators (i.e. better antioxidants) than the corresponding boranophosphate analogues.

As described in the Examples section that follows, exemplary thiophosphate nucleotide analogues according to the present embodiments were found to act as neuroprotectants, rescuing PC12 cells and primary neurons from oxidative stress initiated by FeSO₄, assisting in maintaining the normal morphology of the neurons undergoing oxidative insult, rescuing neurons from A β ₄₂ toxicity and promoting disaggregation of zinc complexes of A β ₄₂. Without being bound by any particular theory, it is assumed that the neuroprotective action of thiophosphate nucleotide analogues according to the present embodiments is due to their activity as antioxidants, both as Fe(II)-chelators and OH radical scavengers in Fenton reaction.

In addition, the thiophosphate nucleotide analogues according to the present embodiments were shown to act as highly potent P2Y₁-R agonists, some of which also activate P2Y₁₁-R and P2Y₁₂-R. The activation of P2Y₁-R is known to result in neuroprotection. The EC₅₀ values of exemplary thiophosphate nucleotide analogues according to the present embodiments were shown to correlate well with a combination of desolvation and docking energies.

The most active thiophosphate nucleotide analogue according to exemplary embodiments of the present invention is denoted herein as Compound **7A**, or, interchangeably, simply as **7A**, or as 2-SMe-ADP(α -S), SK, SK6 or SSA37. See, Table B.

5 Compound **7A** was shown to act in rescuing PC12 cells and primary neurons from oxidative stress initiated by FeSO₄ with EC₅₀ value of 0.04 μ M. Furthermore, Compound **7A** helped maintain the normal morphology of the neurons undergoing oxidative insult.

10 Compound **7A** was shown to be a better Fenton reaction inhibitor than EDTA (Fe(II)-chelator) (IC₅₀ 37 vs. 54 μ M), and a better radical scavenger than Trolox (IC₅₀ 12 vs 18 μ M).

 Compound **7A** was shown to act as a highly potent P2Y1-R agonist which also activates P2Y11-R and P2Y12-R.

15 Compound **7A** was found to be relatively metabolically stable both to isolated ectonucleotidases and in human blood serum.

 Compound **7A** protected primary neuron culture from A β 42 toxicity with IC₅₀ of 0.5 μ M).

20 Compound **7A** showed also high treatment efficacy as indicated by behavioral and biochemical parameters in models of AD treatment. Compound **7A** markedly restored disinhibited behavior in the elevated plus maze (85 % decrease in time spent in the open arms ($p < 0.05$)); and showed improvement in learning and memory, (32 % reduction in the number of days required for learning in the water T maze assay ($p = 0.07$)), and is therefore a highly potent biocompatible multifunctional drug candidate, which may delay or slow down the progression of AD in patients.

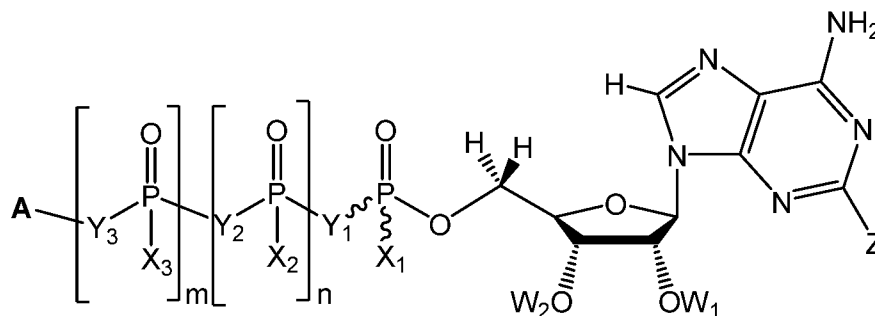
25 Embodiments of the present invention therefore relate to a novel family of compounds featuring an adenosine 5'-phosphorothioate skeleton, and to uses thereof as Fenton reaction inhibitors, P2Y receptor agonists, antioxidants, radical scavengers, and neuroprotectants, and in the treatment of medical conditions associated with oxidative stress, neurodegeneration, brain injuries, and more.

30 ***The compounds:***

 The compounds of the present embodiments are also referred to herein interchangeably as nucleotide analogues, thiophosphate analogues, thiophosphate

nucleotide, nucleoside 5'-thiophosphate analogues, or simply as nucleotides or as analogues, including variations of these terms.

The compounds of the present embodiments can be collectively represented by Formula I:



Formula I

wherein:

the curled lines denote an Rp or Sp configuration (when applicable);

n and m are each independently 0 or 1;

Z is selected from $-SR_1$, $S(=O)R_1$, $S(=O)NHR_1$, OR_1 , NHR_1 , R_1 and halo, preferably from $-SR_1$, $S(=O)R_1$, OR_1 , NHR_1 , R_1 and halo;

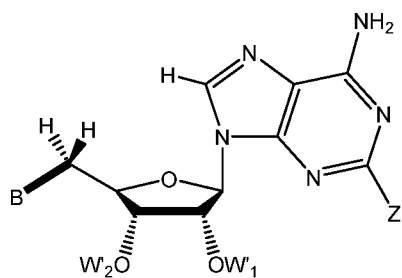
R_1 is alkyl, cycloalkyl, aryl or acyl, preferably alkyl, cycloalkyl or aryl;

W_1 and W_2 are each independently selected from H, alkyl and acyl;

Y_1 , Y_2 and Y_3 are each independently O, S, CH_2 , $C(L)_2$ or NH, wherein L is halo;

X_1 , X_2 and X_3 are each independently -OH or -SH, provided that at least one of X_1 , X_2 and X_3 is SH;

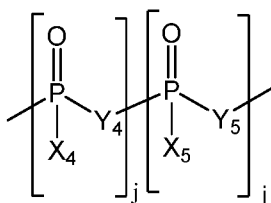
and



A is H or

wherein Z' is selected from $-SR'_1$, $S(=O)R'_1$, $S(=O)NHR'_1$, OR'_1 , $NH'R'_1$, R'_1 and halo, preferably from $-SR'_1$, $S(=O)R'_1$, OR'_1 , NHR'_1 , R'_1 and halo; R'_1 is alkyl, cycloalkyl, aryl or acyl, preferably alkyl, cycloalkyl or aryl; W'_1 and W'_2 are each independently selected from H, alkyl and acyl; and B is absent or is

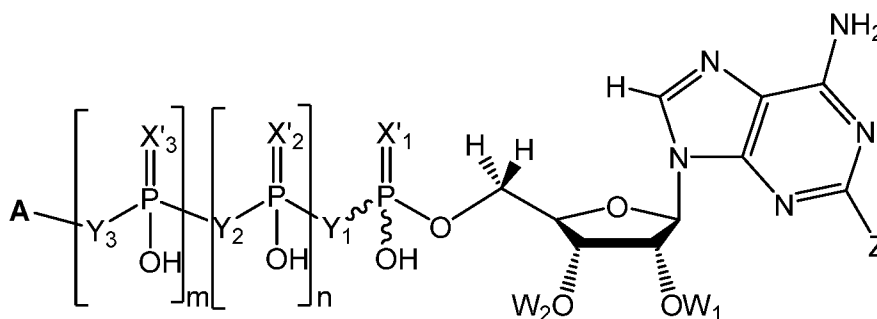
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, wherein Y_4 and Y_5 are each independently O, S, CH_2 , $C(L')_2$ or NH, wherein L' is halo; X_4 and X_5 are each independently -OH or -SH; and j and i are each independently an integer of 0, 1 or 2,

or a pharmaceutically acceptable salt thereof.

- 5 The compounds described herein can otherwise be collectively represented by Formula I*, which presents a tautomeric form thereof:



Formula I*

- 10 wherein:

the curled lines denote an Rp or Sp configuration (when applicable);

n and m are each independently 0 or 1;

Z is selected from $-SR_1$, $S(=O)R_1$, $S(=O)NHR_1$, OR_1 , NHR_1 , R_1 and halo, preferably from $-SR_1$, $S(=O)R_1$, OR_1 , NHR_1 , R_1 and halo;

- 15 R_1 is alkyl, cycloalkyl, aryl or acyl, preferably alkyl, cycloalkyl or aryl;

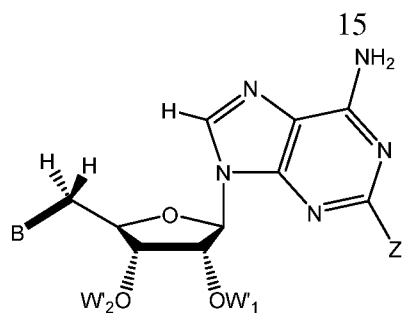
W_1 and W_2 are each independently selected from H, alkyl and acyl;

Y_1 , Y_2 and Y_3 are each independently O, S, CH_2 , $C(L)_2$ or NH, wherein L is halo;

X'_1 , X'_2 and X'_3 are each independently O (oxygen) or S (sulfur), provided that

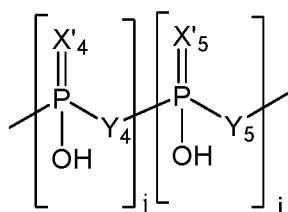
- 20 at least one of X'_1 , X'_2 and X'_3 is S (sulfur);

and



A is H or ,

wherein Z' is selected from $-\text{SR}'_1$, $\text{S}(=\text{O})\text{R}'_1$, $\text{S}(=\text{O})\text{NHR}'_1$, OR'_1 , $\text{NH}'\text{R}_1$, R'_1 and halo, preferably from $-\text{SR}'_1$, $\text{S}(=\text{O})\text{R}'_1$, OR'_1 , NHR'_1 , R'_1 and halo; R'_1 is alkyl, cycloalkyl, aryl or acyl, preferably alkyl, cycloalkyl or aryl; W'_1 and W'_2 are each independently selected from H, alkyl and acyl; and B is absent or is



, wherein Y_4 and Y_5 are each independently O, S, CH_2 , $\text{C}(\text{L}')_2$ or NH, wherein L' is halo; X'_4 and X'_5 are each independently O (oxygen) or S (sulfur); and j and i are each independently an integer of 0, 1 or 2,

or a pharmaceutically acceptable salt thereof.

Herein throughout, whenever a compound is presented as one of its tautomeric forms, it is to be understood that the other tautomeric form is also encompassed. Thus compounds represented by Formulae I and I* are to be regarded as equivalent alternatives to one another.

The phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter ion. In some embodiments, a pharmaceutically acceptable salt exhibits substantially similar biological activity and other properties as the parent compound. In some embodiments, a pharmaceutically acceptable salt is used to modify the solubility or other physical or chemical properties of the parent compound, while not abrogating its biological activity. An example, without limitation, of a pharmaceutically acceptable salt of the compounds described herein is a compound represented by Formula I or I* as described herein in which the phosphate and phosphorothioate groups are in a form of an anion thereof, in which case the compound further comprises a corresponding number of cations (which can be the same or different. Exemplary cations include, but not limited to, monovalent metal cations such

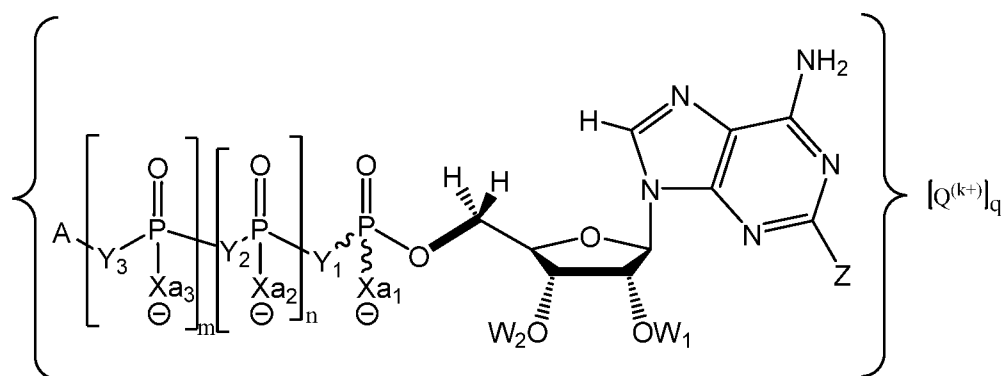
as cations of sodium, potassium, lithium and/or cesium (Na^+ , K^+ , Li^+ and/or Cs^+ , respectively); divalent metal cations such as cations of magnesium, calcium, iron, cobalt, nickel, copper, manganese, cadmium, strontium, and/or zinc (Mg^{2+} , Ca^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Mn^{2+} , Cd^{2+} , Sr^{2+} or Zn^{2+}); trivalent metal cations such as, for example, Fe^{3+} , La^{3+} , Eu^{3+} or Gd^{3+} ; and organic cations such as, for example, ammonium, sulfonium, phosphonium or arsonium. Any other cations are contemplated.

In some embodiments, the cations are monovalent cations, and in some of these embodiments, the cations are sodium cations.

The number of cations is equivalent to the number of phosphate or thiophosphate (thiophosphate) anions in the compound.

The salts described herein comprise negatively charged phosphate and/or thiophosphate anions, and one or more monovalent cations, each interacting with one phosphate or thiophosphate group, or divalent or trivalent cations which interact with more than one phosphate and/or thiophosphate group.

In embodiments where the compound is a salt, the compounds described herein are collectively represented by Formula Ia:

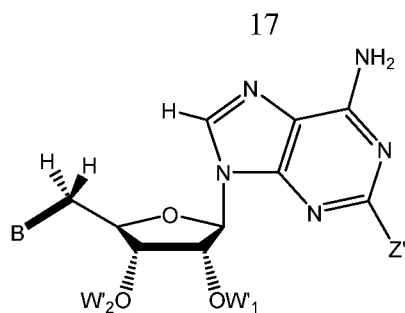


Formula Ia

wherein:

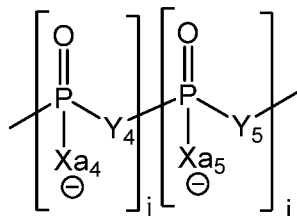
Y_1 , Y_2 , Y_3 , W_1 , W_2 , Z , n , m and the curled lines are as defined for Formula I;

Xa_1 , Xa_2 and Xa_3 are each independently S (sulfur) or O (oxygen), at least one of Xa_1 , Xa_2 and Xa_3 being S (sulfur);



A is absent or is

wherein Z' , W'_1 and W'_2 are as defined for Formula I; and B is absent or is



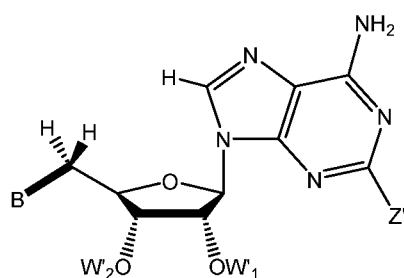
, wherein Y_4 and Y_5 , i and j are as defined herein for Formula I, and Xa_4 and Xa_5 are each independently O (oxygen) or S (sulfur);

- 5 Q is a cation, k is the cation valence, and q is the number of cations, whereby q corresponds to k and is such that the number of cations provide for a positive charge that is equal to the number of negatively charged group in the compound (which in turn corresponds to the values of m , n and A , as described herein).

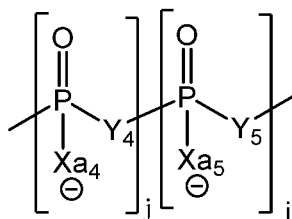
10 In some embodiments, when A is Formula Ia is absent, Y_3 can be a negatively charged group such as S^- , O^- .

For example, when Q is a monovalent cation, as described herein, k is 1, and q is equal to the number of negatively charged groups in the compound. For example, when m and n are each 1, A is absent and Y_3 is a negatively charged group, q is 4. When one of m and n is 0, A is absent and Y_3 is a negatively charged group, q is 3.

15 When m and n are each 1, A is absent and Y_3 is a non-charged group, q is 3. When one of m and n is 0, A is absent and Y_3 is a non-charged group, q is 2. Whenever A is



and B is

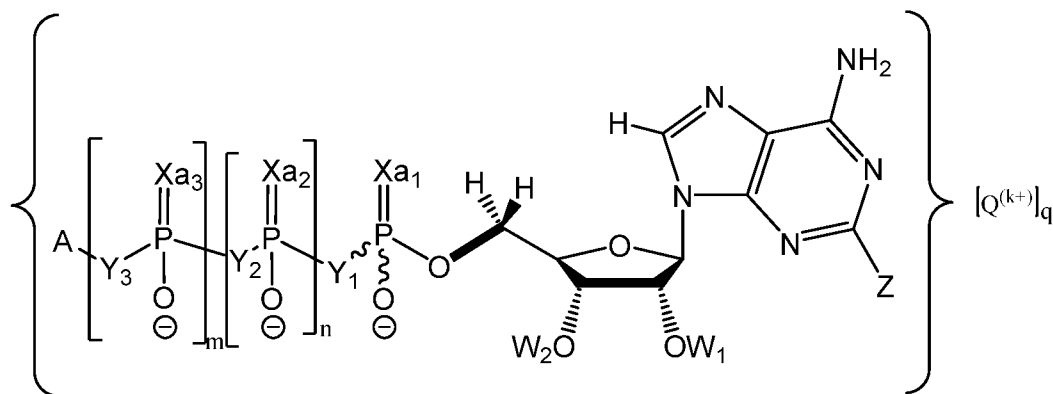


1, q is $m+n+3$, for monovalent cations.

In embodiments where the compound is a salt, the compounds described herein

20 are alternatively collectively represented by Formula I*a:

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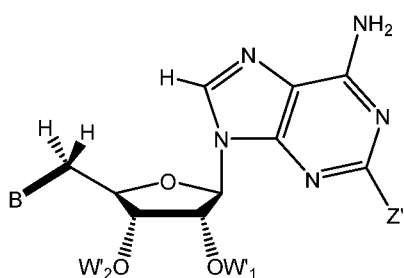


Formula I*a

5 wherein:

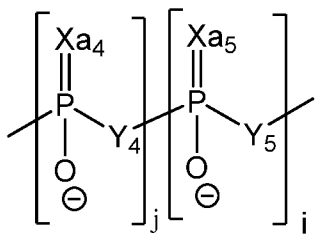
Y_1 , Y_2 , Y_3 , W_1 , W_2 , Z , n , m and the curled lines are as defined for Formula I;

Xa_1 , Xa_2 and Xa_3 are each independently S (sulfur) or O (oxygen), at least one of Xa_1 , Xa_2 and Xa_3 being S (sulfur);



A is absent or is

10 wherein Z' , W'_1 and W'_2 are as defined for Formula I; and B is absent or is



, wherein Y_4 and Y_5 , i and j are as defined herein for Formula I, and Xa_4 and Xa_5 are each independently O (oxygen) or S (sulfur);

15 Q is a cation, k is the cation valence, and q is the number of cations, whereby q corresponds to k and is such that the number of cations provide for a positive charge that is equal to the number of negatively charged group in the compound (which in turn corresponds to the values of m , n and A , as described herein), as described herein for Formula Ia.

In some embodiments, when A is Formula I**a* is absent, Y₃ can be a negatively charged group such as S⁻, O⁻.

In some of any of the embodiments described herein, the salt is a sodium salt, such that Q is sodium and k is 1. The sodium salt can be a trisodium salt, a
5 tetrasodium salt, and so forth, depending on the number of negatively charged groups in the compounds, as described herein.

Any one of the embodiments described herein, and any combination thereof, refer to compounds represented by Formulae I and/or I*, or Ia and/or I**a*, unless otherwise indicated.

10 The present embodiments further encompass any enantiomers, isomers, or diastereomers of the compounds described herein.

As used herein, the term "enantiomer" refers to a stereoisomer of a compound that is superposable with respect to its counterpart only by a complete inversion/reflection (mirror image) of each other. Enantiomers are said to have
15 "handedness" since they refer to each other like the right and left hand. Enantiomers have identical chemical and physical properties except when present in an environment which by itself has handedness, such as all living systems. In the context of the present embodiments, a compound may exhibit one or more chiral centers, each of which exhibiting an *R*- or an *S*-configuration and any combination, and compounds according
20 to some embodiments of the present invention, can have any their chiral centers exhibit an *R*- or an *S*-configuration.

The term "diastereomers", as used herein, refers to stereoisomers that are not enantiomers to one another. Diastereomerism occurs when two or more stereoisomers of a compound have different configurations at one or more, but not all of the
25 equivalent (related) stereocenters and are not mirror images of each other. When two diastereoisomers differ from each other at only one stereocenter they are epimers. Each stereo-center (chiral center) gives rise to two different configurations and thus to two different stereoisomers. In the context of the present invention, embodiments of the present invention encompass compounds with multiple chiral centers that occur in any
30 combination of stereo-configuration, namely any diastereomer.

The compounds as described herein comprise a nucleoside moiety, adenoside, which features a chiral glucose moiety. While in Formulae I, I*, Ia, I**a*, II, and II*,

described herein, the compounds are presented as featuring a certain configuration of the glucose moiety, it to be understood that other configurations of this chiral center are also contemplated.

In some embodiments, for example, when one or both of m and n is 0, the first
5 phosphate (or thiophosphate) group (also referred to herein and in the art as $P\alpha$), also features a chiral center, giving rise to two different diastereomers.

In these embodiments, the configuration of $P\alpha$ can be an R_p or R_s configuration.

In Formula I, an R_p configuration denotes a diastereoisomer in which X_1 points inwards and Y_1 points outwards, and an S_p configuration denotes an inverse
10 stereoisomer in which X_1 points outwards and Y_1 points inwards (see, for example, Table B).

In Formula Ia, an R_p configuration denotes a diastereoisomer in which X_{a1} points inwards and Y_1 points outwards, and an S_p configuration denotes an inverse
15 stereoisomer in which X_{a1} points outwards and Y_1 points inwards (see, for example, Table B).

In Formula I* or I*a, an R_p configuration denotes a diastereoisomer in which OH or O points inwards and Y_1 points outwards, and an S_p configuration denotes an
inverse stereoisomer in which OH or O points outwards and Y_1 points inwards (see, for example, Table B).

20 In some of any of the embodiments described herein, when relevant and/or applicable (namely, when $P\alpha$ features a chiral center), the $P\alpha$ configuration is R_p configuration as described herein, and the curled lines are such that the respective groups point inwards and outward as described herein.

According to some of any of the embodiments described herein, when relevant
25 and/or applicable, the compound exhibits an R_p configuration. In some embodiments the compound exhibits S_p configuration, and in some embodiments, it includes a mixture of the diastereomers.

It is to be noted that when $P\alpha$ does not feature a chiral center, the curled lines in
any of the Formulae described herein denote bonds of non-chiral phosphate or
30 thiophosphate group.

In any of the embodiments described herein, at least one of X_1 , X_2 and X_3 in Formula I is SH, or at least one of X'_1 , X'_2 and X'_3 in Formulae I*, or at least one of Xa_1 , Xa_2 and Xa_3 in Formulae Ia or I*a is S.

That is, either X_1 is SH (for Formula I), Xa_1 is S (for Formulae Ia and I*a) or X'_1 is S (for Formula I*), or one of n and m are not 0, and the corresponding “X” (in Formula I) or “X’” (In Formula I*) or “Xa” (in Formulae Ia and I*a) is SH or S.

In some embodiments, X_1 is SH (for Formula I), Xa_1 is S (for Formulae Ia and I*a) or X'_1 is S (for Formula I*), n is 0 or 1, and m is 0 or 1. In some of these embodiments, each of X_2 and X_3 (or X'_2 and X'_3 for Formula I*, or Xa_2 or Xa_3 for Formulae Ia and I*a), if present, is other than SH (for Formula I) or S (for Formulae Ia and I*a) or X'_1 is other than S (for Formula I*), and in other embodiments, one or both of X_2 and X_3 (or X'_2 and X'_3 for Formula I* or Xa_2 or Xa_3 for Formulae Ia and I*a), if present, is SH (for Formula I) or S.

In some embodiments, X_1 is other than SH (for Formula I) or Xa_1 is other than S (for Formulae Ia and I*a) or X'_1 is other than S (for Formula I*), n is 0 or 1, and m is 1. In some of these embodiments, X_3 is SH (for Formula I) or Xa_3 is S (for Formulae Ia and I*a) or X'_3 is S (for Formula I*). In some of these embodiments, X_2 (or X'_2 for Formula I* or Xa_2 for Formulae Ia and I*a), if present, is also SH (for Formula I) or S (for Formulae Ia and I*a) or X'_2 is S (for Formula I*), or is other than SH (for Formula I) or S.

In some embodiments, X_1 is other than SH (for Formula I) or Xa_1 is other than S (for Formulae Ia and I*a) or X'_1 is other than S (for Formula I*), n is 1, and m is 0 or 1. In some of these embodiments, X_2 is SH (for Formula I) or Xa_2 is S (for Formulae Ia and I*a) or X'_2 is S (for Formula I*). In some of these embodiments, X_3 (or X'_3 for Formula I* or Xa_3 for Formulae Ia and I*a), if present, is also SH (for Formula I) or S (for Formulae Ia and I*a) or X'_3 is S (for Formula I*), or is other than SH (for Formula I) or S.

According to some of any of the embodiments described herein, whenever X_1 , X_2 and/or X_3 is other than SH (for Formula I) or X'_1 , X'_2 and/or X'_3 (for Formula I*) or Xa_1 , Xa_2 and/or Xa_3 (for Formulae Ia and I*a) is other than S, one or both of those X_1 , X_2 and/or X_3 or of those X'_1 , X'_2 and/or X'_3 or of those Xa_1 , Xa_2 and/or Xa_3 is OH (for Formula I) or O.

In some embodiments, X_1 is SH (for Formula I) or X_{a1} is S (for Formulae Ia and I*a) or X'_1 is S (for Formula I*), and each of X_2 and X_3 (or X'_2 or X'_3 for Formula I* or X_{a2} or X_{a3} for Formulae Ia and I*a), when present, is OH (for Formula I) or O.

In some embodiments, X_1 is OH (for Formula I) or X_{a1} is O (for Formulae Ia and I*A), or X'_1 is O (for formula I*), and one or both of X_2 and X_3 (or X'_2 or X'_3 for Formula I* or X_{a2} or X_{a3} for Formulae Ia and I*a), when present, is SH (for Formula I) or S. In some of these embodiments, n is 0, m is 1, and X_3 is SH (for Formula I) or X_{a3} is S (for Formulae Ia and I*a) or X'_3 is S (for Formula I*). In some of these embodiments, n is 1, m is 1, and X_2 is SH (for Formula I) or X_{a2} is S (for Formulae Ia and I*a) or X'_2 is S (for Formula I*). In some of these embodiments, n is 1, m is 1, and X_3 is SH (for Formula I) or X_{a3} is S (for Formulae Ia and I*a) or X'_3 is S (for Formula I*).

According to some of any of the embodiments described herein, A is H, and the compound is a nucleoside (or nucleotide) analog. Alternatively, the compound is dinucleoside, wherein the two nucleoside moieties can be the same or different, as is described in further detail hereinafter.

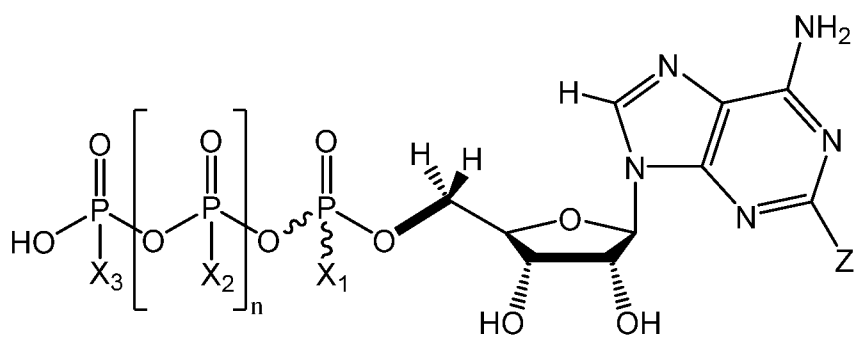
According to some of any of the embodiments described herein, one or more, or all of, Y_1 , Y_2 and Y_3 is O (oxygen).

According to some of any of the embodiments described herein, W_1 and W_2 are each H.

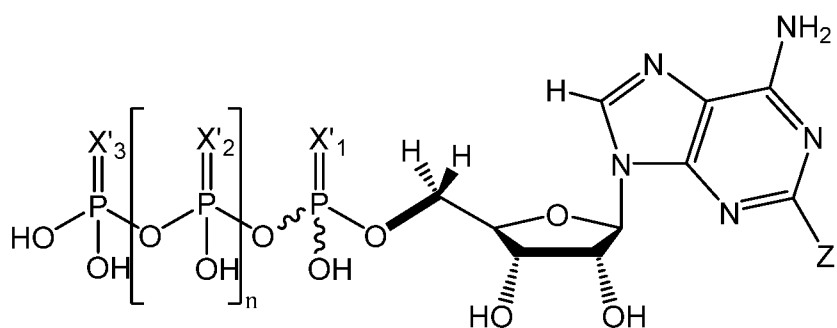
According to some of any of the embodiments described herein, n is 0 and m is 1, and the compound is an analog of ADP. Alternatively, the compound is analog of ATP, wherein m is 1 and n is 1.

In some of any of the embodiments described herein, A is H, W_1 and W_2 are each H, Y_1 , Y_2 and Y_3 are each O, n is 0 or 1, and m is 1. Compounds encompassed by these embodiments can be collectively represented by Formulae II, II*, IIa or II*a:

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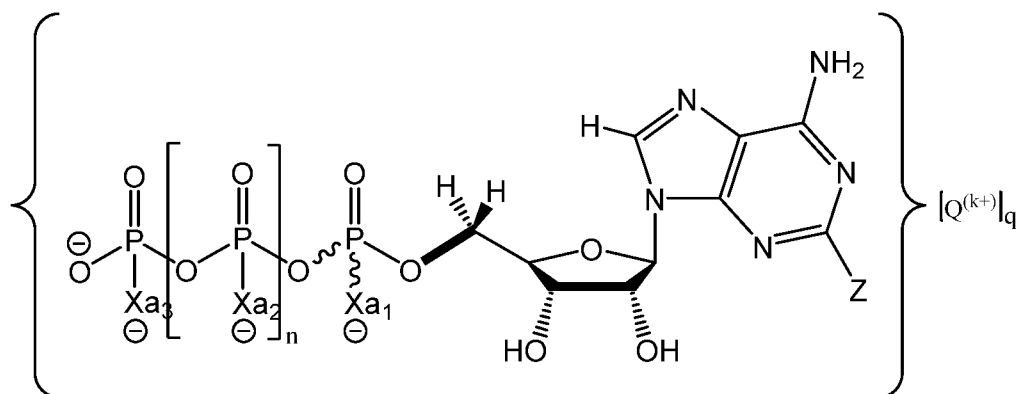


Formula II

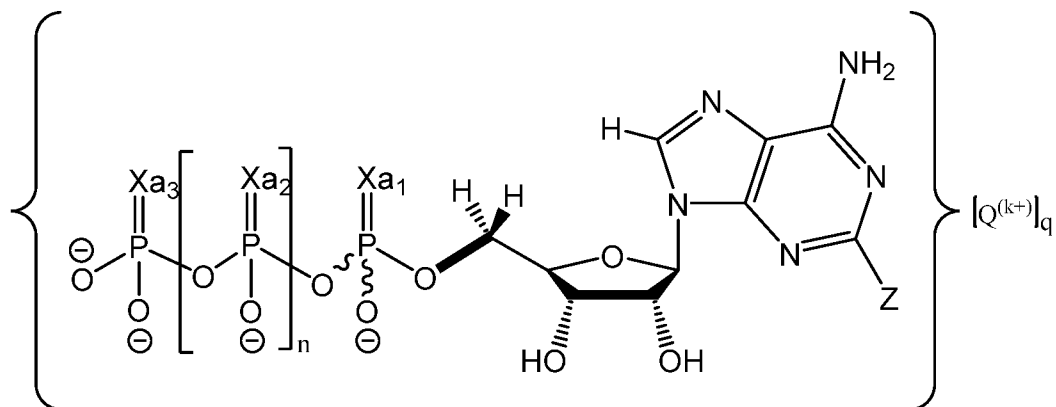


Formula II*

5



Formula IIa



Formula II*a

Wherein n, Z, X₁, X₂, X₃, X'₁, X'₂, X'₃, Xa₁, Xa₂ and Xa₃ are as defined herein in any of the respective embodiments.

According to some of any of the embodiments described herein, X₁ is –SH (for Formula I or II) or Xa₁ is S (for Formulae Ia and I*a, and IIa and II*a) or X'₁ is S (for Formula I* or II*).

In some of these embodiments, n is 0, and m is 1, and the compound is a derivative of ADP-α-S.

According to some of these embodiments, X₃ is –OH (for Formula I) or Xa₃ is O (for Formulae Ia and I*a) or X'₃ is O (for Formula I*).

According to some of any of the embodiments described herein, n is 0, and m is 1, and X₃ is –SH (for Formula I) or Xa₃ is S (for Formulae Ia and I*a) or X'₃ is S (for Formula I*), and the compound is a derivative of ADP-β-S.

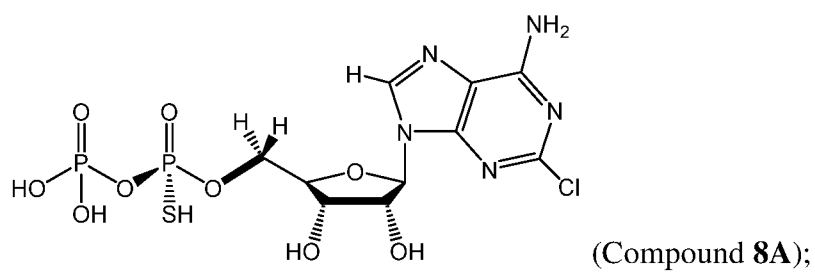
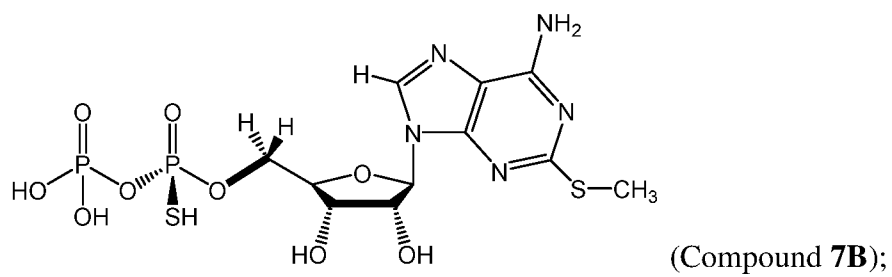
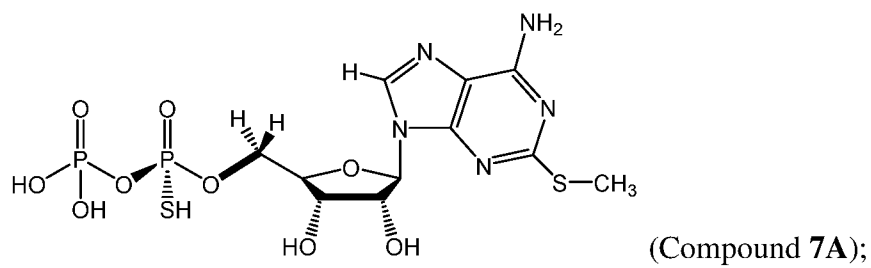
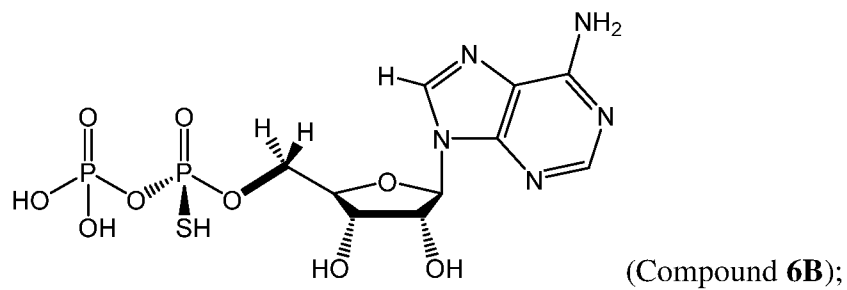
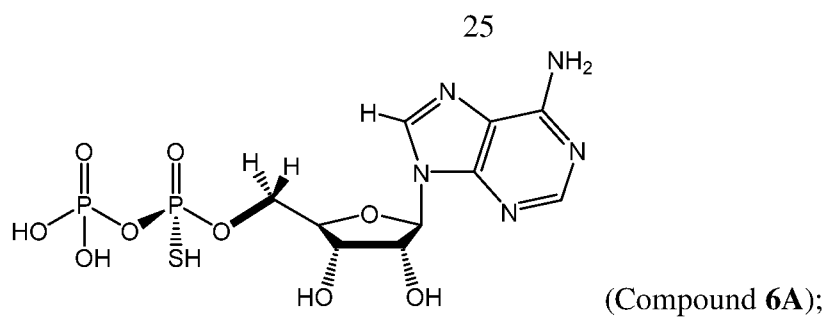
According to some of these embodiments, X₁ is –OH (for Formula I) or Xa₁ is O (for Formulae Ia and I*a) or X'₁ is O (for Formula I*).

According to some of any of the embodiments described herein, Z is –SR₁ or halo (e.g., chloro), and in some embodiments, Z is –SR₁.

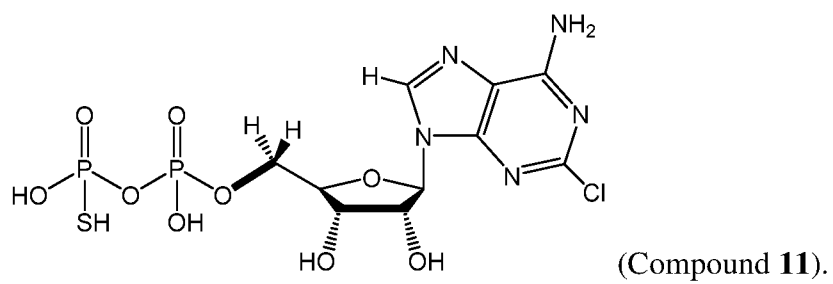
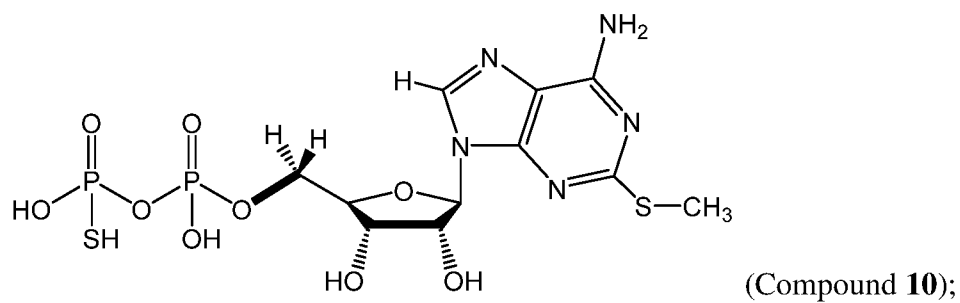
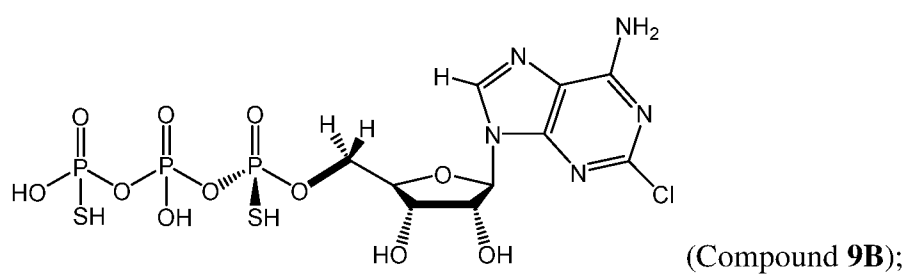
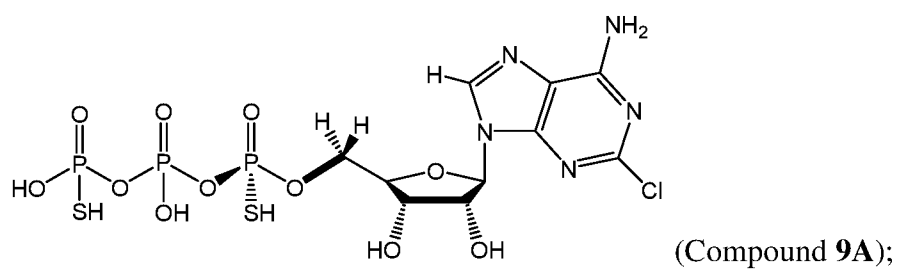
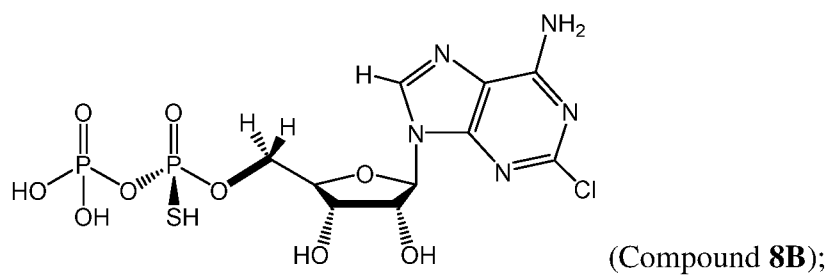
According to some of any of the embodiments described herein, Z is –SR₁ and R₁ is alkyl, preferably methyl.

According to some of any of the embodiments described herein, m is 1, n is 0, A is H, W₁ and W₂ are each H, Y₁, Y₂ and Y₃ are each O, X₁ is SH (for Formula I) or Xa₁ is S (for Formulae Ia and I*a) or X'₁ is S (for Formula I*) and X₃ is OH (for Formula I) or Xa₃ is O (for Formulae Ia and I*a) or X'₁ is O (for Formula I*). In some of these embodiments, Z is SR₁, and preferably, Z is –SMe.

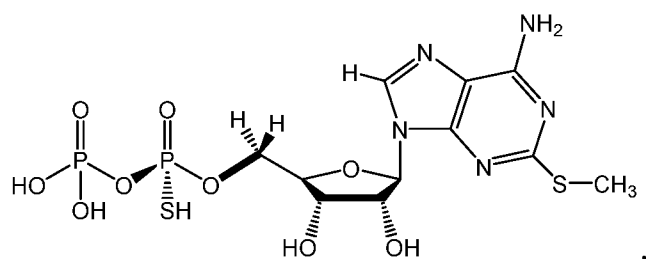
Exemplary compounds according to the present embodiments include, but are not limited to, Compounds **6-11**, as described herein. The following presents the structures of these compounds, in a representation form of Formula I. Table B below presents the structures of the anionic portion of these compounds in a representation form of Formulae IIa and II*a. Any other representation (e.g., of Formula I*), and any corresponding salt, as represented in Formulae Ia and I*a, is encompassed.



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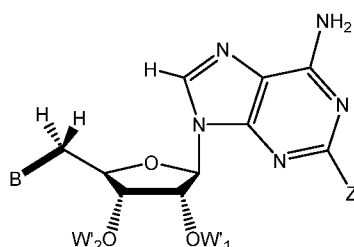


According to some of any of the embodiments described herein, the compound is Compound **7A**:



5 or a pharmaceutically acceptable salt thereof (e.g., a trisodium salt thereof).

According to some of any of the embodiments described herein, A is or



comprises another nucleoside moiety, , which can be the same or different from the corresponding moiety in the compound. Thus, Z' can be the same as or different from Z, W₁' can be the same as or different from W₁, and W₂' can be the same as or different from W₂.

When B is absent, and A is a nucleoside moiety as described herein, the two nucleoside moiety are bridged (linked therebetween) by a di- or tri-phosphate bridge (in which one phosphate group is a phosphothioate group).

15 Alternatively, B is as described herein, and the two nucleoside groups are linked therebetween via a higher phosphate bridge (in which one or more phosphate groups is a phosphothioate group).

The present embodiments further encompass any hydrates and solvates of the compounds described hereinabove.

20 The term “solvate” refers to a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on), which is formed by a solute (the compound of present embodiments) and a solvent, whereby the solvent does not interfere with the biological activity of the solute. Suitable solvents include, for example, ethanol, acetic acid and the like.

The term “hydrate” refers to a solvate, as defined hereinabove, where the solvent is water.

In some of any of the embodiments described herein, a compound as described herein is capable of activating, or activates, a P2Y₁ receptor, for example, hP2Y₁ receptor. Activation of a P2Y₁ receptor can be measured by assays known in the art, for example, as described herein in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of activating, or activates, a P2Y₁ receptor, a P2Y₁₁ receptor, a P2Y₁₂ receptor and/or a P2Y₁₃ receptor, for example, hP2Y₁, hP2Y₁₁, hP2Y₁₂ and/or hP2Y₁₃ receptor. Activation of these receptors can be measured by assays known in the art, for example, as described herein in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein inhibits, or is capable of inhibiting, Fenton reaction.

Herein and in the art, “Fenton reaction” describes a reaction in which Iron(II) is oxidized by hydrogen peroxide to iron(III), forming a hydroxyl radical and a hydroxide ion in the process. Iron(III) is then reduced back to iron(II) by another molecule of hydrogen peroxide, forming a hydroperoxyl radical and a proton. The net effect is a disproportionation of hydrogen peroxide to create two different oxygen-radical species, with water ($H^+ + OH^-$) as a byproduct. The Fenton reaction thus involves generation of free radicals in the presence of transition-metal ions such as iron.

Inhibition of Fenton reaction can be measured, for example, by ESR measurements, as exemplified in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of radical scavenging, or scavenges free radicals. Radical scavenging can be measured by assays well known in the art, for example, as ABTS colorization assay, as described in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of chelating metal ions, or chelates metal ions, namely, is a metal ion chelator, preferably, of transition metal ions such as ions of iron and copper, preferably iron. In some embodiments, the metal ion is Fe(II). Chelation of metal ions such as Fe(II) can be measured by methods known in the art, as exemplified in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein inhibits, or is capable of inhibiting production of reactive oxygen species (ROS) in neuronal cells.

As used herein and in the art, reactive oxygen species (ROS) include oxygen-containing molecules and/or ions in which an oxygen atom is in a free radical form (having an unpaired electron) or molecules or ions that readily generate species featuring one or more oxygen free radicals or oxygen in singlet state. Examples include, without limitations: ozone, peroxides, $\text{RO}\cdot$, and $\text{ROO}\cdot$, in which R is an organic moiety or hydrogen. In the presence of water or any other protic solvent, ROS typically generate hydrogen peroxide.

Assays for measuring production of ROS are known in the art and some are described in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is resistant to enzymatic hydrolysis, for example, to hydrolysis by enzymes of the ecto-nucleotide pyrophosphatase / phosphodiesterase (eNPP) family, or any other blood serum enzymes. Assays for measuring level of enzymatic hydrolysis are known in the art and some are described in the Examples section that follows. By “resistant” it is meant, for example, that a rate of hydrolysis of the compound in the presence of an indicated enzyme or blood serum (e.g., human blood serum) is less than 50 % within 1 hour, or less than 40 % within 1 hour, or less than 30 % within one hour, or less than 20 % within one hour, at the body temperature, which values are dependent on the assay. In some embodiments, the compound is characterized by a half life in human blood serum higher than 1 hour, or higher than 2 hours, preferably higher than 3 hours, or higher than 4 hours, or higher than 5 hours, or higher than 6 hours, or higher than 7 hours, or higher than 8 hours, or higher than 9 hours, or higher than 10 hours, or higher than 11 hours, or higher than 12 hours, or higher than 13 hours, or higher than 14 hours, under assay conditions as described in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of protecting, or protects, neurons from Fe(II) insult. Such a property can be determined, for example, by determining neuronal cells viability in the presence of Fe(II) ions, as exemplified in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of protecting, or protects, neurons from amyloid beta ($A\beta_{42}$) toxicity and/or is capable of disintegrating, or disintegrates complexes of amyloid beta with zinc. Such a property can be determined by assays known in the art, for example, as exemplified in the Examples section that follows.

In some of any of the embodiments described herein, a compound as described herein is capable of, or usable in, treating medical conditions in which one or more of the above characteristics is beneficial, such as medical conditions associated with oxidative stress and/or neurodegeneration, as is described in further detail hereinafter.

Therapeutic/Pharmacological Application:

According to an aspect of some embodiments of the present invention, a compound as described is for use in activating P2Y₁ (e.g., hP2Y₁) receptor or a phylogenetically related receptor, for example, in a subject in need thereof.

According to an aspect of some embodiments of the present invention, a compound as described is for use in treating a medical condition treatable by activating P2Y₁ (e.g., hP2Y₁) receptor or a phylogenetically related receptor.

According to an aspect of some embodiments of the present invention, there is provided a method of activating P2Y₁ (e.g., hP2Y₁) receptor or a phylogenetically related receptor, which is effected by contacting cells expressing the receptor with a compound as described herein. In some embodiments, contacting is effected *in vitro*. In some embodiments, contacting is effected *in vivo*, by administering the compound to a subject, e.g., a subject in need of activating the receptor.

According to an aspect of some embodiments of the present invention, there is provided a method of treating a medical condition treatable by activating P2Y₁ (e.g., hP2Y₁) receptor or a phylogenetically related receptor and/or a medical condition in which activating a P2Y₁ (e.g., hP2Y₁) receptor is beneficial, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

According to some of any of the embodiments described herein, the medical condition is treatable also by activating P2Y₁₁ receptor and/or P2Y₁₂ receptor and/or P2Y₁₃ receptor.

According to an aspect of some embodiments of the present invention, a compound as described is for use in inhibiting Fenton reaction, for example, in a subject in need thereof.

According to an aspect of some embodiments of the present invention, a
5 compound as described is for use in treating a medical condition treatable by inhibiting Fenton reaction.

According to an aspect of some embodiments of the present invention, there is provided a method of inhibiting Fenton reaction, which is effected by contacting a medium containing Fenton reagents with a compound as described herein. In some
10 embodiments, contacting is effected *in vitro*.

According to an aspect of some embodiments of the present invention, a compound as described is for use in scavenging free radicals, and/or chelating metal ions and/or inhibiting or reducing ROS production, for example, in a subject in need thereof.

According to an aspect of some embodiments of the present invention, a
15 compound as described is for use in treating a medical condition in which scavenging free radicals, and/or chelating metal ions and/or inhibiting or reducing ROS production is beneficial.

According to an aspect of some embodiments of the present invention, there is
20 provided a method of scavenging free radicals, and/or chelating metal ions and/or inhibiting or reducing ROS production, in a subject in need thereof, which is effected by administering an effective amount of a compound as described herein to the subject.

According to an aspect of some embodiments of the present invention, there is provided a method of treating a medical condition in which scavenging free radicals,
25 and/or chelating metal ions and/or inhibiting or reducing ROS production is beneficial, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

According to an aspect of some embodiments of the present invention, a compound as described is for use in reducing toxicity of amyloid beta to neuronal cells
30 and/or disintegrating zinc complexes of amyloid beta, for example, in a subject in need thereof.

According to an aspect of some embodiments of the present invention, a compound as described is for use in treating a medical condition in which reducing toxicity of amyloid beta to neuronal cells and/or disintegrating zinc complexes of amyloid beta is beneficial.

5 According to an aspect of some embodiments of the present invention, there is provided a method of reducing toxicity of amyloid beta to neuronal cells and/or disintegrating zinc complexes of amyloid beta, in a subject in need thereof, which is effected by administering an effective amount of a compound as described herein to the subject.

10 According to an aspect of some embodiments of the present invention, there is provided a method of treating a medical condition in which reducing toxicity of amyloid beta to neuronal cells and/or disintegrating zinc complexes of amyloid beta is beneficial, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

15 According to an aspect of some embodiments of the present invention, a compound as described is for use in treating a medical condition associated with oxidative stress in a subject in need thereof.

According to an aspect of some embodiments of the present invention, there is provided a method of treating a medical condition associated with oxidative stress in a
20 subject in need thereof, which is effected by administering to the subject a therapeutically effective amount of a compound as described herein.

Medical conditions associated with oxidative stress include a wide-ranging variety of diseases, such as, but not limited to, cardiovascular, neurological, metabolic, infectious, hepatic, pancreatic, rheumatoid, malignant and immunological diseases, as well as
25 conditions such as sepsis, cataract, amyotrophic lateral sclerosis and congenital diseases such as Down syndrome, multiple organ dysfunction and cystic fibrosis.

Oxidative stress is involved in many neurodegenerative diseases and disorders including, but not limited to, Parkinson's disease, Alzheimer's disease, Creutzfeldt-Jakob disease, multiple sclerosis, spongiform encephalopathies, degenerative diseases of the
30 basal ganglia, motoneuron diseases and memory loss, cerebral ischemia, scrapies, neurological viral diseases, post-surgical neurological dysfunction loss or memory impairment, Restless Legs Syndrome (RLS), Migraine, Huntington's disease (HD),

amyotrophic lateral sclerosis (ALS), ataxias, and traumatic brain injuries (such as concussion, blast injury, combat-related injury) or spinal cord injuries (such as partial or total spinal cord transection).

Oxidative stress is also involved in diabetes, cataract formation, and cancer.

5 Oxidative stress is also involved in infectious diseases, including, but not limited to, hepatitis C, AIDS, influenza and diseases caused by various neurotropic agents.

Oxidative stress is also involved in neurological dysfunction following cardiac surgery such as coronary bypass surgery, following multiple infarctions, such as impairment of brain function and memory.

10 Oxidative stress is also involved in cardiovascular diseases such as atherosclerosis, hypertension, stroke, cerebral ischemia, and restenosis.

In some embodiments, medical conditions associated with oxidative stress include gastric diseases and disorders such as dyspepsia, gastritis, gastric ulcers, gastric cancer and gastroparesis (e.g., diabetic gastroparesis), as well as other disease and disorders such as cardiovascular disease (e.g., atherosclerosis), diabetes (e.g., diabetic cataracts, diabetic nephropathy, diabetic neuropathy, diabetic accelerated atherosclerosis), neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Creutzfeld-Jacob disease), inflammatory diseases (e.g., osteoarthritis, lupus erythematosus, asthma, acute lung inflammation, allergic airway inflammation, primary biliary cirrhosis, viral hepatitis and alcoholic hepatitis), age-related disorders and cancer (e.g., colon cancer), rheumatoid arthritis, cataract, Down syndrome, cystic fibrosis, acute respiratory distress syndrome, asthma, post-surgical neurological dysfunction, amyotrophic lateral sclerosis, atherosclerotic cardiovascular disease, hypertension, post-operative restenosis, pathogenic vascular smooth muscle cell proliferation, pathogenic intra-vascular macrophage adhesion, pathogenic platelet activation, pathogenic lipid peroxidation, myocarditis, stroke, multiple organ dysfunction, complication resulting from inflammatory processes, cancer, aging, bacterial infection, sepsis; viral disease, such as AIDS, hepatitis C, an influenza and a neurological viral disease.

25 Any of the herein described medical conditions is encompassed by embodiments of the present invention.

According to an aspect of some embodiments of the present invention, a compound as described is for use in the treatment of a neurodegenerative disease or disorder, as described herein.

According to an aspect of some embodiments of the present invention, a
5 compound as described is for use in the treatment of Alzheimer's disease.

According to an aspect of some embodiments of the present invention, there is provided a method of treating a neurodegenerative disease or disorder, as described herein, which comprises administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

10 According to an aspect of some embodiments of the present invention, there is provided a method of treating Alzheimer's, which comprises administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

According to some embodiments, a compound as described herein is usable in methods of treating medical conditions such as, but not limited to, Alzheimer's disease,
15 Parkinson's Disease, Huntington's Disease, Prion Disease, and Amyotrophic lateral sclerosis; traumatic injuries to the brain (such as concussion, blast injury, combat-related injury) or spinal cord (such as partial or total spinal cord transection); malnutrition; toxic neuropathies; meningoencephalopathies; vascular diseases, such as loss of brain mass resulting from a stroke; genetic disorders aging; and myocardial
20 infarction.

According to an aspect of some embodiments of the present invention, there is provided a use of a compound as described herein in the manufacture of a medicament.

According to some embodiments, the medicament is for treating any of the medical conditions described herein.

25 The term "treating" refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or condition) and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction,
30 remission or regression of a pathology.

As used herein, the term “preventing” refers to keeping a disease, disorder or condition from occurring in a subject who may be at risk for the disease, but has not yet been diagnosed as having the disease.

As used herein, the term “subject” includes mammals, preferably human beings at any age which suffer from the pathology. Preferably, this term encompasses individuals who are at risk to develop the pathology (e.g., a medical condition as described herein).

In some embodiments, in any of the methods and uses described herein, the compound of the present embodiments can be used (e.g., co-administered) in combination with an additional active agent usable in treating the medical condition or pathology.

Pharmaceutical Compositions:

In any of the methods and uses described herein, the compound as described herein can be utilized per se or as a part of a pharmaceutical composition, which further comprises a pharmaceutically acceptable carrier.

According to an aspect of some embodiments of the present invention, there is provided a pharmaceutical composition comprising the compound as described herein, and a pharmaceutically acceptable carrier.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term “active ingredient” refers to the compounds accountable for the biological effect of the composition, e.g., a compound as described herein in any of the respective embodiments.

Hereinafter, the phrases “physiologically acceptable carrier” and “pharmaceutically acceptable carrier” which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term “excipient” refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient.

Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in
5 “Remington’s Pharmaceutical Sciences,” Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct
10 intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

In any of the methods and uses described herein, suitable routes of
15 administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

20 Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the
25 BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active
30 agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems,

potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

5 Alternately, one may administer the compound or composition in a local rather than systemic manner, for example, via injection of the compound or composition directly into a tissue region of a patient.

The term “tissue” refers to part of an organism consisting of cells designed to perform a function or functions. Examples include, but are not limited to, brain tissue,
10 retina, skin tissue, hepatic tissue, pancreatic tissue, bone, cartilage, connective tissue, blood tissue, muscle tissue, cardiac tissue brain tissue, vascular tissue, renal tissue, pulmonary tissue, gonadal tissue, hematopoietic tissue.

Pharmaceutical compositions of embodiments of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional
15 mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with present embodiments thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of
20 the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, for example, in physiologically compatible buffers such as Hank’s solution, Ringer’s solution, or physiological salt buffer. For
25 transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be
30 formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and

processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to embodiments of the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be
5 suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection
10 suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethylcellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the
15 solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of embodiments of the present invention may
20 also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present embodiments include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically
25 effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of a disorder or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of embodiments of the invention, the
30 therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a

desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures (e.g., as exemplified herein in the Examples section) or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl, *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma or brain levels of the active ingredient sufficient to exhibit the desired effect (minimal effective concentration, MEC), for example, activate a receptor as indicate and/or reduce oxidative stress. The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of embodiments of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of

pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation described herein formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is detailed herein.

Thus, in an optional embodiment of the present invention, the pharmaceutical composition is packaged in a packaging material and identified in print, on or in the packaging material, for use in the treatment or prevention of medical conditions, according to any of the respective embodiments described herein.

In some embodiments, a pharmaceutical composition as described herein further comprises an additional active agent, suitable for treating a medical condition as described herein.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as

individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

The term "alkyl" describes a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. In some embodiments, the alkyl group has 1-10 carbon atoms. In some embodiments, the alkyl group has 1-4 carbon atoms. Exemplary alkyl groups include, but are not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, heptadecyl, octadecyl and nonadecyl.

The term "cycloalkyl" describes an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane.

The term "aryl" describes an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted.

5 The term "hydroxy" describes an -OH group.

The term "alkoxy" describes both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

The term "thiol" describes a -SH group.

10 The term "thioalkoxy" describes both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

The term "halogen" or "halo" describes fluoro, chloro, bromo or iodo atom.

The term "acyl" describes a $-C(=O)-R'$ group, wherein R' can be H, alkyl, cycloalkyl, aryl, alkoxy, or carboxy, as defined herein.

15 The term "carboxy" describes a $-O-C(=O)-R'$ or $-C(=O)-O-R'$ group, wherein R' is as defined herein.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided
20 separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated
25 hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the
30 above descriptions illustrate some embodiments of the invention in a non limiting fashion.

EXAMPLE 1**CHEMICAL SYNTHESSES*****Materials and Methods:***

All air and moisture sensitive reactions were carried out in flame-dried, argon
5 flushed, two-neck flasks sealed with rubber septa, and the reagents were introduced by syringe.

Progress of reactions was monitored by TLC on precoated Merck silica gel
plates (60F-254). Visualization was accomplished by UV light.

Flash chromatography was carried out on silica gel (Davisil Art. 1000101501).

10 Separations were carried out using also an HPFC automated flash purification system (Biotage SP1 separation system (RP)).

Compounds were characterized by NMR using Bruker AC-200, DPX-300 or
DMX-600 spectrometers. ^1H NMR spectra were recorded at 200, 300, 600 or 700
MHz. Chemical shifts are expressed in ppm downfield from Me_4Si (TMS), used as an
15 internal standard.

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 Premier (Waters
UK) spectrometer by chemical ionization.

20 Nucleotides were analyzed under ESI (electron spray ionization) conditions on a Q-TOF micro-instrument (Waters, UK).

Primary purification of the nucleotides was achieved on a LC (Isco UA-6)
system using a Sephadex DEAE-A25 column, swollen in 1M NaHCO_3 at 4 °C for 1
day. The resin was washed with deionized water before use. The LC separation was
25 monitored by UV detection at 280 nm. A buffer gradient of NH_4HCO_3 was applied as detailed below.

Final purification of the nucleotides was achieved on an HPLC (Merck-Hitachi)
system, using a semi-preparative reverse-phase column (Gemini 5u C-18 110A,
250×10.00 mm, 5 micron, Phenomenex, Torrance, USA). The purity of the nucleotides
30 was evaluated with an analytical reverse-phase column system (Gemini 5u C-18 110A,
150 mm ×4.60 mm; 5 μm ; Phenomenex, Torrance, CA) using two solvent systems:
solvent system I, (A) 100 mM triethylammonium acetate (TEAA), pH 7:(B) CH_3CN ;

solvent system II, (A) 0.01 M KH_2PO_4 , pH = 4.5:(B) CH_3CN . The details of the solvent system gradients used for the separation of each product are given below. The purity of the nucleotides was generally $\geq 90\%$.

All commercial reagents were used without further purification, unless otherwise noted.

All reactants in moisture sensitive reactions were dried overnight in a vacuum oven.

All phosphorylation reactions were carried out in flame-dried, argon-flushed, two-neck flasks sealed with rubber septa.

Nucleosides were dried in-vacuo overnight.

Phosphorus oxychloride was distilled and kept under nitrogen.

The tri-*n*-butylammonium pyrophosphate and tri-*n*-butylammonium phosphate solutions were prepared as previously described [Burnstock et al., *Drug Dev. Res.* **1994**, *31*, 206-219].

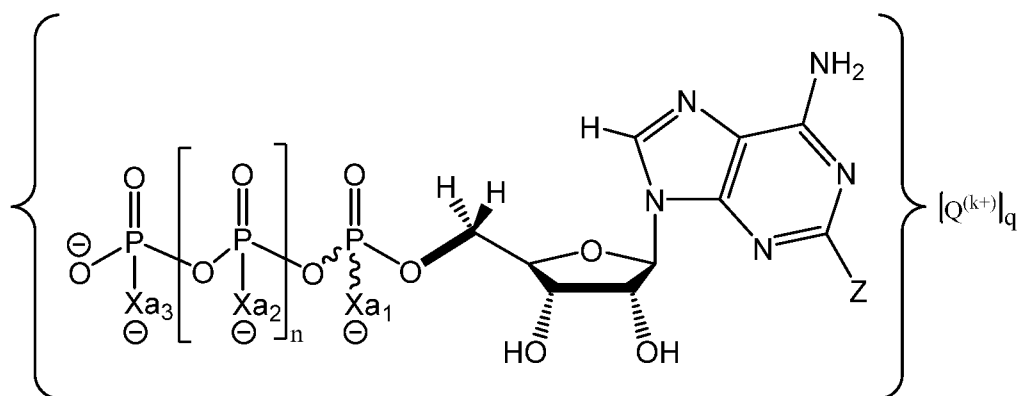
General:

As discussed hereinabove, ATP- γ -S, **3**, was found to be a potent antioxidant, inhibiting OH radical production in the Fe(II)- H_2O_2 system. In addition, it was found that ATP- γ -S is a highly promising neuroprotectant, rescuing primary neurons from insults such as FeSO_4 and $\text{A}\beta_{42}$. The potential of adenosine 5'-phosphorothioate analogues as antioxidants and neuroprotectants was therefore further explored.

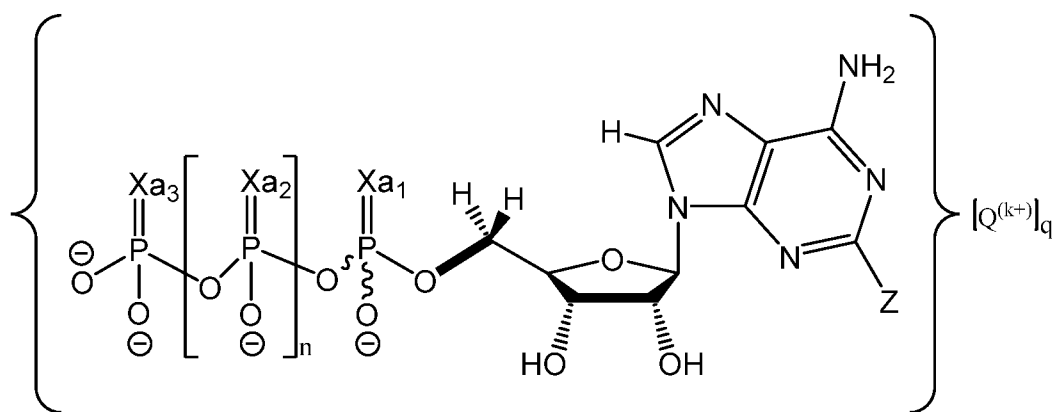
A series of adenosine 5'-phosphorothioate analogues, **6-11** was synthesized, and their antioxidant and neuroprotective activity as well as metabolic stability, and structure-activity relationship, was studied. Specifically, the following modifications were studied: (1) the position of the phosphorothioate moiety (e.g., ADP- α -S, **6**, vs. ADP- β -S, **5**); (2) the presence of electron donating group on the adenine C2-position vs. electron withdrawing group (e.g., 2-SMe-ADP- α -S, **7**, vs. 2-Cl-ADP- α -S, **8**); (3) the presence of di- vs. tri-phosphate group (e.g., 2-Cl-ADP- α -S, **8**, vs. 2-Cl-ATP- α -S, **9**); and (4) the effect of β -phosphorothioate analogue bearing different C2-substituents (2-SMe-ADP- β -S, **10**, vs. 2-Cl-ADP- β -S, **11**). SMe and Cl substitutions at the adenine C2 position of analogues **7**, **10** and **8**, **9**, **11**, respectively, are selected as representative examples of substituents that may improve selectivity and potency of the ligand at the P2Y_1 receptor.

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The structures of the tested compounds are encompassed by Formula IIa or, in the alternative, by Formula II*a, as shown in the following table A:



Formula IIa



Formula II*a

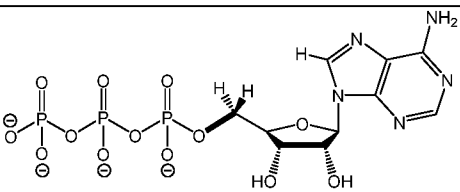
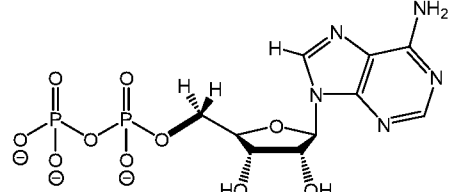
wherein $Q^{(k+)}$ is Na^+ , and q is 3 when $n=0$, and is 4 when $n=1$.

Table A

Compound No.	Z	n	Xa ₁	Xa ₂	Xa ₃	Pα Configuration
1	H	1	O	O	O	----
2	H	0	O	---	O	----
3	H	1	O	O	S	----
4	S-Me	0	O	---	O	----
5	H	0	O	---	S	----
6A	H	0	S	---	O	R _p
6B	H	0	S	---	O	S _p
7A	S-Me	0	S	---	O	R _p
7B	S-Me	0	S	---	O	S _p
8A	Cl	0	S	---	O	R _p
8B	Cl	0	S	---	O	S _p
9A	Cl	1	S	O	O	R _p
9B	Cl	1	S	O	O	S _p
10	S-Me	0	O	---	S	----
11	Cl	0	O	---	S	----

Table B below presents the chemical structures of the anionic portion of the tested compounds. The cationic portion can be, for example, 3 or 4 monovalent cations, as described herein, for example, sodium cations.

Table B

Compound No.	Structure according to Formula I or IIa	Structure according to Formula I* or II*a
1		
2		

Compound No.	Structure according to Formula I or IIa	Structure according to Formula I* or II*a
3		
4		
5		
6A		
6B		
7A		
7B		

Compound No.	Structure according to Formula I or IIa	Structure according to Formula I* or II*a
8A		
8B		
9A		
9B		
10		
11		

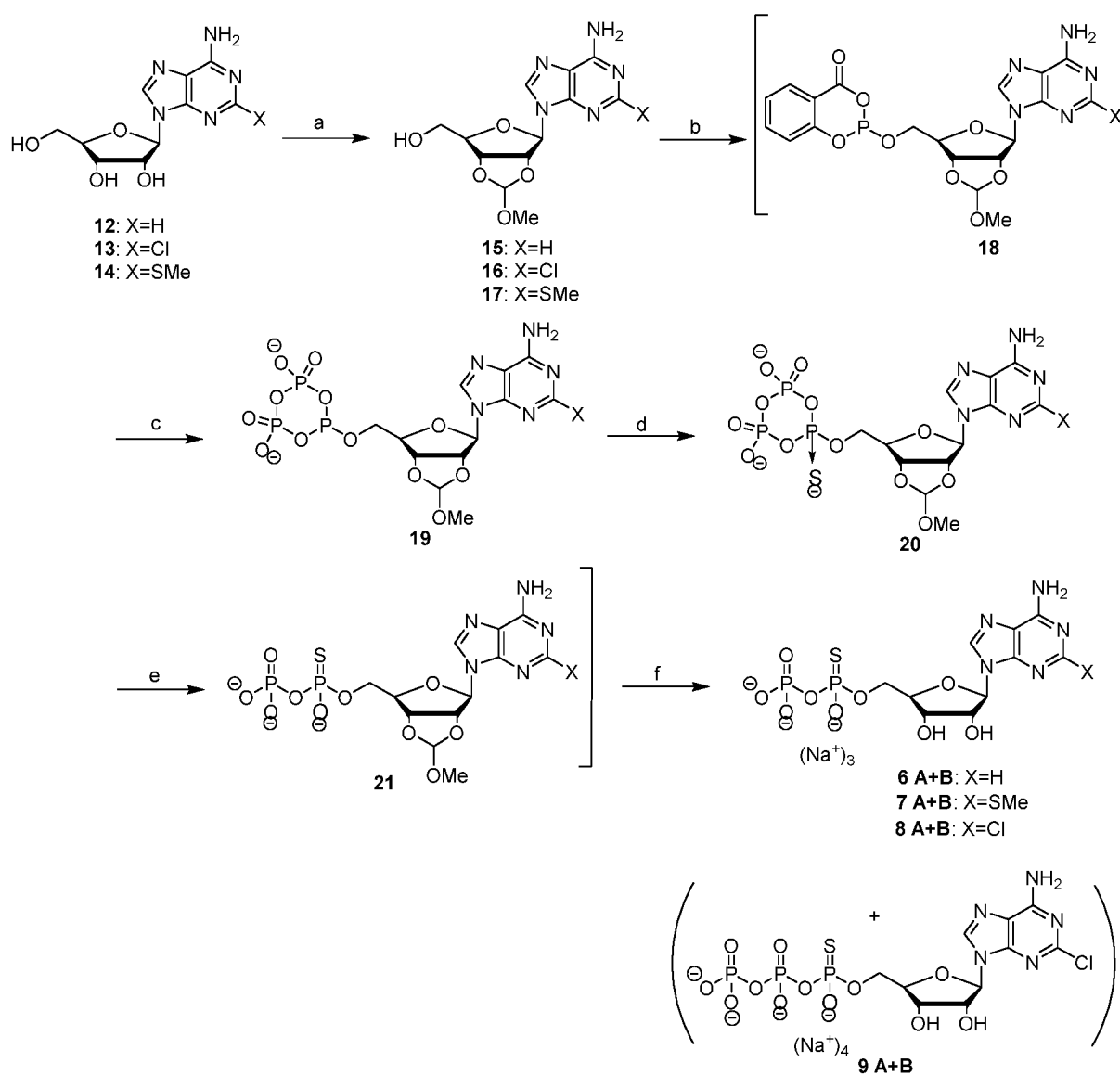
(Table B Cont.)

Syntheses of nucleoside-5'-phosphorothioate analogues:

ADP(α -S), **6**, 2-SMe-ADP(α -S), **7**, 2-Cl-ADP(α -S), **8**, and 2-Cl-ATP(α -S), **9**, were synthesized in a 4-step one-pot reaction from C2-modified methoxymethylidene protected adenosine analogues, **15**, **16** and **17**, upon addition of 2-Cl-1,2,3-benzodioxaphosphorin-4-one in dry dioxane and dry pyridine to give phosphites, **18** (see, Scheme 1). The latter

were treated with pyrophosphate tributylammonium salt in dry DMF to generate the cyclic intermediates, **19**. Treatment of **19** with S_8 led to cyclic thiotriphosphate intermediates **20**. Subsequently, ethylenediamine was added to generate **21** analogues upon elimination of cyclic phosphorodiamidate. Removal of the methoxymethylidene group involved a hydrolysis step at pH 2.3 and then at pH 9. Compounds ADP(α -S), **6A/B**, 2-SMe-ADP(α -S), **7A/B** and 2-Cl-ADP(α -S), **8A/B**, were obtained in 51 %, 47 % and 42 % yield, respectively, after LC separation. By-product 2-Cl-ATP(α -S), **9A/B**, was obtained in 8 % yield.

Scheme 1

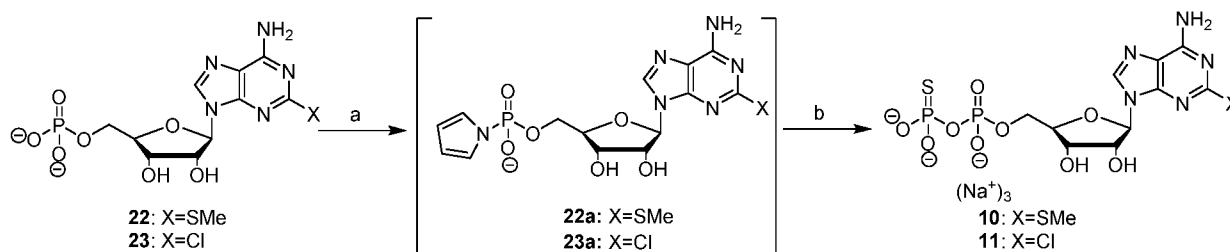


Reaction conditions:

- (a) (1) HC(OMe)_3 , *p*-TsOH, room temperature, overnight; and (2) Dowex MWA-1 (weak base), room temperature, 3 hours, 96 % yield;
 (b) 2-Cl-1,3,2-benzdioxaphosphorin-4-one, dry pyridine, dry dioxane, room temperature, 10 minutes;
 5 (c) 0.5 M $\text{P}_2\text{O}_7\text{H}_2^{2-}(\text{Bu}_3\text{N}^+\text{H})_2$ in dry DMF, Bu_3N , room temperature, 5 minutes;
 (d) S_8 , room temperature, 1 hour;
 (e) ethylenediamine, room temperature, 10 minutes;
 (f) (1) 10 % HCl, pH 2.3, room temperature, 3 hours; and (2) 24 % NH_4OH , pH 9, room temperature, 45 minutes.

10 2-SMe-ADP(β -S), **10**, and 2-Cl-ADP(β -S), **11**, were obtained from 2-SMe-AMP, **22**, and 2-Cl-AMP, **23**, tributylammonium, trioctylammonium salts, respectively, in a 2-step one-pot reaction (see, Scheme 2). The latter AMP analogues were activated with carbonyldiimidazole (CDI) to give **22a** and **23a**, respectively, and then reacted with thiophosphate salt (bistributylammonium) in the presence of ZnCl_2 for preventing
 15 conjugation via the sulfur atom. This synthetic method proved highly efficient as compared to a two-step reaction described for the preparation of related compounds. This one pot synthesis resulted in products **10** and **11** in 75 % and 50 % yields, respectively, after LC separation.

Scheme 2



Reaction conditions:

- (a) (1) CDI, DMF, room temperature, 3 hours; (2) MeOH, 8 minutes, room temperature;
 25 (b) (1) $[\text{PSO}_3\text{H}]^{2-}(\text{Bu}_3\text{NH}^+)_2$, ZnCl_2 , DMF, room temperature, 2 hours; (2) EDTA.

Compounds **6-9** were obtained as pairs of diastereoisomers, due to a chiral center at Pa . These diastereoisomers were separated on HPLC showing 3-5 minutes difference of their retention times. The first and second eluting diastereoisomers were denoted as A- and B-diastereoisomers, respectively. Without being bound by any particular theory, it is
 30 believed that in the first eluting isomer of **6-9**, H8 is less shielded, and is the *Rp* isomer, and the second eluting isomer is the *Sp* isomer.

Exemplary procedure for preparation of adenosine nucleoside 5'-diphosphate- α -S derivatives:

2-Chloro-2',3'-O-methoxymethylidene adenosine, **16**, (140 mg, 0.41 mmol, 1 equivalent) was dissolved in DMF (3.9 mL), and a freshly prepared solution of 2-chloro-1,3,2-benzodioxaphosphorin-4-one (0.452 mmol, 1.1 equivalent) in dry dioxane and dry pyridine was added. After stirring for 10 minutes, a freshly prepared 0.5 M solution of bis(tri-n-butylammonium) pyrophosphate (0.61 mmol, 1.5 equivalent) in DMF and tri-n-butylamine (1.64 mmol, 4 equivalents) were simultaneously added. Precipitation occurred immediately after the addition of the reagents but disappeared with further stirring. Sulfur (4.1 mmol, 10 equivalents) was added, and the mixture was stirred for 15 minutes. Ethylenediamine (2.05 mmol, 5 equivalents) was then added. A brown precipitate was immediately formed. After stirring for 60 minutes, deionized water (0.13 mL) was added and the brown precipitate gradually dissolved. The reaction mixture was filtered through Buchner funnel and then diluted with deionized water and washed twice with di-ethyl ether. The aqueous layer was then freeze-dried. Methoxymethylidene protecting group was removed by acidic hydrolysis (10 % HCl solution was added until pH 2.3 was obtained). After 3 hours at room temperature, the pH was rapidly raised to 9 by addition of 24 % NH₄OH solution (pH 11), and the solution was stirred at room temperature for 45 minutes and then freeze-dried. The residue was subjected to ion-exchange chromatography (on DEAE A25 Sephadex, swollen overnight in 1M NaHCO₃ at 4 °C). The column was eluted with an ammonium bicarbonate gradient of 0-0.2 M (300 mL each), and then 0.2-0.4 M (150 mL each). Finally, relevant fractions containing the products were separated on HPLC, as described below, and thereafter passed through a Dowex 50WX8-200 ion-exchange resin Na⁺-form column and eluted with deionized water to obtain the corresponding sodium salts after freeze-drying.

Separation of 7A and 7B:

The separation of diastereoisomers **7A** and **7B** was accomplished using a semipreparative reverse-phase Gemini 5u column and isocratic elution with 87:13 (A) 100 mM TEAA, pH 7:(B) CH₃CN, at a flow rate of 4 mL/minute. Fractions containing purified isomers [Rt: 9.2 minutes (**7A**); 10.8 minutes (**7B**)] were collected and freeze-dried. Excess buffer was removed by repeated freeze-drying cycles, with the solid

residue dissolved each time in deionized water. Diastereoisomers **7A** and **7B** were obtained at 23 % overall yield (36 mg) after LC separation.

Characterization of 7A:

¹H NMR (D₂O, 200 MHz): δ = 8.51 (s, 1H, H-8), 6.15 (d, J=6.2 Hz, 1H, H-1'),
5 4.7 (m, H-2' signal is hidden by the water signal), 4.61 (m, 1H, H-3'), 4.4 (m, 1H, H-4'),
4.28 (m, 2H, H-5', H-5''), 2.61 (m, 3H, CH₃) ppm.

³¹P NMR (D₂O, 200 MHz) δ = 43.46 (d, J=31.1 Hz, P_α-S), -10.76 (d, J=31.1 Hz, P_β) ppm.

HR MALDI (negative): calcd for C₁₁H₁₆N₅O₉P₂S₂ 458.9859, found 487.9870.

10 Purity data obtained on an analytical column: Rt: 4.62 minutes (98 % purity) using solvent system I (isocratic elution of 93:7 A:B over 20 minutes at a flow rate of 1 mL/minutes). Rt: 7.53 min (93 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Characterization of 7B:

15 ¹H NMR (D₂O, 200 MHz): δ = 8.48 (s, 1H, H-8), 6.15 (d, J=4.2 Hz, 1H, H-1'),
4.69 (m, H-2', H-3'), 4.33 (m, 1H, H-4'), 4.25 (m, 2H, H-5', H-5''), 2.61 (m, 3H, CH₃) ppm.

³¹P NMR (D₂O, 200 MHz): δ = 41.33 (d, J=31.3 Hz, P_α-S), -5.916 (d, J=31.1 Hz, P_β) ppm.

20 HR MALDI (negative): calcd for C₁₁H₁₆N₅O₉P₂S₂ 487.9859, found 487.985.

Purity data obtained on an analytical column: Rt: 7.86 minutes (96 % purity) using solvent system I (isocratic elution of 93:7 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 10.28 minutes (92 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

25 ***Separation of 8A and 8B:***

The separation of diastereoisomers **8A** and **8B** was accomplished using a semipreparative reverse-phase Gemini 5u column and isocratic elution with 90.5:9.5 (A) 100 mM TEAA, pH 7:(B) MeOH, at a flow rate of 4.5 mL/minute. Fractions containing purified isomers [Rt: 8.49 minutes (**8A**); 11.69 minutes (**8B**)] were collected
30 and freeze-dried. Excess buffer was removed by repeated freeze-drying cycles, with the solid residue dissolved each time in deionized water. Diastereoisomers **8A** and **8B** were obtained at 42 % overall yield (84 mg) after LC separation.

Characterization of 8A:

^1H NMR (D_2O , 200 MHz): δ = 8.58 (s, 1H, H-8), 6.03 (d, J =5.8 Hz, 1H, H-1'), 4.6 (m, 1H, H-2'), 4.54 (m, 1H, H-3'), 4.39 (m, 1H, H-4'), 4.24 (m, 2H, H-5', H-5'') ppm.

^{31}P NMR (D_2O , 200 MHz): δ = 44.51 (d, J =30.4 Hz, $\text{P}_\alpha\text{-S}$), -3.425 (d, J =30.2 Hz, P_β) ppm.

HR MALDI (negative): calcd for $\text{C}_{10}\text{H}_{13}\text{Cl}_1\text{N}_5\text{O}_9\text{P}_2\text{S}_1$ 475.9592, found 475.964.

Purity data obtained on an analytical column: Rt: 3.92 minutes (90 % purity) using solvent system I (isocratic elution of 93:7 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 5.16 minutes (93 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Characterization of 8B:

^1H NMR (D_2O , 200 MHz): δ = 8.55 (s, 1H, H-8), 6.01 (d, J =4.8 Hz, 1H, H-1'), 4.7 (m, 1H, H-2' signal is hidden by the water signal), 4.63 (m, 1H, H-3'), 4.39 (m, 1H, H-4'), 4.26 (m, 2H, H-5', H-5'') ppm.

^{31}P NMR (D_2O , 200 MHz): δ = 41.44 (d, J =30.78 Hz, $\text{P}_\alpha\text{-S}$), -5.91 (d, J =31.2 Hz, P_β) ppm.

HR MALDI (negative): calcd for $\text{C}_{10}\text{H}_{13}\text{Cl}_1\text{N}_5\text{O}_9\text{P}_2\text{S}_1$ 475.9592, found 475.9630.

Purity data obtained on an analytical column: Rt: 8.96 minutes (91 % purity) using solvent system I (isocratic elution of 92:8 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 10.12 minutes (90 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Preparation of 2-Cl-adenosine- 5'-triphosphate- α -S, 9:

This analogue was obtained as a by-product from the above described synthesis of **8**. After LC separation, the relevant fractions were pooled and freeze-dried three times to yield a white solid. Final separation of the diastereomers and purification of the relevant fractions was carried out on an HPLC system, using a semi-preparative reverse-phase column, under the conditions described below. The purity of the nucleotides was evaluated on an analytical reverse-phase column system, in two solvent systems as described below. Finally, aqueous solutions of the products were passed through a Dowex 50WX8-200 ion-exchange resin Na^+ -form column and the products

were eluted with deionized water to obtain the corresponding sodium salts after freeze-drying.

Separation of 9A and 9B:

The separation of diastereoisomers **9A** and **9B** was accomplished using a semipreparative reverse-phase Gemini 5u column and isocratic elution with 90.5:9.5 (A) 100 mM TEAA, pH 7:(B) MeOH, at a flow rate of 4.5 mL/minute. Fractions containing purified isomers [Rt: 10.75 minutes (**9A**); 15.49 minutes (**9B**)] were collected and freeze-dried. Excess buffer was removed by repeated freeze-drying cycles, and the solid residue was dissolved each time in deionized water. Diastereoisomers **9A** and **9B** were obtained at 8 % overall yield (20 mg) after LC separation.

Characterization of 9A:

^1H NMR (D_2O , 200 MHz): δ = 8.66 (s, 1H, H-8), 6.05 (d, J=6 Hz, 1H, H-1'), 4.3 (m, 2H, H-2', H-3'), 3.97 (m, 1H, H-4'), 3.7 (m, 2H, H-5', H-5'') ppm.

^{31}P NMR (D_2O , 200 MHz): δ = 44.4 (d, J=30 Hz, $\text{P}_\alpha\text{-S}$), -5.23 (d, J=18.9 Hz, P_β), -21.753 (m, P_γ) ppm.

HR MALDI (negative): calcd for $\text{C}_{10}\text{H}_{13}\text{Cl}_1\text{N}_5\text{O}_9\text{P}_2\text{S}_1$ 555.9256, found 555.9261.

Purity data obtained on an analytical column: Rt: 6.67 minutes (90 % purity) using solvent system I (isocratic elution of 93:7 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 5.69 minutes (92 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Characterization of 9B:

^1H NMR (D_2O , 200 MHz): δ = 8.64 (s, 1H, H-8), 6.03 (d, J=5.4 Hz, 1H, H-1'), 4.68 (m, 2H, H-2', H-3'), 4.56 (m, 1H, H-4'), 4.33 (m, 2H, H-5', H-5'') ppm.

^{31}P NMR (D_2O , 200 MHz): δ = 43.55 (d, J=30 Hz, $\text{P}_\alpha\text{-S}$), -5.21 (d, J=20.3 Hz, P_β), -21.86 (dd, J=20.4, 26 Hz, P_γ) ppm.

HR MALDI (negative): calcd for $\text{C}_{10}\text{H}_{13}\text{Cl}_1\text{N}_5\text{O}_9\text{P}_2\text{S}_1$ 555.9256, found 555.9243.

Purity data obtained on an analytical column: Rt: 9.71 minutes (91 % purity) using solvent system I (isocratic elution of 93:7 A:B over 20 minutes at a flow rate of 1

mL/minute). Rt: 9.29 minutes (90 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Exemplary procedure for preparation of adenosine 5'-monophosphate derivatives:

5 A solution of 2-Cl-adenosine, **13**, (200 mg, 0.365 mmol) in dry trimethyl phosphate (2 mL) was cooled to -15°C using an ethylene glycol-dry ice bath; then Proton Sponge® (427 mg, 1.99 mmol, 3 equivalents) was added. After 20 minutes, distilled phosphorus oxychloride (91 µL, 0.96 mmol, 2 equivalents) was added dropwise, and stirring was continued for 3 hours at -15 °C. TLC on a silica gel plate
10 (isopropanol: 25 % NH₄OH: H₂O 11:2:7), indicated the disappearance of the starting material and the formation of a more polar product. 1M TEAB solution (8 mL, pH 8) was then added until neutralization, and the clear solution was stirred at room temperature for 45 minutes. The solution was freeze-dried overnight. The semisolid obtained after freeze-drying was chromatographed on an activated Sephadex DEAE-
15 A25 column. The resin was washed with deionized water and loaded with the crude reaction residue dissolved in a minimal volume of water. The separation was monitored by UV detection at 280 nm. A buffer of 0-0.2 M NH₄HCO₃ was used. The relevant fractions were collected and freeze-dried three times until a constant weight was obtained, to yield the product as a white solid (220 mg, 79 %).

20 ***Preparation of [PSO₃H]²⁻(Bu₃NH⁺)₂ salt:***

[PSO₃H]²⁻(Bu₃NH⁺)₂ salt was prepared from the corresponding sodium salt (tribasic hydrate). The thiophosphate sodium salt was passed through a column of activated Dowex 50WX-8 200 mesh, H⁺ form. The column elutante was collected in an ice-cooled flask containing tributylamine (1 equivalent) and EtOH. The resulting
25 solution was freeze-dried to yield the salt as a colorless oil. The oil was dried by repeated evaporation with absolute EtOH (3 times), followed by co-evaporation with anhydrous DMF for three times to obtain colorless oil. [PSO₃H]²⁻(Bu₃NH⁺)₂ salt was stored in a desiccator at -20 °C.

30 ***Exemplary procedure for preparation of adenosine 5'-diphosphate-α-S derivatives:***

2-Cl-AMP (Bu₄N⁺) salt (0.26 mmol, 1 equivalent) and anhydrous DMF (2.5 mL) were stirred in a two-necked flask to form a colorless solution. CDI (214 mg, 1.32

mmol, 5 equivalents) was added to the solution. The reaction was stirred at room temperature for 3 hours. Next, dry MeOH (0.053 mL, 1.325 mmol, 5 equivalents) was added to the reaction flask and the solution was stirred for 8 minutes. Subsequently [PSO₃H]²⁻(Bu₃NH⁺)₂ salt (1.59 mmol, 6 equivalents) dissolved in anhydrous DMF (5 mL) and anhydrous ZnCl₂ (283 mg, 2.12 mmol, 8 equivalents) were added to form a colorless solution. After 2 hours, the reaction was quenched by addition of EDTA solution (0.93 grams, 2.01 mmol in 30 mL of deionized water) and 1M TEAB was added until pH 8 was attained. The solution was then freeze-dried. The resulting white solid residue was separated on a Sephadex DEAE-A25 column applying a linear gradient of water (200 mL) to 0.2 M TEAB (200 mL) and then 0.2 M-0.4 M TEAB (total volume of 800 mL). The solution was freeze-dried at least 4 times to yield a yellowish solid in 50 % yield (65 mg). Final purification was carried out on an HPLC system, using a semi-preparative reverse-phase column. The purity of the nucleotides was evaluated on an analytical reverse-phase column system, in two solvent systems as described below. The products, obtained as triethylammonium salts, generally featured a purity of 90 % or higher. Finally, aqueous solutions of the products were passed through a sodium form Dowex 50WX8-200 ion-exchange resin column and the products were eluted with deionized water to obtain the corresponding sodium salts after freeze-drying.

Purification of 10:

Analogue **10** was obtained in a 75 % overall yield (142 mg) after LC separation.

Characterization of 10:

¹H NMR (D₂O, 200 MHz): δ = 8.38 (s, 1H, H-8), 6.1 (d, J=4.6 Hz, 1H, H-1'), 4.68 (m, 2H, H-2', H-3'), 4.64 (m, 1H, H-4'), 4.25 (m, 2H, H-5', 5'') ppm.

³¹P NMR (D₂O, 200 MHz): δ = 33.88 (d, J=31.3 Hz, P_α-S), -11.11 (d, J=31.3 Hz, P_β) ppm.

HR MALDI (negative): calcd for C₁₁H₁₆N₅O₉P₂S₂ 487.9850, found 487.9863.

Purity data obtained on an analytical column: Rt: 8.47 minutes (90 % purity) using solvent system I (isocratic elution of 92:8 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 6.59 minutes (95 % purity) using solvent system II (isocratic elution of 97:3 A:B over 20 minutes at a flow rate of 1 mL/minute).

Purification of 11:

Purification of analogue **11** was accomplished using a semipreparative reverse-phase Gemini 5u column and isocratic elution with 92:8 (A) 100 mM TEAA, pH 7:(B) CH₃CN at a flow rate of 4.5 mL/minute. The fraction containing the purified analogue
5 (Rt 15.89 minutes) was freeze-dried. Excess buffer was removed by repeated freeze-drying cycles, and the solid residue was dissolved each time in deionized water. Analogue **11** was obtained in a 50 % overall yield (65 mg) after LC separation.

Characterization of 11:

¹H NMR (D₂O, 200 MHz): δ = 8.49 (s, 1H, H-8), 6.01 (d, J=5.8 Hz, 1H, H-1'),
10 4.60 (m, 2H, H-2', H-3'), 4.36 (m, 1H, H-4'), 4.20 (m, 2H, H-5', 5'') ppm.

³¹P NMR (D₂O, 200 MHz): δ = 36.97 (d, J=30 Hz, P _{α} -S), -11.47 (d, J=30 Hz, P _{β}) ppm.

HR MALDI (negative): calcd for C₁₀H₁₃Cl₁N₅O₉P₂S₁ 475.9592, found 475.9600. Purity data obtained on an analytical column: Rt: 4.8 minutes (89 % purity)
15 using solvent system I (isocratic elution of 92:8 A:B over 20 minutes at a flow rate of 1 mL/minute). Rt: 3.8 minutes (96 % purity) using solvent system II (isocratic elution of 96:4 A:B over 20 minutes at a flow rate of 1 mL/minute).

EXAMPLE 2***IN VITRO STUDIES***

20

Experimental Methods:***Calcium Measurements:***

1321N1 astrocytoma cells transfected with the respective plasmid for P2Y-R-GFP expression plated on coverslips (22 mm diameter) and grown to approximately 80
25 % density, were incubated with 2 μ M fura 2/AM and 0.02 % pluronic acid in Na-HBS buffer (Hepes buffered saline: 145 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 25 mM glucose, 20 mM Hepes/Tris pH 7.4) for 30 minutes at 37 °C. The cells were superfused (1 mL/minute, 37 °C) with different concentrations of nucleotide in Na-HBS buffer. The nucleotide-induced change of [Ca²⁺]_i was monitored by detecting
30 the respective emission intensity of fura 2/AM at 510 nm with 340 nm and 380 nm excitations. The average maximal amplitude of the responses and the respective standard errors were calculated from ratio of the fura 2/AM fluorescence intensities

with excitations at 340 nm and 380 nm. Only GFP-labelled cells were analyzed. Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and SigmaPlot (SPSS Inc., Chicago, IL, USA) were used to derive the concentration-response curves and EC₅₀ values from the average response amplitudes obtained in at least three independent experiments. Only cells with a clear GFP-signal and with the typical calcium response kinetics upon agonist pulse application were included in the data analysis. The GFP-tagged P2Y receptors are suitable for pharmacological and physiological studies, as previously reported.

ABTS radical cation decolorization assay:

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is a water-soluble analog of vitamin E, which acts as antioxidant, and is marketed by Hoffman-LaRoche. Trolox equivalent antioxidant capacity (TEAC) is a measurement of antioxidant strength based on Trolox, measured in units called Trolox Equivalents (TE), e.g. micromolTE/100 grams. Trolox equivalency is most often measured using the ABTS decolorization assay. Ferric reducing ability of plasma (FRAP) is an antioxidant capacity assays which uses Trolox as a standard.

ABTS radical cation (ABTS^{•+}) was produced by reacting 7 mM aqueous ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The radical was stable under these conditions for more than two days. ABTS^{•+} solution was diluted with water, pH 7.4, to an absorbance of 0.80 at 734 nm. After addition of 180 µL of diluted ABTS^{•+} solution to 2.5-48 µL of antioxidant compounds or Trolox standard (final concentration 0–60 µM) in water, the absorbance reading at 734 nm was taken exactly 7 minutes after initial mixing. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times, in triplicate. The percentage of inhibition is calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data.

ESR OH radical assay:

ESR settings for OH radicals detection were as follows: microwave frequency, 9.76 GHz; modulation frequency, 100 KHz; microwave power, 6.35 mW; modulation amplitude, 1.2 G; time constant, 655.36 ms; sweep time 83.89 s; and receiver gain 2×10^5 in experiments with Fe(II).

1 mM Ammonium iron(II) sulfate (10 μ L) was added to 5-500 μ M tested compound (1-10 μ L) solutions. Afterwards, 1 mM Tris buffer, pH 7.4, (60-70 μ L) was added to the mixture. After mixing for 30 seconds, 100 mM DMPO (10 μ L) were quickly added followed by the addition of 100 mM H_2O_2 (10 μ L). Final sample pH values for the Fe(II) systems ranges between 7.2 and 7.4. Each ESR measurement was performed 150 seconds after the addition of H_2O_2 . All experiments were performed at room temperature, at a final volume of 100 μ L.

Determination of the relative binding of ferrous ions to analogue 7A vs. 2:

The relative binding of ferrous ions to nucleotides was determined by a competitive assay as described in Decker and Welch [J. Agr. Food Chem. **1990**, 38, 674-677]. Briefly, $FeSO_4$ (75 μ M, 38 μ L, final concentration: 10 μ M) was mixed with ferrozine (140 μ M, 70 μ L, final concentration: 20 μ M) and with the nucleotide analogue in DDW at final concentrations of 25-200 μ M, and agitated for 10 minutes at room temperature. The absorbance of the formed colored Fe(II)-ferrozine complex in the presence or absence of nucleotides was measured at 562 nm.

Determination of ROS production in cultured PC12 cells:

PC12 cells were grown in Dulbecco's modified Eagle's medium and seeded into medium in 96-well tissue culture plates for 24 hours. A stock solution of 2',7'-dichlorofluorescein-diacetate (DCFH-DA) was prepared by dissolving 2 mg/mL of the material in ethanol, and kept in the dark at -20 $^{\circ}$ C. For the experiments, the DCFH-DA stock solution was diluted 100 times in PBS, and 25 μ L of the diluted solution was added to each well in the microplate, incubated for 20 minutes, and removed from the cells. After DCFH-DA was removed, the nucleotides were added to the cells at a final concentration of 0.2-200 μ M. Oxidation was initiated by the addition of $FeSO_4$ (2 μ M, 12 μ L, final concentration: 0.16 μ M) to the wells. The plates were incubated for 1 hour at 37 $^{\circ}$ C, during which time absorbance was read by a Tecan fluorometer at 485/530 nm.

Evaluation of the resistance of 7 A/B to hydrolysis by eNPP1,3:

The percentage of hydrolysis of analogue **7A/B** by human eNPP1,3 was evaluated as follows: human eNPP1 or eNPP3 extract 19 μ g or 32 μ g, respectively, was added to 0.473 mL of the incubation mixture (1 mM $CaCl_2$, 200 mM NaCl, 10 mM KCl, and 100 mM Tris, pH 8.5) and pre-incubated at 37 $^{\circ}$ C for 3 minutes. Reaction was

initiated by the addition of 4 mM **7A/B** (0.012 mL). The reaction was stopped after 2 hours or 3 hours for NPP1 or NPP3, respectively, by adding ice-cold 1 M perchloric acid (0.350 mL). These samples were centrifuged for 1 minute at 10000 g. Supernatants were neutralized with 2 M KOH (140 mL) in 4 °C and centrifuged for 1 minute at 10000 g. The reaction mixture was filtered and freeze-dried. Each sample was dissolved in HPLC-grade water (200 µL), and 20 µL was injected to an analytical HPLC column (Gemini analytical column (5µ C-18 557 110A; 150 mm × 4.60 mm)). The samples were separated on an analytical reverse-phase HPLC using isocratic elution applying 90 % 100 mM TEAA (pH 7) and 10 % CH₃CN, flow rate 1 mL/minute. The hydrolysis rates of Compounds **7A** and **7B** by eNPP1 or eNPP3 were determined by measuring the change in the integration of the HPLC peaks for each analogue over time versus ATP as control. To determine the percentage of degradation due to enzymatic hydrolysis, each of the samples was compared to a control to which acid, but no enzyme, was added. The percentage of degradation was calculated from the area under the curve of the nucleoside monophosphate peak, after subtraction of the control, which is the amount of the nucleoside monophosphate formed due to acidic hydrolysis.

Determination of involvement of P2Y₁₂-R in antioxidant activity of compound 7A:

PC12 or Ntera-2 cells were grown in Dulbecco's modified Eagle's medium and seeded into medium in 96-well tissue culture plates for 24 hours. 50 µM 2-MeS-AMP (3.75 µL) was added to each well in the microplate and incubated for 20 minutes. Determination of ROS production in cultured PC12 cells, was performed with DCFH-DA with or without the addition of Compound **7A** at a range of concentrations (0.04 - 100 µM), and initiation of oxidation by addition of FeSO₄ (2 µM, 12 µL, final concentration: 0.16 µM) to the wells. ROS production was evaluated by the DCFH-DA assay as detailed above.

Determination of cell viability by MTT assay:

Primary cortical neurons were seeded into grown in Dulbecco's modified Eagle's: F12 medium in 96-well tissue culture plates for 5 days. After 5 days, the tested compounds were added to the well. After 24 hours, the culture medium was removed. Cell viability was assessed using an MTT assay which is a marker of mitochondrial

activity. MTT (1 mg/mL) in PBS, pH 7.4, was added to the wells, following incubation (2 hours at 37 °C). MTT solution was removed, and the formazan product was dissolved in DMSO. Absorbance was read at 550 nm using a Tecan spectrophotometer, plate reader. Data are presented as a percentage relative to their vehicle controls.

5 ***Preparation of primary neuron cell cultures:***

Rat brain (1-day old) were removed under sterile conditions, the cortex was dissected and separated from the remaining brain, roughly homogenised by repeating pipetting and then trypsin was added. The trypsin was removed from the dissociated cells by centrifugation at 4000 rpm, and dissociated cells were plated at a density of
10 4x10⁵/mL into 96 multi-well plates (Nunc, Naperville, IL, USA) that had previously been pre-coated with poly-ornithine (15 µg/mL). Cells were cultured in a serum-free medium composed of a mixture of Dulbecco's modified Eagle's medium and F12 nutrient (1:1 v/v) supplemented with 10 % B-27 (Gibco, BRL), 5 % glutamine, 1 % penicillin-streptomycin-nystatin. After 24 hours the medium was replaced with fresh
15 medium. After 72 hours the cells were treated with Ara-C (Cytosine β-D-arabinofuranoside, inhibitor of DNA replication) at 50 mM for 48 hours, which resulted in 90 % neuronal cell culture. The medium was then replaced with fresh medium and cells were ready for experiments.

Neuroprotection assay:

20 Primary cortical neurons were seeded in 96-well plates and treated with FeSO₄ at final concentrations of 3 µM for 24 hours. Prior to exposure to FeSO₄ the cells were treated with Compound **7A** or **7B** at various concentrations (0.01-5 µM). After 24 hours cells were tested for cell viability by the MTT assay.

Other assays are described within the results section below.

25 **Results:**

Evaluation of Compounds 6-11 as P2Y_{1/11}-Rs agonists:

The activity of nucleotide analogues **6-11** at the phylogenetically related P2Y_{1/11}-Rs, was studied by measuring [Ca²⁺]_i mobilization induced by these analogues and comparing it to that of the endogenous agonists of P2Y₁-R and P2Y₁₁-R, ADP (**2**),
30 and ATP (**1**), respectively. These studies were performed in 1321N1 astrocytoma cells stably expressing the human P2Y_{1/11} receptors. Concentration-response curves were derived for a range of nucleotide concentrations and are presented in Figures 1A-C and

2A-C. The resulting EC₅₀ values for the compounds evaluated are summarized in Table 1. Potencies of nucleotide analogues **6-11** at hP2Y_{1/11}-R. EC₅₀ values for [Ca²⁺]_i elevation were obtained from concentration-response curves based on fura 2/AM F340 nm/ F380 nm ratio measurements.

Table 1

Agonist	EC ₅₀ values (μM)			
	P2Y ₁ -R	s.e.m.	P2Y ₁₁ -R	s.e.m.
ATP (1)	0.85	0.047	6.7	0.87
ADP (2)	0.024	0.02	1.7	0.45
2-MeS-ADP (4)	0.013	0.0045	n.m.	
ADP(β-S) (5)	1.27	0.34	32.7	1.85
ADP(α-S) (6A)	0.080	0.0078	4.5	0.55
ADP(α-S) (6B)	0.13	0.022	8.7	0.3
2-SMe-ADP(α-S) (7A)	0.0026	0.000025	3.2	0.5
2-SMe-ADP(α-S) (7B)	0.017	0.0037	1	0.03
2-Cl-ADP(α-S) (8A)	0.030	0.003	1.4	0.24
2-Cl-ADP(α-S) (8B)	0.15	0.0025	0.5	0.013
2-Cl-ATP(α-S) (9A)	0.73	0.018	9.1	1.43
2-Cl-ATP(α-S) (9B)	0.88	0.034	1.1	0.08
2-SMe-ADP(β-S) (10)	0.037	0.004	0.9	0.054
2-Cl-ADP(β-S) (11)	0.090	0.025	1	0.125

n.m. - not measured, s.e.m. -standard error of the mean.

Each data point represents mean values of at least 40 cells measured.

s.e.m of the EC₅₀ values was determined from n= 3 concentration-response curves.

At the P2Y₁-R, ADP(α-S) isomers **6A** and **6B**, (EC₅₀ 80 and 130 nM, respectively) were less active than ADP, **2**, (EC₅₀ 24 nM) (see, Table 1 and Figures 1A-C), indicating that the replacement of the P_α non-bridging oxygen atom by sulfur atom did not improve the potency of the compound at the P2Y₁-R. Modification at the C2 position by Cl/SMe group, i.e., compounds 2-SMe-ADP(α-S), **7A** and **7B**, and 2-Cl-ADP(α-S), **8A** and **8B**, improved the agonist activity of **6**. SMe group substitution of **6**

resulted in a highly active agonist, **7A**, (EC_{50} 2.6 nM) being 5-fold more potent than 2-MeS-ADP, **4**, (EC_{50} 13 nM), thus making **7A** the most potent P2Y₁-R agonist currently known. Isomer **7B** was less active than the **7A** isomer.

For all P α -S modified nucleotide analogues the A isomer was more active than the B isomer at P2Y₁-R. Modification at the C2 position of **5**, by Cl/SMe to give 2-SMe-ADP(β -S), **10**, and 2-Cl-ADP(β -S), **11** (EC_{50} 90 and 37 nM, respectively) resulted in 34- and 14-fold, respectively, improved agonist activity at the P2Y₁-R vs. ADP- β -S, **5** (EC_{50} 1270 nM).

At P2Y₁₁-R, compounds **6A** and **6B** (EC_{50} 4500 and 8700 nM, respectively) were less active than ADP (1700 nM) indicating that the sulfur atom at the P α -position is not tolerated by P2Y₁₁-R (Table 1 and Figures 2A-C). Compound **7** containing SMe modification showed a similar activity to that of ADP for the **7B** isomer (EC_{50} 1000 nM), and reduced agonist activity for **7A** isomer (EC_{50} 3200 nM). Compound **8B** (EC_{50} 500 nM) substituted by 2-Cl was 2-fold more active than ADP, while the **8A** isomer was equipotent to ADP (EC_{50} 1400 nM).

The triphosphate analogues **9A** and **9B** showed reduced potency for the **9A** isomer (EC_{50} 9100 nM), as compared to ADP (EC_{50} 1700 nM) and ATP (EC_{50} 6700 nM), and improved potency for the **9B** isomer (EC_{50} 1100 nM) as compared to ADP and ATP.

A clear preference was observed for the B isomer over the A isomer at the P β -S modified nucleotides tested at P2Y₁₁-R. ADP(β -S), **5**, showed low activity, while C2-Cl/SMe modification at **10** and **11** resulted in EC_{50} of 900 and 1000 nM, respectively. These results suggest that the enhancement of P2Y₁₁-R agonist activity is mainly attributed to the Cl/SMe modification at the C2 position of the adenosine ring. Compounds **7B**, **8A**, **9B** and **10** were about 2-fold more active than ADP at P2Y₁₁-R but were not P2Y₁₁-R selective since they also have agonist activity at P2Y₁-R. In addition, compounds **8B** and **11** were 3.4- and 2-fold respectively, more active than ADP at P2Y₁₁-R, while their agonist activity at the P2Y₁-R was low. The most active agonist at P2Y₁-R, **7A**, was 2-fold less active than ADP at the P2Y₁₁-R.

Evaluation of nucleotide analogues 6-11 as Fenton reaction inhibitors:

ESR was used to monitor the modulation of OH radical ($\cdot$ OH) formation from H₂O₂ in the Fe(II)-induced Fenton reaction by Compounds **6-11**. As the hydroxyl

radical formed in the reaction is extremely short-lived, 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) was used as a spin trap. DMPO-OH adduct was then detected by ESR. The addition of nucleotides to Fe(II)-H₂O₂ mixture lowered DMPO-OH signal, presumably due to metal-ion chelation and/or radical scavenging.

5 Table 2 below presents the obtained data, showing that the nucleoside-5'-thiophosphate analogues are good Fenton reaction inhibitors, being 2.5-8-fold more active than ADP and EDTA, respectively, and far more active than ATP.

Antioxidant IC₅₀ values represent the compound's concentration that inhibits 50 % of the OH radical amount produced in the control reaction.

Table 2

Compound	IC ₅₀ (μM)
EDTA	54±5
ATP (1)	n.a
ADP (2)	170±6.36
ADP(β-S) (5)	19±2.5
ADP(α-S) (6A)	31±1.55
ADP(α-S) (6B)	36±3
2-SMe-ADP(α-S) (7A)	37±2.1
2-SMe-ADP(α-S) (7B)	38±3
2-Cl-ADP(α-S) (8A)	36±2.8
2-Cl-ADP(α-S) (8B)	39±4
2-Cl-ATP(α-S) (9A)	37±0.7
2-Cl-ATP(α-S) (9B)	27±2
2-SMe-ADP(β-S) (10)	21±0.07
2-Cl-ADP(β-S) (11)	21±2.1

10 n.a. = Not available, the minimal amount of radical production exceeds 50 %.

Without being bound by any particular theory, it is assumed that the differences between the activity of compounds **10-11** containing a sulfur atom at the P_β position (IC₅₀ 19-20 μM), and compounds **6-8** containing a sulfur atom at the P_α position (IC₅₀ 31-39 μM) may be related to the fact that under Fenton reaction conditions oxidation of
 15 ADP-α-S moiety to the corresponding disulfide dimer provides two terminal distant

phosphate groups for Fe(II)-coordination, while oxidation of the ADP- β -S moieties provides four neighboring phosphate groups for Fe(II) coordination. It was previously found that under Fenton reaction conditions, phosphorothioate compounds underwent rapid oxidation to form the corresponding disulfide dimers [Richter et al., 2006, *supra*].

5 It was found that ATP- γ -S, **3**, disulfide-dimer has six phosphate coordination sites and may form an octahedral complex with Fe(II). Occupation of all Fe(II) coordination sites by this chelator precludes the binding of H₂O₂ to Fe(II)-chelates and the subsequent Fenton reaction. ATP- α -S, however, was found to be a 30-fold weaker inhibitor as compared to ATP- γ -S, and this may be due to the fact that ATP- α -S dimer
10 contains only four phosphate coordination sites.

As shown in Figures 2A-C, it was found that there was no difference in the activity between both isomers of the P- α -S compounds, e.g., IC₅₀ values of ADP- α -S, **7A** and **7B** isomers are 37 and 38 μ M, respectively. This result supports the suggestion above that the disulfide dimer is the actual Fenton reaction inhibitor.

15 ADP binds Fe(II) preferentially in a "closed" structure in which the coordination to the metal-ion is both through the phosphate chain and the adenine N7-nitrogen atom [Sigel, H. *Chem. Soc. Rev.* **1993**, 22, 255-267]. Therefore, substitution of an electron withdrawing- or donating-group on the nucleobase is expected to affect electron density on N7-nitrogen atom and hence, affinity to metal-ion. However, comparison of C2-
20 modified nucleotides to the corresponding parent compounds, ADP- α -S, showed that substitution of C2 by an electron withdrawing group, such as Cl, or an electron donating group, as SMe, had no influence on the antioxidant activity. Namely, the modified adenine moiety is probably not directly involved (although an outer sphere coordination is possible) in Fe(II)-chelation, and it is probably the disulfide dimer that is the actual
25 chelator via the two terminal phosphate groups.

Evaluation of the radical scavenging activity of nucleotide analogues 6-11 using ABTS decolorization assay:

Fenton reaction can be inhibited by both metal-ion chelators and radical scavengers. Hence, Compounds **6-11** were evaluated also as radical scavengers by the
30 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) decolorization assay. ABTS^{•+} is formed by the oxidation of ABTS with potassium persulfate, and absorbs at 645, 734 and 815 nm. The scavenging of ABTS^{•+} radical is determined as a function of

the antioxidant concentration, and is calculated relative to the reactivity of Trolox as a standard.

The Scavenging of ABTS^{•+} by nucleotide analogues **6-11** vs. Trolox is presented in Table 3 below. As shown therein, the thiophosphate analogues **6-11** are good radical scavengers with IC₅₀ values around 12 μM as compared to IC₅₀ of 18 and 30 μM for Trolox and ADP, respectively. This improved antioxidant activity of Compounds **6-11** is probably due to the thiophosphate group which can donate an electron to ABTS^{•+} and subsequently form a disulfide dimer.

Table 3

Compound	IC ₅₀ (μM)
Trolox	18±1.4
ATP (1)	60±0.49
ADP (2)	30±0.54
ADP(β-S) (5)	15±0.8
ADP(α-S) (6A)	20±0.6
ADP(α-S) (6B)	22±0.3
2-SMe-ADP(α-S) (7A)	12.5±0.01
2-SMe-ADP(α-S) (7B)	12±0.02
2-Cl-ADP(α-S) (8A)	11±0.7
2-Cl-ADP(α-S) (8B)	12±0.35
2-Cl-ATP(α-S) (9A)	25±0.6
2-Cl-ATP(α-S) (9B)	24±0.4
2-SMe-ADP(β-S) (10)	15±0.7
2-Cl-ADP(β-S) (11)	13±0.7

Inhibition of ROS production in PC12 cells under oxidizing conditions by Compounds 6-11:

The modulation of Fe(II)-induced oxidative stress in PC12 cells by nucleotides and dinucleotides, using DCFH-DA, a radical sensitive indicator, was previously studied [Halbfinger et al., *J. Med. Chem.* **1999**, 42, 5325-5337]. When DCFH-DA is oxidized by ROS, it is converted to 2',7'-dichlorofluorescein (DCF), and emits green fluorescence. The fluorescence intensity, a function of the ROS concentration in the cells, is measured quantitatively by a spectrofluorometer.

Compounds **6-11** were measured as potential inhibitors of ROS formation in PC12 cells treated with FeSO₄. Table 4 summarizes IC₅₀ values obtained for

phosphorothioate analogues **6-11** vs. the corresponding parent nucleotides (ATP (**1**), ADP (**2**) and ADP- β -S (**5**)), in inhibiting Fe(II)-induced ROS production in PC12 cells.

Table 4

Compound	IC ₅₀ (μ M)
Trolox	20 $\pm$ 2.4
ATP (1)	n.a
ADP (2)	21 $\pm$ 1.5
ADP(β -S) (5)	20 $\pm$ 2.3
ADP(α -S) (6A)	38 $\pm$ 1.2
ADP(α -S) (6B)	39 $\pm$ 2.5
2-SMe-ADP(α -S) (7A)	0.04 $\pm$ 0.002
2-SMe-ADP(α -S) (7B)	0.6 $\pm$ 0.02
2-Cl-ADP(α -S) (8A)	0.2 $\pm$ 0.04
2-Cl-ADP(α -S) (8B)	0.11 $\pm$ 0.02
2-Cl-ATP(α -S) (9A)	106 $\pm$ 3.8
2-Cl-ATP(α -S) (9B)	100 $\pm$ 2
2-SMe-ADP(β -S) (10)	12 $\pm$ 1.9
2-Cl-ADP(β -S) (11)	5 $\pm$ 0.7

Values represent mean $\pm$ S.D. of three experiments (P < 0.05).

n.a - The nucleotide did not inhibit 50% of ROS production.

A reduction of 50 % of total ROS concentration, was observed at 21 μ M ADP, **2**, while replacement of P $_{\alpha}$ of ADP by thiophosphate in ADP(α -S), **6**, resulted in slightly less active analogues, IC₅₀ 31 and 36 μ M for isomers **6A** and **6B**, respectively. However, substitution of the adenine C2-position by SMe in addition to thiophosphate at P $_{\alpha}$, 2-SMe-ADP(α -S), **7A**, improved ADP's antioxidant activity 500-fold (IC₅₀ 0.04 μ M). Analogue **7A** was 15-fold more potent than **7B** (IC₅₀ 0.6 μ M). In addition, substitution of Cl at the C2 position in 2-Cl-ADP(α -S), **8** improved antioxidant activity vs. ADP 100- and 200- times for **8A** and **8B** isomers, respectively. Replacement of the P $_{\beta}$ -non-bridging oxygen atom by sulfur atom, **5**, has not changed the antioxidant activity of ADP (IC₅₀ 20 μ M). Substitution of 2-Cl/SMe in addition to thiophosphate at P $_{\beta}$ resulted in improved antioxidant activity. Specifically, 2-Cl-ADP(β -S), **11** and 2-SMe-ADP(β -S), **10**, inhibited ROS production with IC₅₀ values of 5 and 12 μ M vs. 20 μ M for **5**. The least active compound in this series was 2-Cl-ATP(α -S), **9**, with IC₅₀ of 100 μ M for both isomers.

Analysis of the SAR of the tested compounds at PC12 cells under oxidizing conditions revealed that substitution of Cl/SMe at the C2 position of the adenine ring improves antioxidant activity. 2-SMe substitution at ADP- α -S, **6A**, improves antioxidant activity of **7A** 950-fold. 2-Cl substitution in **8A** resulted in a 190-fold more active compound. These results support the above suggestion that the mechanism of the antioxidation activity also involves P2Y-R activation. This finding also supports that anti-oxidant activity of ADP analogues involves activation of ADP-binding-P2Y-Rs (e.g. P2Y₁-R and P2Y₁₂-R).

Resistance of Compounds 7A and 7B to hydrolysis by ecto-nucleotidases:

The therapeutic merit of nucleotide analogues is dependent on their metabolic stability, specifically on their resistance to hydrolysis. Ecto-nucleotide pyrophosphatase/phosphodiesterase (eNPP) family is one of the principal enzyme families that metabolize extracellular nucleotides. Since Compound **7A** was demonstrated as highly potent antioxidant and P2Y₁-R agonist, the resistance of Compounds **7A** and **7B** to hydrolysis by eNPP1/3, was tested. The hydrolytic stability of **7A** and **7B** was compared to that of its parent nucleotide ADP, and the primary endogenous substrate of eNPP1/3, ATP.

Table 5 below presents the data obtained for hydrolysis of ADP, **2**, and 2-SMe-ADP(α -S), **7A** and **7B**, by human-ectonucleotidases, eNPP1/3, as percentages of hydrolysis relative to ATP, meaning ATP was calculated as being 100% hydrolyzed.

As shown in Table 5, while Compounds **7A** and **7B** did undergo hydrolysis by eNPP1 and eNPP3 (23.7 - 42.0 %), both **7A** and **7B** were considerably more stable than ADP (74.5 and 106 % hydrolysis of ADP by eNPP1 and eNPP3, respectively). The **7B** analogue was found to be more resistant to enzymatic hydrolysis, being four times more stable than ATP and 3- and 4- times more stable than ADP at NPP1 and eNPP3, respectively. As eNPP1 and eNPP3 hydrolyze the phosphate chain mostly between P α -P β , it is suggested that the replacement of the P α non-bridging oxygen by a sulfur atom has a role in inhibiting hydrolysis of **7A** and **7B** as well as in possibly enabling stronger binding to the zinc containing catalytic site.

Table 5

relative hydrolysis (% $\pm$ SD of ATP hydrolysis)			
human ectonucleotidase	7A	7B	ADP
eNPP1	36.6% $\pm$ 2.4	23.7% $\pm$ 4.4	74.52% $\pm$ 2.6
eNPP3	42.0% $\pm$ 1.8	25.6% $\pm$ 2.6	106.3% $\pm$ 2.5

Resistance to hydrolysis in human blood serum:

The usage of nucleotides as therapeutic agents is limited due to their rapid dephosphorylation by enzymes in physiological systems. Blood serum contains such enzymes and, therefore, provides a good model system for estimation of the *in vivo* stability of nucleotide analogues.

The stability of 2-SMe-ADP(α -S), **7A**, in human blood serum as compared to ADP, **2**, and ADP- α -S, **6A**, was tested. These analogues were incubated in human blood serum and RPMI-1640 at 37 °C for 0.5–24 hours. The hydrolysis rate of the nucleotide analogues was determined by measuring the change in the integration of the HPLC peaks for each analogue over time.

The results are presented in Figure 4. ADP was hydrolyzed to AMP, followed by further degradation to adenosine, with a half-life of 1.5 hours. Analogue **7A** was hydrolyzed with a half-life of 15 hours, and analogue **6A** displayed a half-life of 14.5 hours, indicating that the major contribution to stability enhancement of ADP is the thiophosphate substitution.

P2Y₁₂-R activation:

Like P2Y₁-R, P2Y₁₂ purinergic receptor is activated by endogenous ADP and a synthetic agonist, 2-MeS-ADP. The activity of these nucleotides at P2Y₁₂-R is blocked by 2-MeS-AMP.

The possible involvement of P2Y₁₂-R in protection of cells against oxidative stress by 2-SMe-ADP(α -S), **7A**, was investigated. For this purpose, the effect of **7A** on PC12 cells, known to express P2Y₁₂-R, under oxidizing conditions, with or without antagonist, was measured. PC12 cells were selected for this study, rather than primary neurons, since the former express P2Y₁₂-R, but not P2Y₁-R. PC12 cells were treated with 2-MeS-AMP and then with DCFH-DA, and oxidation was initiated by addition of FeSO₄.

Figure 5A presents the obtained data and show that the effect of the antagonist (2-MeS-AMP) on percentage of inhibition of ROS formation (vs. control not containing 2-MeS-AMP) is negligible (about 2 %). When this experiment was repeated with analogue **7A**, added at a final concentration of 0.04-100 μ M, up to 70 % decrease in the antioxidant activity of **7A** in the presence of 2-MeS-AMP was measured. These results suggest that the antioxidant activity of **7A** also involves the activation of P2Y₁₂-R in PC12 cells.

The involvement of P2Y₁-R in the antioxidant activity of **7A**, was studied also using Ntera-2 cells, under oxidizing conditions, with or without the antagonist, MRS2179. Ntera-2 cells were selected since these cells endogenously express a functional P2Y₁ receptor, while other P2Y subtypes, except perhaps P2Y₄, are not functionally expressed. Therefore, Ntera-2 cells provide a useful neuronal-like cellular model for studying the precise signaling pathways and physiological responses mediated by a native P2Y₁ receptor.

The results are presented in Figure 5B, and show that **7A** inhibited 50 % of ROS formation at 0.2 μ M. When Ntera-2 cells were incubated with the antagonist MRS2179, prior to treatment with **7A**, the antioxidant activity was reduced by 35 %. MRS2179 alone did not affect the ROS reduction.

These results suggest that the antioxidant activity of **7A** also involves the activation of P2Y₁-R and P2Y₁₂-R.

Neuroprotective effect:

The neuroprotective effect of Compound **7A** was evaluated. Primary cortical neurons were treated with 3 μ M FeSO₄ and 0.01-25 μ M **7A** or **7B** for 24 hours, and cell viability was measured by MTT assay.

The results are presented in Figure 6, and show that ADP, **2**, protects primary neurons in the presence of FeSO₄, with EC₅₀ 19 μ M, while **7A** was found to be a 475-time more potent neuroprotectant (EC₅₀ 0.04 μ M). In addition, **7A** was 15-fold more active than the **7B** isomer. These results are in good correlation with the higher potency of **7A** at P2Y₁-R, thus implying the involvement of this receptor in protection of primary neurons. In addition, P2Y₁₂-R may possibly be also involved in the neuroprotective activity of **7A**.

Neuron morphology:

In order to establish the effects of 2-SMe-ADP(α -S), **7A**, on cell morphology, especially on cytoskeleton elements, **7A** and FeSO₄ were co-applied to primary cortical neurons, and the cells were viewed by light microscopy.

5 The obtained images are shown in Figures 7A-E. Viewing cells after treatment with 3 μ M FeSO₄ for 24 hours (Figure 7B) as compared to control (Figure 7A), clearly showed a change in the morphology of the primary cortical neuron cells. After treatment with FeSO₄, the amount of cells decreased dramatically. In addition, the cytoskeleton elements were damaged and the cells lost their extensions as compared to
10 control. When cortical neurons were treated with increasing amounts of 2-SMe-ADP(α -S), **7A** (0.2, 5 and 100 μ M) (Figures 7C, 7D and 7E), the number of vital neuronal cells increased correspondingly. Furthermore, the morphology of the cells at 5 and 100 μ M 2-SMe-ADP(α -S) was almost similar to that of the native neurons (Figures 7D and 7E).

Evaluation of the relative binding of Fe(II) ions:

15 In order to evaluate the ability of Compound **7A** to chelate Fe(II)-ions competitive studies of the relative binding of Fe(II) by **7A** vs. ferrozine were performed. Specifically, the absorbance of the formed colored Fe(II)-ferrozine complex in the presence or absence of **7A** and ADP was measured.

The obtained data is presented in Figure 8. Compound **7A** inhibited 50 % of the
20 formation of Fe(II)-ferrozine complex at 80 μ M, while, ADP at maximal concentration of 200 μ M inhibited only 40 % of Fe(II)-ferrozine complex formation. These findings support the assumption that a sulfur atom replacing oxygen in the phosphate moiety improves the antioxidant activity of the molecule due to improved affinity to Fe(II) and improved chemical and metabolic stability of the compounds.

Rescue of neurons from A β toxicity:

25 Iron chelators have been shown to protect neuron cells against the pro-apoptotic signaling of amyloid beta [Kuperstein F, Yavin E. J Neurochem 2003;86:114-25]. In order to study the involvement of apoptotic processes in A β 42-induced cell death and the neuroprotective actions of SSA-37, a primary mixed culture of neurons and
30 astrocytes was used to create conditions resembling those in animal models [Maiti et al. Biochem J 2011;433:323-32]. A β 42 oligomers [Amir et al. Dalton Transactions 2012;41:8539-49] induced a concentration-dependent decrease in cell viability, as

assessed by dyeing the cells with trypan blue and counting the vital cells following 48 h of exposure [Danino et al. Biochem Pharmacol. 2014 Apr 1;88(3):384-92. doi: 10.1016/j.bcp.2014.02.001. Epub 2014 Feb 15].

Hence, the neuroprotective effect of **7A** was evaluated in a primary mixed culture exposed to 50 μ M A β 42 for 48 hours.

Primary neuronal cells were cultured in 96-well plates. The cells were treated with 50 μ M A β 42 and various concentrations of **7A** (0.04-25 μ M) for 48 hours. Cell viability was measured by dyeing the cells with trypan blue and counting the vital cells. The obtained data is presented in Figure 9A. The results shown are representative from three independent experiments performed in triplicate (* P < 0.05 vs. A β 42 treatment).

As shown in Figure 9A, co-application of 5 μ M **7A** with A β 42 resulted in almost 80 % protection. The IC₅₀ value obtained for Compound **7A** was 0.5 μ M.

From the culture media of the above assay, the level of LDH, which elevate in cell death, was also tested.

The media from the A β 42 experiment was tested for LDH level. 25 μ L from the media was transferred into a 96-well plate together with 100 μ L of LDH testing reagent. The amount of LDH released to the extracellular fluid was measured spectrometrically at 30 °C at a wavelength of 340 nm. The obtained data is presented in Figure 9B. The results shown are the mean $\pm$ S.D. of three independent experiments performed in triplicate (*P < 0.05).

As shown in Figure 9B, after treatment with 50 μ M of A β 42, LDH level was elevated by 50 % as compared to the control. Treatment with Compound **7A** at 1 μ M reduces the level of LDH to the basal level.

By measuring the percentage of vital cells and LDH assay, it is demonstrated that **7A** improved the cell viability of this culture: 50 % of the culture remained vital after treatment with 0.5 μ M **7A** and 50 μ M A β 42. The result was confirmed by using LDH assay, which is a marker for cell death. Compound **7A** reduced the level of LDH to the basal level at 1 μ M.

Disaggregation of A β 42-Zn²⁺ by 7A:

Both Cu(II) and Zn(II) are released from vesicles of neurons during synaptic transmission, reaching concentrations as high as 15 μ M and 300 μ M, respectively. This homeostasis is disrupted in AD and the concentrations of

Cu(II) and Zn(II) can rise up to about 1 mM, and consequently promote A β aggregation.

Aggregation of A β -M(II) complexes results in a turbid solution, which affects the light scattering of the sample. The difference in the solution's turbidity is determined by the absorbance at 405 nm ($A_{405\text{nm}}$), which indicates the degree of A β -Cu(II)/Zn(II) aggregation [Storr et al., *Dalton Transactions* **2009**, 3034-3043; Huang et al., *Journal of Biological Chemistry* **1997**, 272, 26464-26470].

The potential of Compound **7A** to prevent aggregation of A β_{42} -Zn²⁺ was therefore tested. In addition, the ability of **7A** to disassemble A β_{42} -Zn²⁺ by adding **7A** to A β_{42} -Zn²⁺ aggregate (2 hours after mixing A β_{42} and Zn²⁺) was tested.

Freeze-dried A β_{42} was dissolved in 50 mM Tris-HCl (pH 7.4) to obtain a 50 μ M stock solution. From this mixture, controls and A β_{42} -M(II) aggregates were prepared by the addition of 50 μ M ZnCl₂ in H₂O: 1) 50 μ M A β_{42} ; 2) 50 μ M A β_{42} -Zn(II); 3) 50 μ M A β_{42} -Zn(II)+**7A** at 150 μ M; 4) 50 μ M A β_{42} -Zn(II)+**7A** at 150 μ M (**7A** was added to 50 μ M A β_{42} -Zn(II) after 2 hours of incubation). These solutions were incubated at 37 °C for 6 hours to form aggregates. The final volume of all samples was 200 μ L at 96 microplate. The absorbance of triplicates was measured at 405 nm at RT. Tris-HCl buffer was used as the blank.

The obtained data is presented in Figure 9C, and show that Compound **7A** at 50 μ M effectively disaggregates 50 μ M A β_{42} -Zn²⁺ (by 40 %) and is a less efficient inhibitor of aggregation (by 20 %).

Mechanistic Insights:

The data presented herein indicates that the nucleotide analogs of the present embodiments exhibit multiple activities, and target several causes of AD pathology. The nucleotide analogs act as neuroprotectants which rescue neurons from oxidative stress by antioxidation activity which involves Fe(II)-chelation and radical scavenging; and by activating P2Y-receptors involved in neuroprotection. In addition, the nucleotide analogs described herein further act by rescuing neurons from Amyloid beta (A β_{42}) toxicity. This effect involves disassembly of A β_{42} -Zn(II) aggregates via metal-ion chelation exhibited by the nucleotide analogs.

EXAMPLE 3***Docking Studies******Computational methods:***

Curcumin enol tautomer was built using the small molecules module in
5 Discovery Studio (DS) structure-based design software, version 4.0 (BIOVIA/Accelrys Inc., San Diego, CA, USA) running on a HP xw4600 workstation. Molecules were minimized by 1000 steps each of steepest descent followed by conjugate gradient minimization using an implicit solvent model generalized Born smooth switching function (GBSW) and CHARMM force field.

10 The ligands were described using the CHARMM force field with an implicit water solvent model (generalized Born with molecular volume).

Energy minimization of the ligands was performed using the smart minimizer (1000 steps of the steepest descent method followed by the conjugate gradient method).

Prior to docking the ligands into the receptor, a library of various ligand
15 conformations (maximum 255) was generated using the FAST conformation generation method with a maximum energy threshold of 20 kcal/mol. All the conformations were subjected to docking using a high throughput protocol designed within Pipeline Pilot [*Pilot, P.*, Version 9.2; Biovia. Inc.: San Diego, CA, 2014; Wu et al. *J. Comput. Chem.* **2003**, *24*, 1549-1562]. The grid-based molecular docking program CDOCKER [Wu et al.
20 al., 2003, *supra*] with the CHARMM force field [Brooks et al. *J. Comput. Chem.* **2009**, *30*, 1545-1614] was employed to dock all the generated conformations into the P2Y₁R. The docked pose with the highest negative interaction energy (i.e. lowest energy) was considered for further analysis. The solvation energy of the ligands was calculated using the Delphi program [Sitkoff et al. *J. Phys. Chem.* **1994**, *98*, 1978-1988]. The solvation
25 energy of each ligand is computed by averaging the solvation energy of the five lowest energy conformations obtained from the conformational search mentioned above.

To facilitate comparison with experiment, EC₅₀ values were predicted using multi-regression analysis of the solvation energy, the CDOCKER interaction energy and of both the solvation energy and the CDOCKER interaction energy. The crystal
30 structures of P2Y₁R were obtained from protein data bank (pdb codes: 4XNV and 4XNW) [Zhang et al. *Nature* **2015**, *520*, 317-321; Zhang et al. *Nature* **2014**, *509*, 119-

122]. All calculations employed the Discovery Studio (DS 4.0) modeling platform (Biovia, Inc.) [*Studio, D.*, Version 4.1.; Biovia: San Diego, CA, 2015].

EC₅₀ values of P2Y₁-R agonists, Compounds 1-11, are correlated with the docking score and desolvation energies:

5 To rationalize the difference of binding interactions of P2Y₁R agonists **1-11**, docking studies of these nucleotides into crystal structures of P2Y₁R were performed.

Two crystal structures of the human P2Y₁R (pdb codes: 4XNV and 4XNW) are available [Zhang, D., et al., *Nature* **2015**, 520, 317-321]. The crystal structure 4XNV is a complex of P2Y₁R with a non-nucleotide antagonist, 1-(2-(2-(tert-butyl)phenoxy)pyridin-
10 3-yl)-3-(4-(trifluoromethoxy)phenyl)urea (BPTU). In this structure, BPTU binds outside the protein, at the protein-lipid interface in the transmembrane domain (see, Figure 10A). The other crystal structure, 4XNW, is a complex with a nucleotide-based antagonist (1'R,2'S,4'S,5'S)-4-(2-Iodo-6-methylaminopurin-9-yl)-1-[(phosphato)methyl]-
2(phosphato)bicycle[3.1.0]-hexane (MRS2500). In this complex, MRS2500 binds at the
15 top of extracellular side of the protein within the loops and alpha helices (see, Figure 10B).

The crystal structure of human P2Y₁R with agonists has not been reported to date. The human P2Y₁₂R complex with an agonist (2-SMe-ADP) is available (pdb code: 4PXZ) [Zhang, J., et al., *Nature* **2014**, 509, 119-122]. In this complex, the agonist binds deep
20 inside the receptor transmembrane region, with the purine base interacting with hydrophobic and polar residues, while the phosphate moiety interacts with several positively charged residues. Modeling of P2Y₁₁R using the homology modeling based on the P2Y₁R or P2Y₁₂R structures cannot be performed since the sequence identities and similarities (see, Table 6) between these receptors are not high enough to obtain
25 meaningful homology models.

Table 6 below presents sequence similarities and sequence identities between P2Y₁R, P2Y₁₁R and P2Y₁₂R.

Table 6

Receptor-pairs	Sequence Identity	Sequence similarity
P2Y ₁₁ -P2Y ₁₂	19.3%	30.9%
P2Y ₁₁ -P2Y ₁	25.4%	40.4%
P2Y ₁ -P2Y ₁₂	20.8%	36.6%

Both the 4XNV and 4XNW receptor structures were considered for docking Compounds **1-11**. In the 4XNW receptor, all the ligands show binding to the receptor, with high docking scores (see, Table 7). As shown in Figure 10D, all the ligands bind at the top of the extracellular side of the receptor within the loop region. These binding poses are similar to that in the crystal structure with the antagonist MRS2500.

In the 4XNV receptor, all ligands bind inside the bundle of alpha helices, as shown in Figure 10C. In this receptor, the adenine moiety of the ligands makes contact with hydrophobic residues, whereas the phosphate chain interacts with positively charged residues. In the 4XNV receptor, all the ligands bind in similar binding poses deeper into the receptor pocket than MRS2500 (in 4XNW). These binding poses are rather similar to the experimental binding pose of agonist 2-SMe-ADP in P2Y₁₂R, although not quite as deep inside the transmembrane helical bundle.

A difference in the 4XNW and 4XNV structures is seen in the position of Asp204. In the former crystal structure, this residue protrudes deeper into the transmembrane region than in the latter, possibly to minimize repulsion with the antagonist phosphate chain. As a result, a binding pocket deeper inside the transmembrane region is partially blocked in 4XNW, hence not allowing agonist binding. In the 4XNV structure, this alternative binding pocket is available for agonist binding, and indeed, is occupied in the docking simulations. It is suggested that the position of Asp204 is variable, depending on the ligand.

Nonetheless, the calculated interaction energies for some of the ligands were found to be unfavorable in the 4XNV structure, and it is assumed that this is due to repulsion between Asp204 and the ligand phosphate moieties (see, Table 7). The Asp204 was therefore rotated by 90 degrees and the ligands re-docked. In the modified receptor (4XNV_{MOD}) high docking scores (favorable binding) were found for all the ligands. The

CDOCKER interaction energies of all ligands with 4XNV, 4XNW and 4XNV_{MOD} are shown in Table 7.

Table 7 below presents CDOCKER interaction energies of ligands **1-11** with the P2Y₁ receptor and absolute configuration of the phosphorous atoms of the docked ligands.

Table 7

Ligand	CDOCKER Interaction energy (kcal/mol)			Absolute configuration
	4XNV	4XNW	4XNV _{MOD}	
ATP (1)	-19.47	-81.22	-60.40	-
ADP (2)	-10.65	-101.02	-45.93	-
2-MeS-ADP (4)	-9.67	-105.77	-46.20	-
ADP- β -S (5)	0.72	-89.23	-40.51	-
ADP- α -S (6A)	0.17	-81.22	-34.88	R _P
ADP- α -S (6B)	0.17	-71.89	-34.88	S _P
2-SMe-ADP(α -S) (7A)	1.89	-85.80	-34.38	R _P
2-SMe-ADP(α -S) (7B)	1.90	-85.92	-34.65	S _P
2-Cl-ADP(α -S) (8A)	-1.60	-85.28	-36.09	R _P
2-Cl-ADP(α -S) (8B)	-2.67	-79.92	-37.56	S _P
2-Cl-ATP(α -S) (9A)	-18.48	-107.48	-50.72	R _P
2-Cl-ATP(α -S) (9B)	-2.74	-106.65	-55.66	S _P
2-SMe-ADP(β -S) (10)	-5.50	-90.7	-37.47	-
2-Cl-ADP(β -S) (11)	6.87	-86.3	-38.59	-

To understand the experimental trends of the EC₅₀ values, the EC₅₀ values were predicted using regression analysis with solvation energy (P_{Sol}), with CDOCKER interaction energies (P_{CDOCK}) and with both solvation and CDOCKER interaction energies (P_{CDOCK+Sol}). Based on the desolvation energies alone, the difference between several ligands can be rationalized. For instance, the following desolvation trend was obtained: ATP > 2-Cl-ATP(α -S) ~ ADP ~ 2-SMe-ADP > ADP(α -S) ~ 2-SMe-ADP(α -S) (ATP has highest desolvation energy). This suggests that ATP pays a greater free energy penalty on binding to P2Y₁R than ADP. On the other hand, the P2Y₁-R binding site has evolved to bind ADP, and hence is likely unable to fully compensate for ATP's loss of solvent

interactions via binding site interactions. Hence, ATP is a poorer agonist than ADP. Similarly, ADP(α/β -S) is easier to desolvate than ADP, due to weaker interaction of the thiophosphate moiety with water compared to phosphate.

Attempts to correlate P_{CDOCK} values only with EC_{50} suggest that the optimal fit is obtained using the 4XNV_{MOD} structure for docking (see, Figure 11B). Nonetheless, the P_{Sol} values and P_{CDOCK} values alone do not reproduce the experimental trends of EC_{50} (Figures 11A-D). $P_{\text{CDOCK+Sol}}$ values for the 4XNV_{MOD} receptor show similar EC_{50} trends as that obtained experimentally.

Figures 12A-B show the binding pocket of 4XNV_{MOD} receptor with ADP (**2**) (Figure 12A) and 2-SMe-ADP(α -S) (**7A**) (Figure 12B). The positive residues Arg128 and Arg310 are found to interact with all the ligands as shown for ADP and 2-SMe-ADP [Jacobson et al. *WIREs Membr Transp Signal* 2012, 1:815–827]. Mutagenesis of P2Y₁-R also suggests these to be active in ligand binding. Gln307 and Asp204 are also suggested to participate in ligand recognition based on mutagenesis data [Jacobson et al. 2012, *supra*] and these residues are also found to interact with our docked ligands in the 4XNV_{MOD} receptor.

Various ligand-receptor interactions observed in the herein described docking simulations are in agreement with previous predictions by modeling studies done in the absence of crystal structures [Major, D. T.; Fischer, B., *J. Med. Chem.* 2004, 47, 4391-4404; Major et al., *J. Med. Chem.* 2004, 47, 4405-4416].

The herein described studies suggest that the EC_{50} values of P2Y₁R agonists **1-11** can be explained on the basis of a combination of ligand interaction energy and desolvation energy.

The binding of agonists inside the modified protein pocket is clearly different from that of antagonists in the crystal structures. The adenine moiety of the agonists interacts with hydrophobic residues via π - π interaction (Phe131) and the phosphate-chain interacts with positively charged residues (Arg128 and Arg310). This is in agreement with mutagenesis data [Jacobson et al. 2012, *supra*) and other studies [Major, D. T.; Fischer, B., *J. Med. Chem.* 2004, 47, 4391-4404; Major et al., *J. Med. Chem.* 2004, 47, 4405-4416]. The predicted EC_{50} values clearly distinguish between the ATP-based, ADP-based and sulfur containing ADP and ATP analogues, and correlate with

desolvation and docking energies. The docking simulations are not sufficiently accurate to distinguish between diastereoisomers, e.g., **7A** and **7B**.

EXAMPLE 3

IN VIVO STUDIES

The 5xFAD model of Alzheimer's Disease (www.jax.org/strain/006554) was used in *in vivo* studies for evaluating the effect of Compound **7A** (SK, SSA37A). 5xFAD mice were injected daily IP (0.1 ml) with 1 mg/Kg SSA37A or with saline (IP administration) for 45 days (from the age of 6 weeks). Wild type mice (n=10) were injected with saline. At the age of 8 months the mice were subjected to behavioral tests. The study protocol is presented in Figure 13.

Elevated Plus Maze test:

A plus-shaped maze containing two dark and enclosed arms and two open and illuminated arms, elevated 100 cm above ground was used, as depicted in Figure 14A.

The arms were 30 × 5 cm and the walls of the closed arms were 40 cm high.

Mice were placed in the center of the maze, tracked for 5 minutes and then returned to their home cage. The time spent in the open arms, the numbers of entries into the open arms and latency to enter the open arms were scored using the Ethovision video tracking system. Data is presented as average +/- SE, ANOVA, and is shown in Figure 14B, for female mice and in Figure 14C for male mice. As shown in Figures 14B-C, Compound **7A** (SK) restores normal inhibition.

T Maze memory test:

The T-maze apparatus consisted of 3 arms (9 × 50 cm each) filled with water to a depth of 15 cm, as depicted in Figure 15A. The mice were given time to find the transparent platform hidden at the end of the right arm. The mouse was placed at the stern of the T-maze and the time taken to reach the hidden platform was recorded and analyzed. Data is presented as average, +/- SE, ANOVA, and is shown in Figure 15B for female mice and in Figure 15C for male mice. As shown therein, Compound **7A** (SK) restores normal inhibition in T Maze memory test.

Fear Conditioning test:

Mice were subjected to an unconditioned electric stimulus (one foot shock; 1 s/1 mA) in a training pre-session, as shown in Figure 16C. Twenty four hours later, a fear

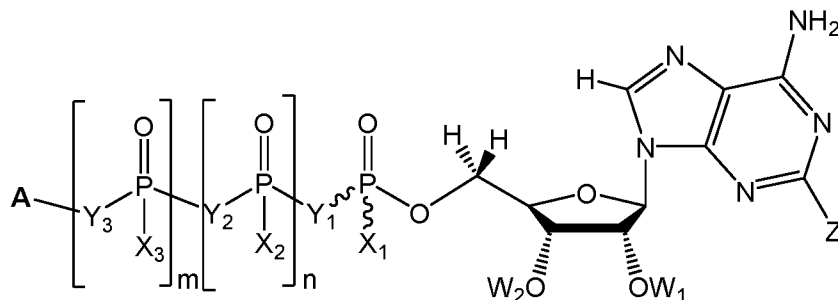
conditioning test was performed by scoring freezing behavior, e.g., the absence of all but respiratory movement was monitored for 180 seconds using the FreezeFrame automated scoring system (Coulbourn Instruments, PA, USA). Data is presented as average $\pm$ SE, ANOVA, and is shown in Figures 16A and 16B, and show that
5 Compound **7A** (SK) restores the long term cognitive memory in the Fear Conditioning test.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all
10 such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and
15 individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A compound represented by Formula I:



Formula I

wherein:

the curled lines denote an Rp or Sp configuration, when relevant;

n and m are each independently 0 or 1;

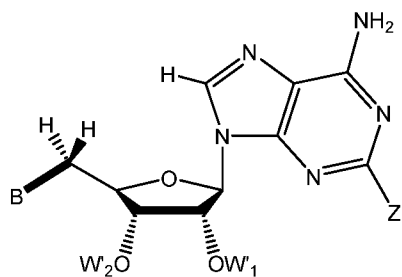
Z is selected from $-SR_1$, $S(=O)R_1$, $S(=O)NHR_1$, OR_1 , NHR_1 , R_1 and halo;

R_1 is alkyl, cycloalkyl, aryl or acyl;

W_1 and W_2 are each independently selected from H, alkyl and acyl;

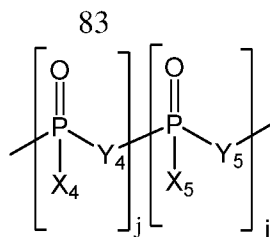
Y_1 , Y_2 and Y_3 are each independently O, S, CH_2 , $C(L)_2$ or NH, wherein L is halo;

X_1 , X_2 and X_3 are each independently -OH or -SH, provided that at least one of X_1 , X_2 and X_3 is SH; and



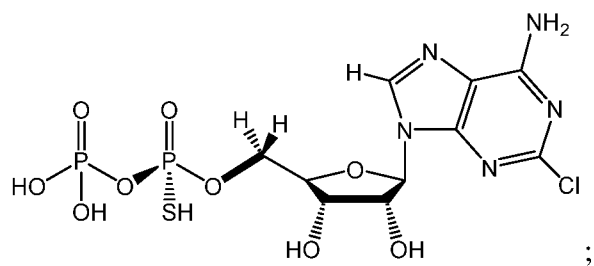
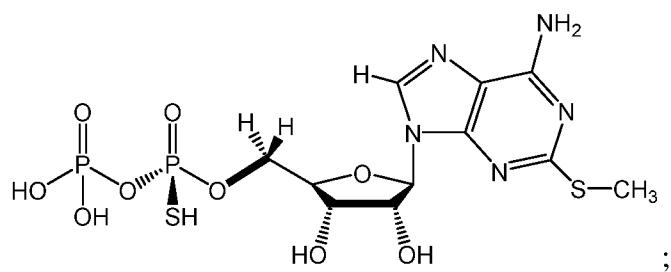
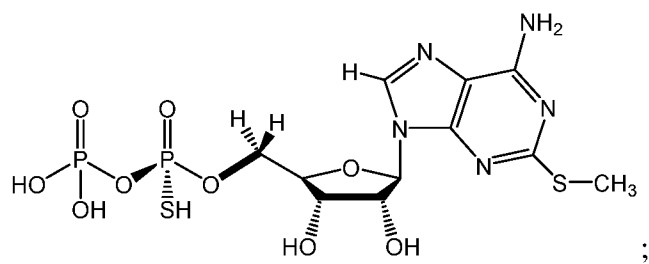
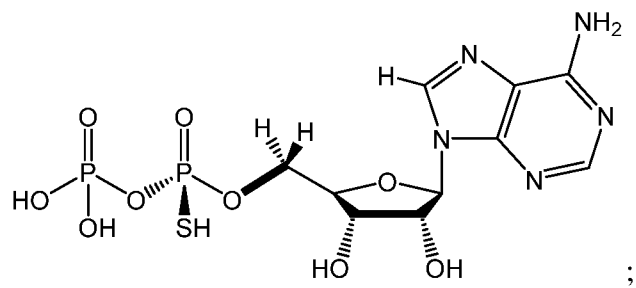
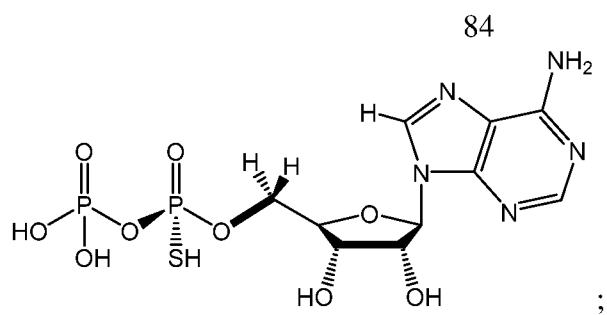
A is H or

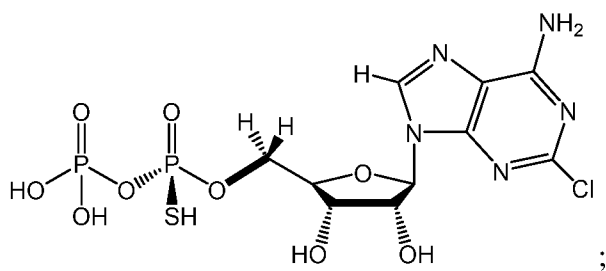
wherein Z' is selected from $-SR'_1$, $S(=O)R'_1$, OR'_1 , NHR'_1 , R'_1 and halo; R'_1 is alkyl, cycloalkyl, aryl or acyl; W'_1 and W'_2 are each independently selected from H,



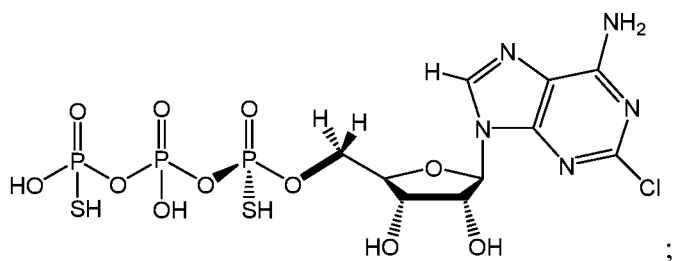
alkyl and acyl; and B is absent or is C(L')_2 , wherein L' is halo; X_4 and X_5 are each independently -OH or -SH; and j and i are each independently an integer of 0, 1 or 2, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein A is H.
3. The compound of claim 1 or 2, wherein Y_1 , Y_2 and Y_3 are each O.
4. The compound of any one of claims 1-3, wherein m is 0.
5. The compound of any one of claims 1-4, wherein X_1 is -SH.
6. The compound of claim 5, wherein X_2 is -OH.
7. The compound of any one of claims 1-6, wherein Z is $-\text{SR}_1$.
8. The compound of any one of claims 1-7, wherein Z is $-\text{SR}_1$ and R_1 is alkyl.
9. The compound of claim 8, wherein R_1 is methyl.
10. The compound of any one of claims 4-8, wherein the curled lines denote an Rp configuration.
11. The compound of any one of claims 1-9, wherein W_1 and W_2 are each H.
12. The compound of claim 1, being selected from:

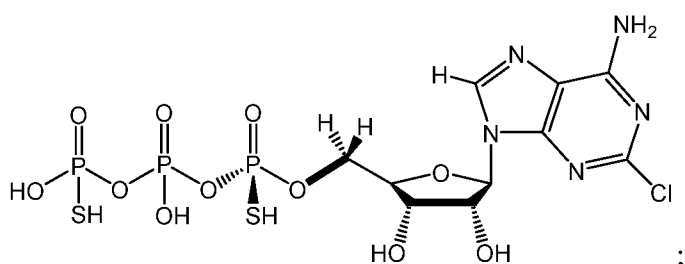




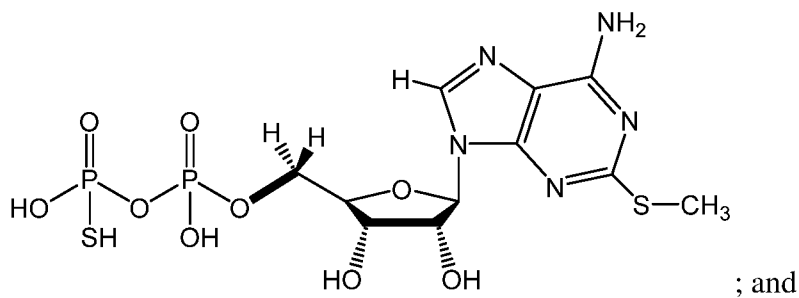
;



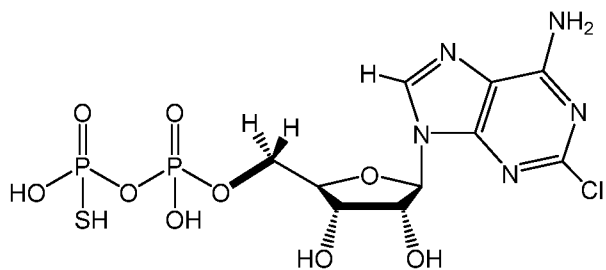
;



;



; and

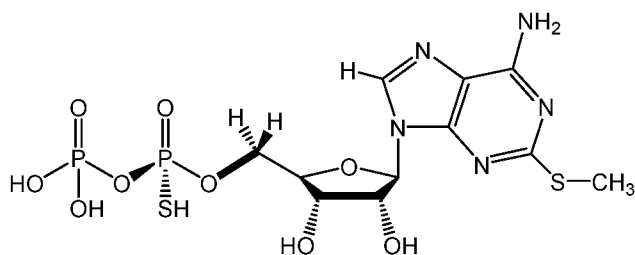


,

86

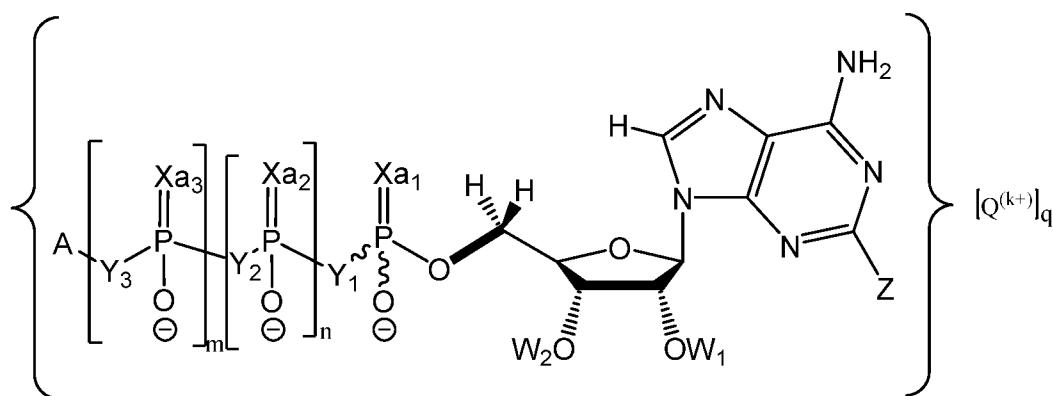
and pharmaceutically acceptable salts thereof.

13. The compound of claim 1, being:



or a pharmaceutically acceptable salt thereof.

14. The compound of claim 1, being a pharmaceutically acceptable salt represented by Formula I*a:

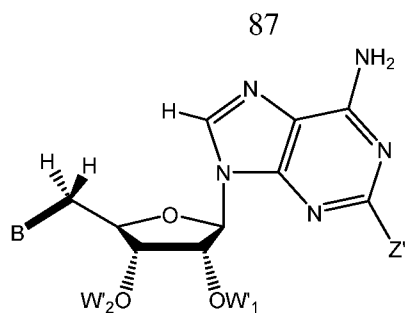


Formula I*a

wherein:

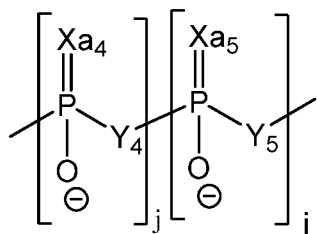
Y_1 , Y_2 , Y_3 , W_1 , W_2 , Z , n , m and the curled lines are as defined for Formula I;

Xa_1 , Xa_2 and Xa_3 are each independently S (sulfur) or O (oxygen), at least one of Xa_1 , Xa_2 and Xa_3 being S (sulfur);



A is absent or is

wherein Z' , W'_1 and W'_2 are as defined for Formula I; and B is absent or is



, wherein Y_4 and Y_5 , i and j are as defined for Formula I, and Xa_4 and Xa_5 are each independently O (oxygen) or S (sulfur);

Q is a cation, k is the cation valence, and q is the number of cations, whereby q corresponds to k and is such that the number of cations provide for a positive charge that is equal to the number of negatively charged groups in the compound.

15. The compound of claim 14, wherein $Q^{(k+)}$ is selected from Na^+ , K^+ , Li^+ , and Cs^+ .

16. A pharmaceutical composition comprising the compound of any one of claims 1-13, and a pharmaceutically acceptable carrier.

17. The compound of any one of claims 1-13 or the composition of claim 16, for use in activating $hP2Y_1$ receptor in a subject in need thereof.

18. The compound of any one of claims 1-13 or the composition of claim 16, for use in treating a medical condition treatable by activating $hP2Y_1$ receptor.

19. The compound or composition of claim 17, wherein said medical condition is treatable also by activating $hP2Y_{11}$ and/or $hP2Y_{12}$ receptor.

20. The compound of any one of claims 1-13 or the composition of claim 16, for use in the treatment of a medical condition associated with oxidative stress.

21. The compound of any one of claims 1-13 or the composition of claim 16, for use in the treatment of a neurodegenerative disease or disorder.

22. The compound of any one of claims 1-13 or the composition of claim 16, for use in the treatment of Alzheimer's disease.

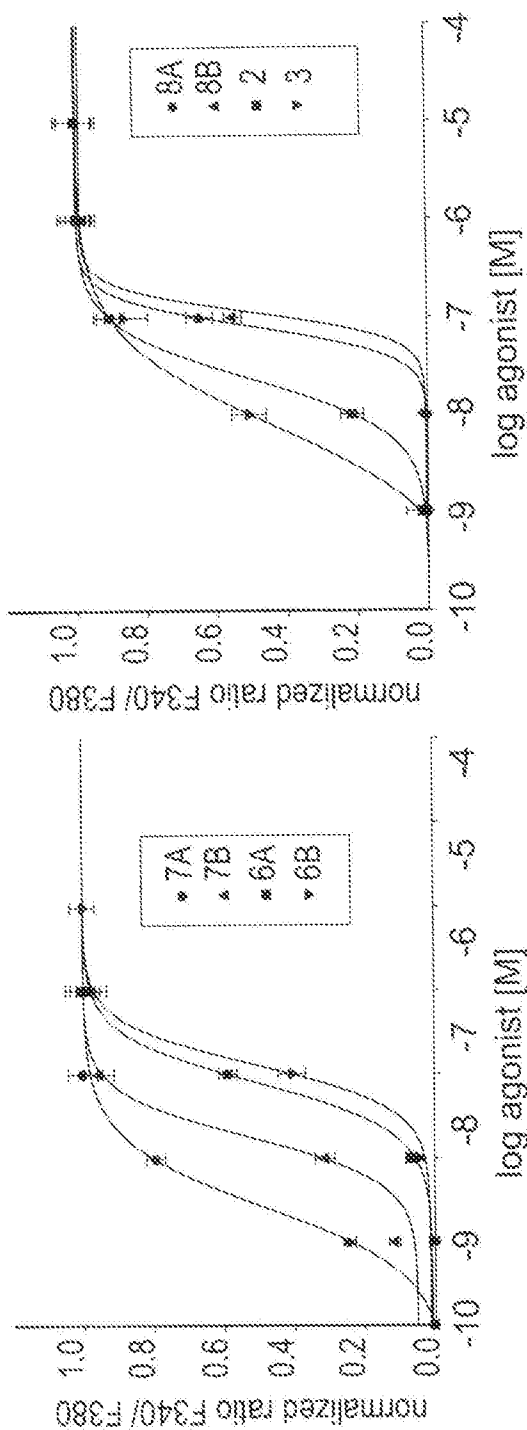


FIG. 1B

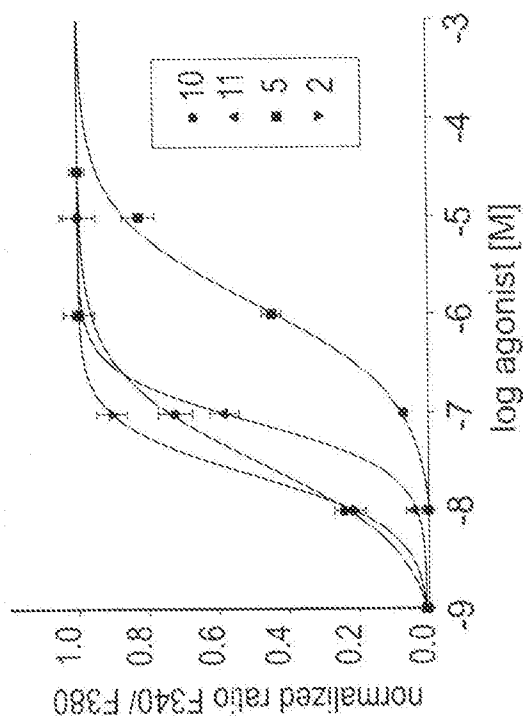
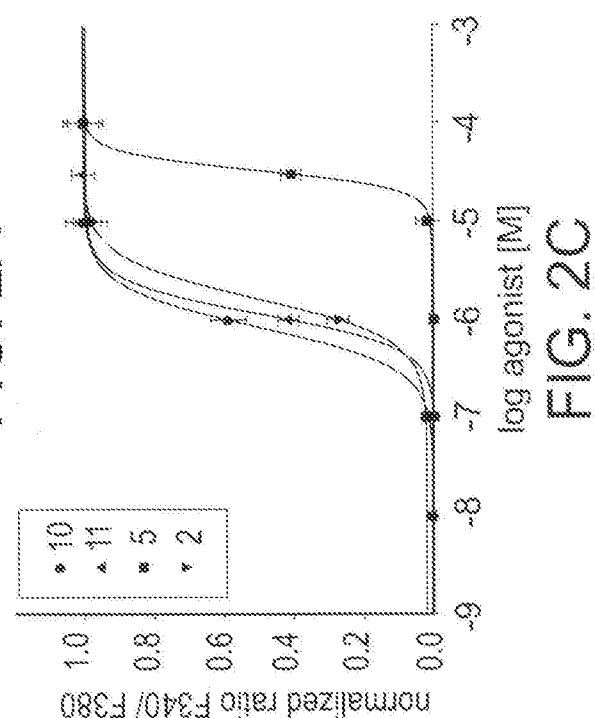
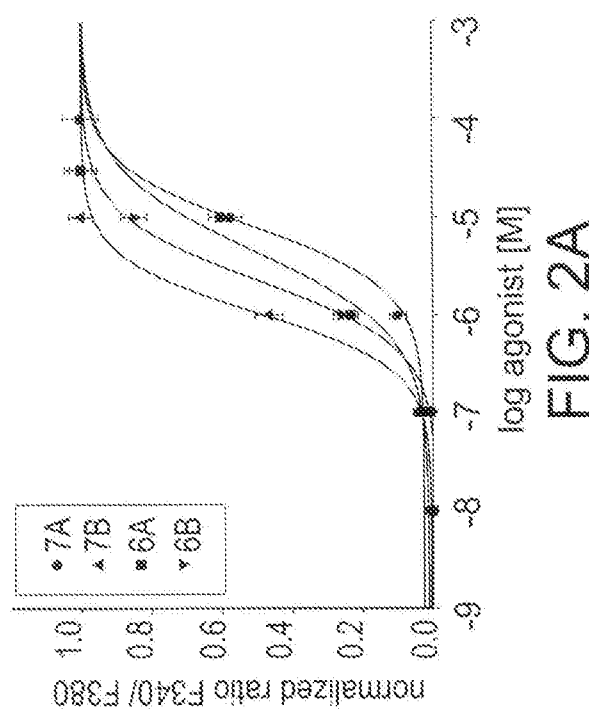
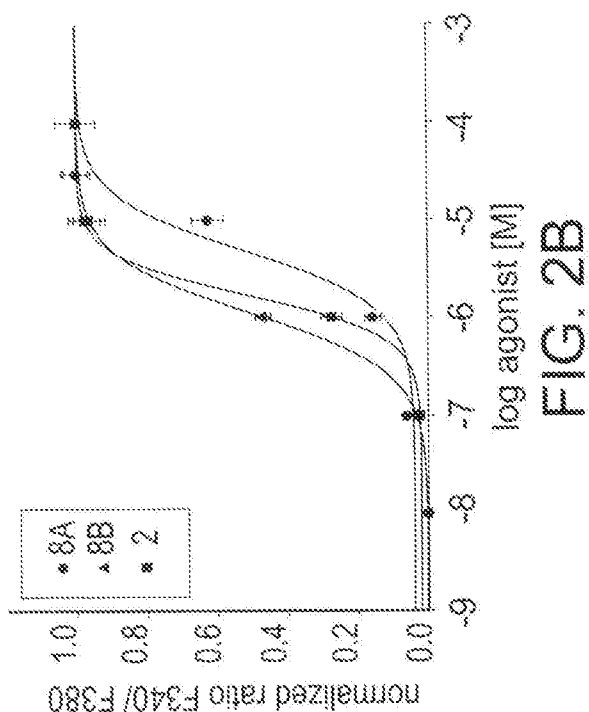


FIG. 1C



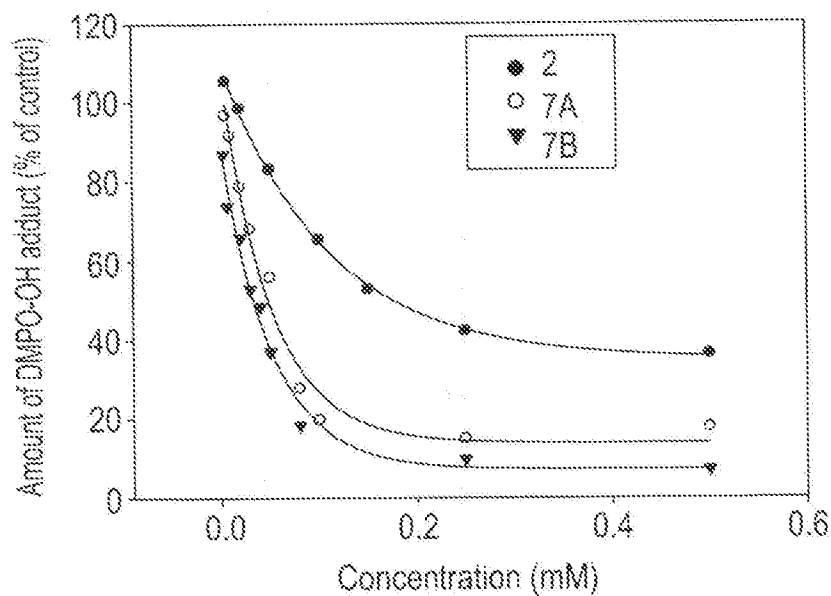


FIG. 3

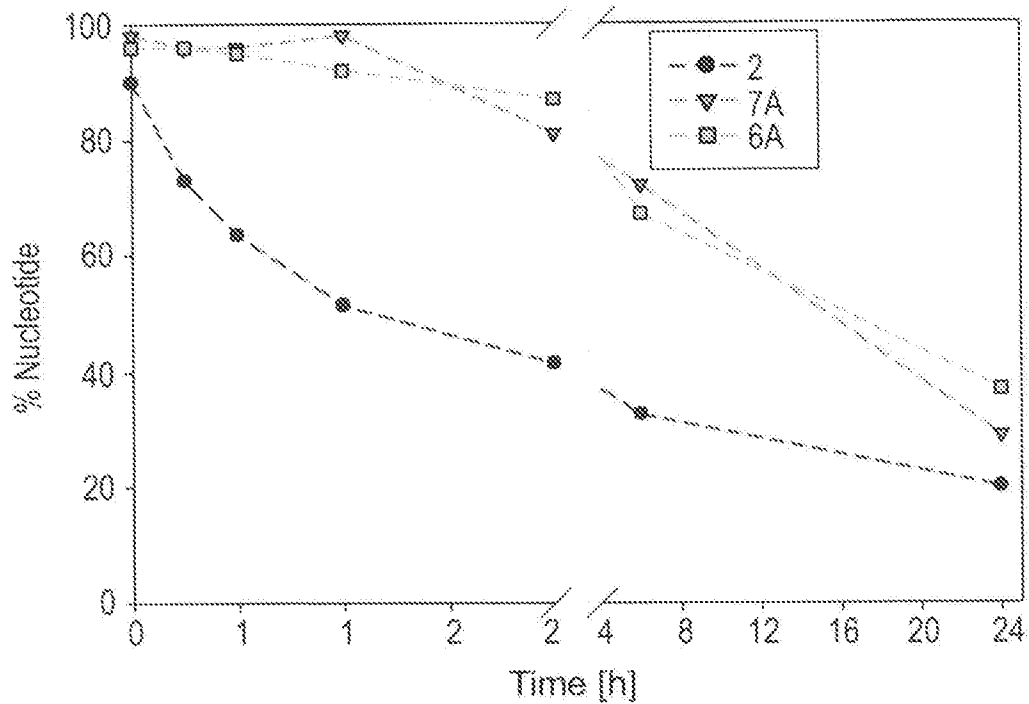


FIG. 4

FIG. 5A

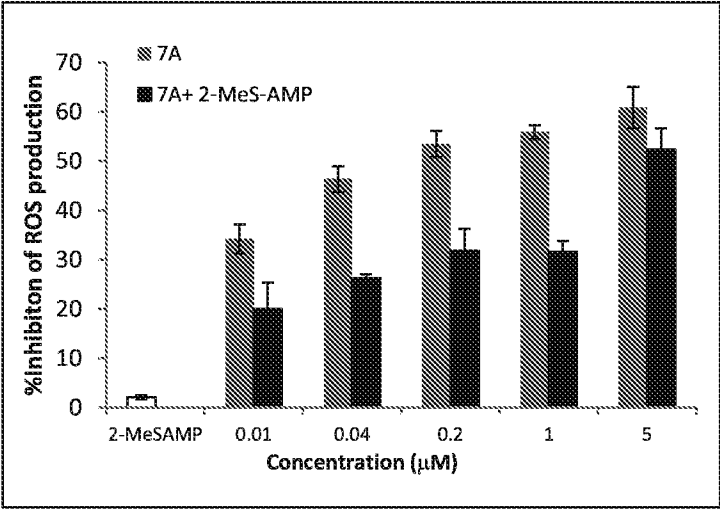
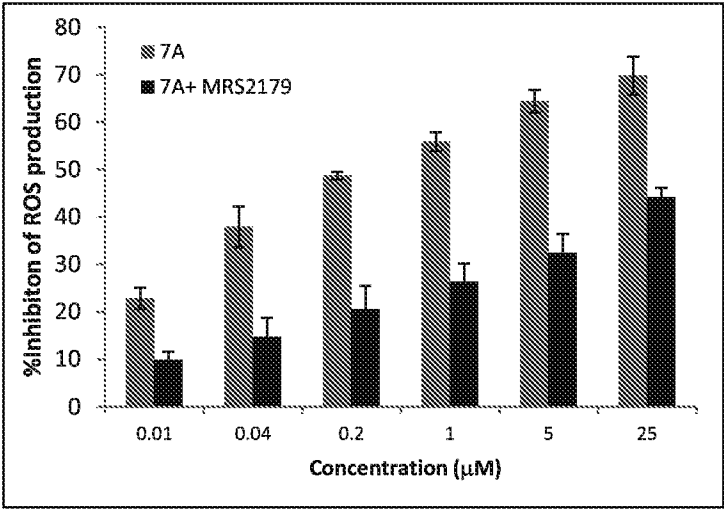


FIG. 5B



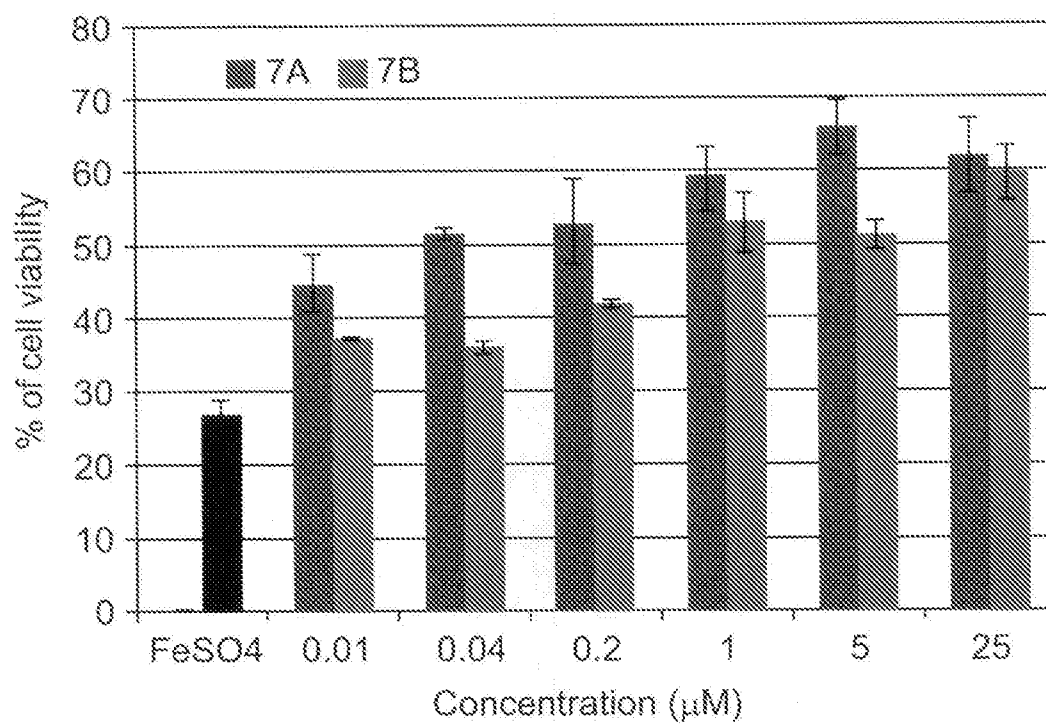


FIG. 6

FIG. 7A

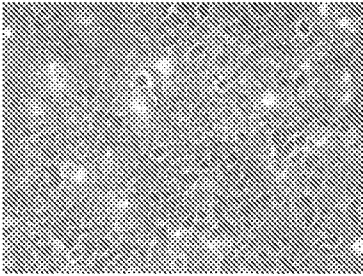


FIG. 7B

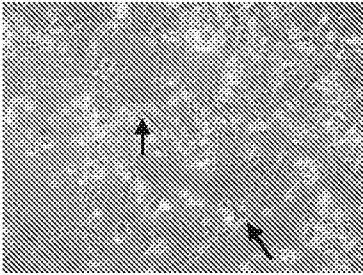
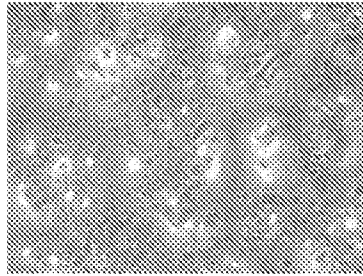


FIG. 7C

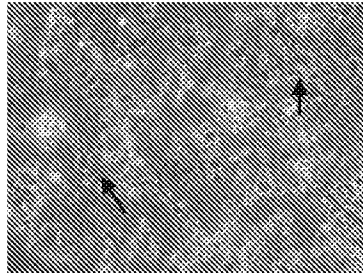


FIG. 7D

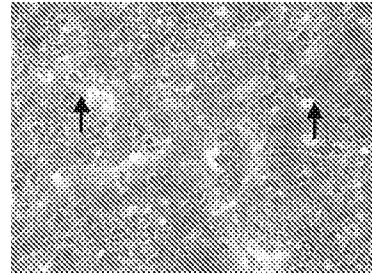


FIG. 7E

7/16

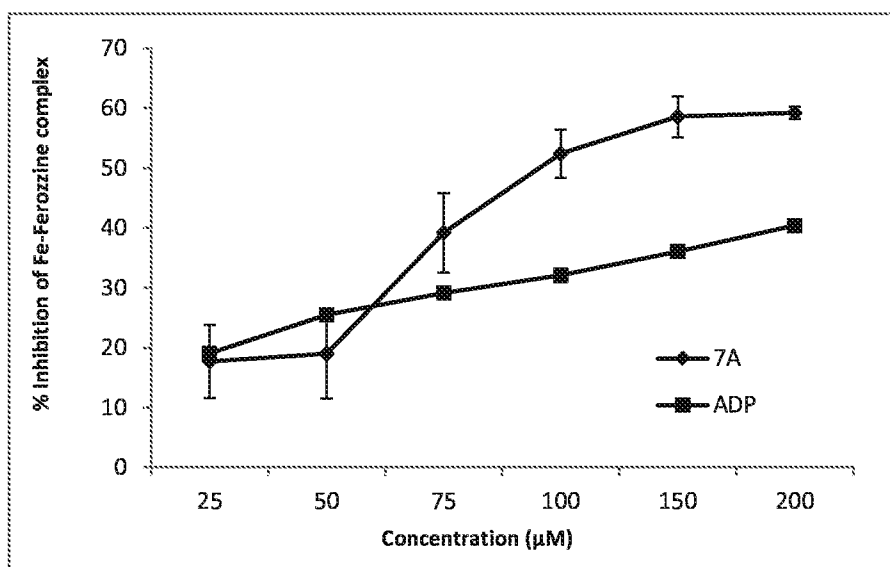


FIG. 8

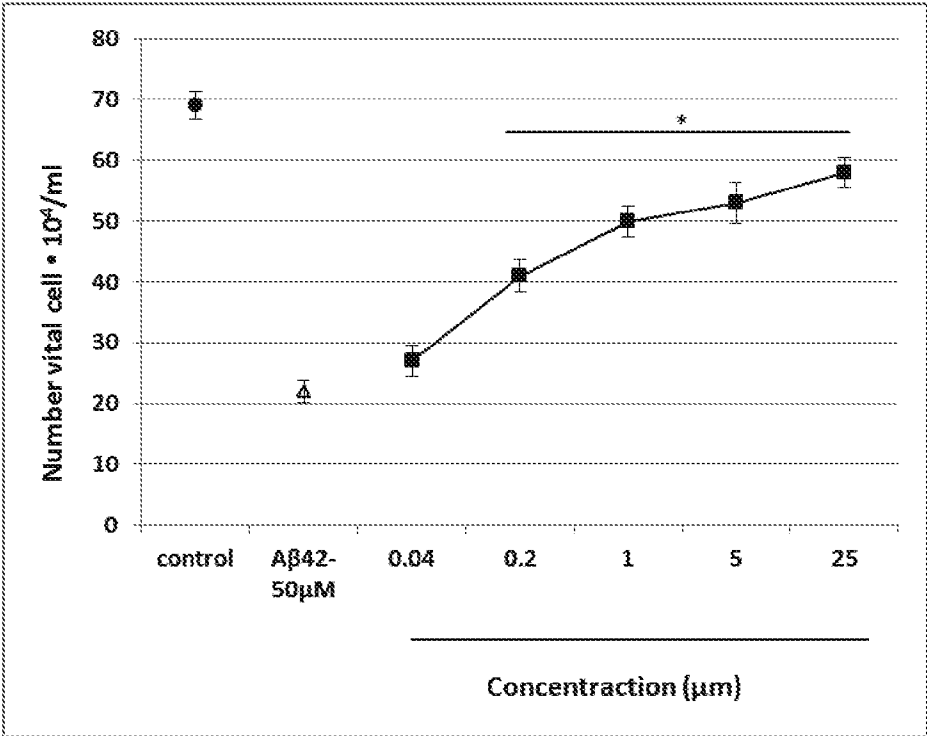


FIG. 9A

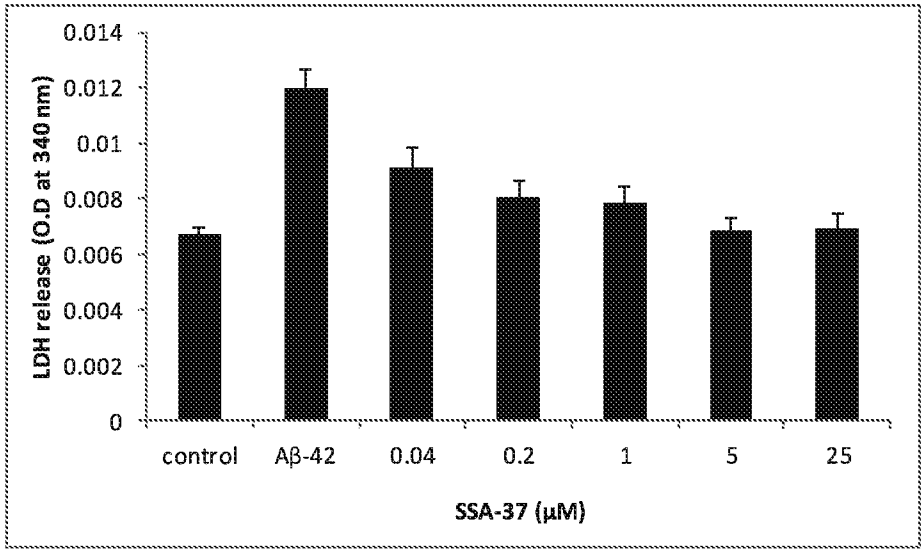


FIG. 9B

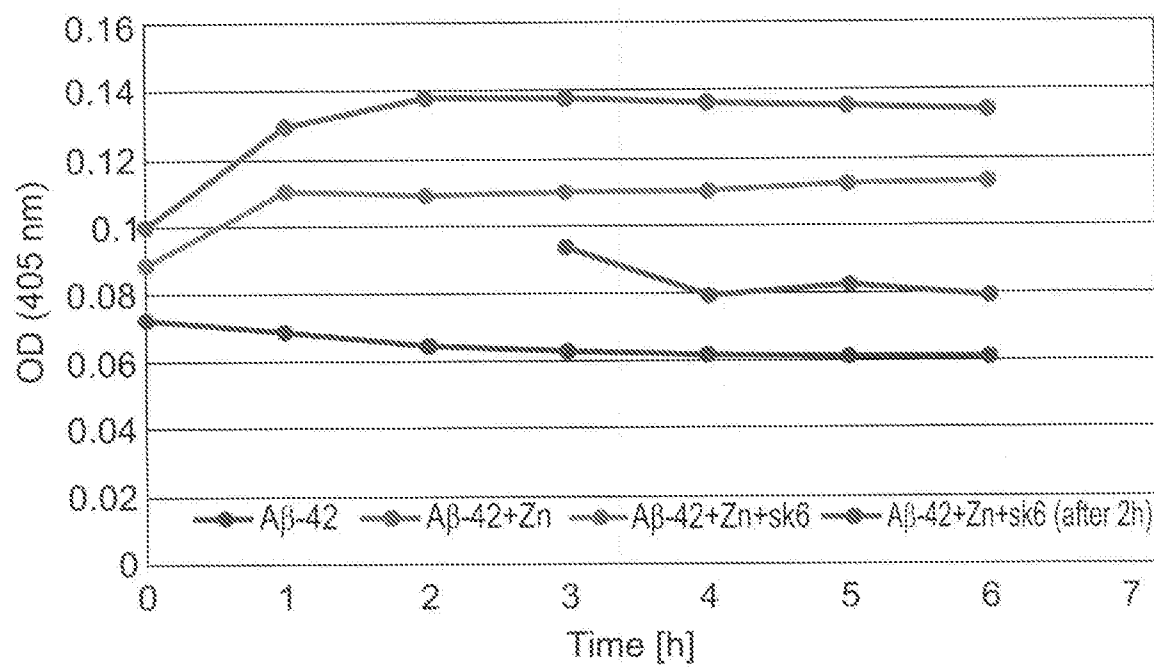


FIG. 9C

FIG. 10A

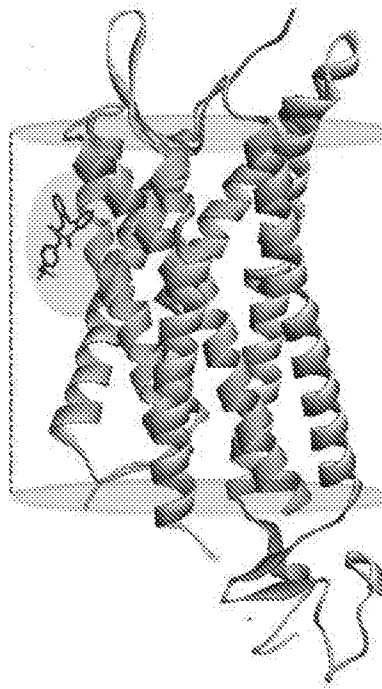


FIG. 10B

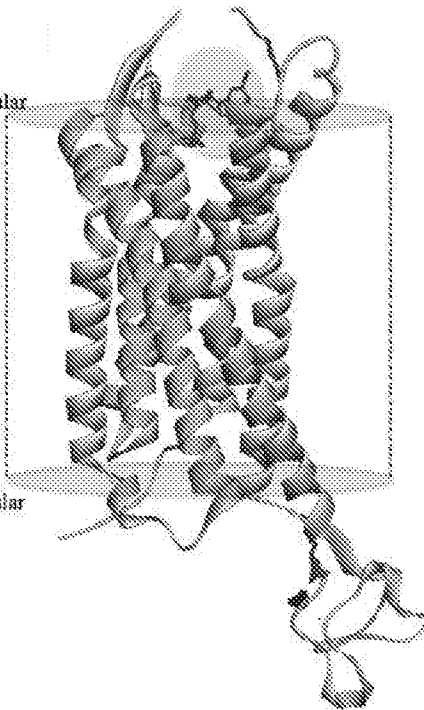


FIG. 10C

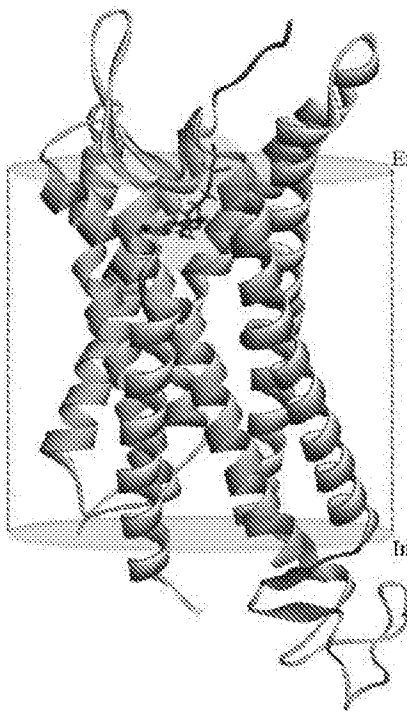
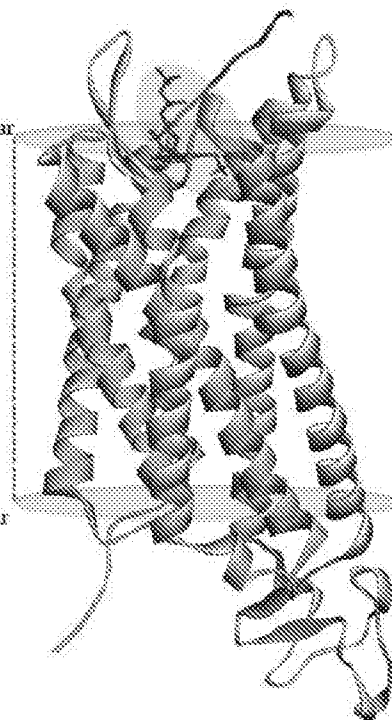
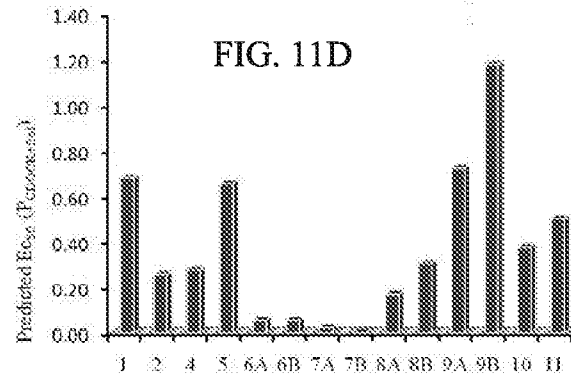
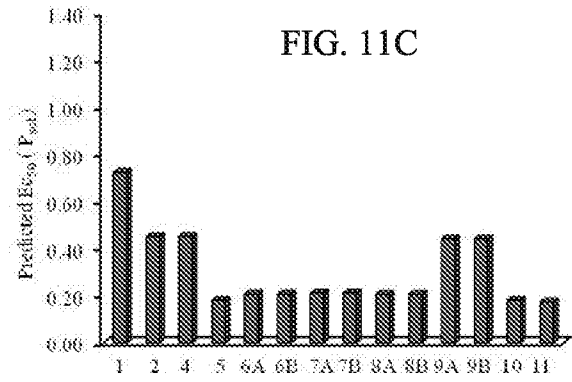
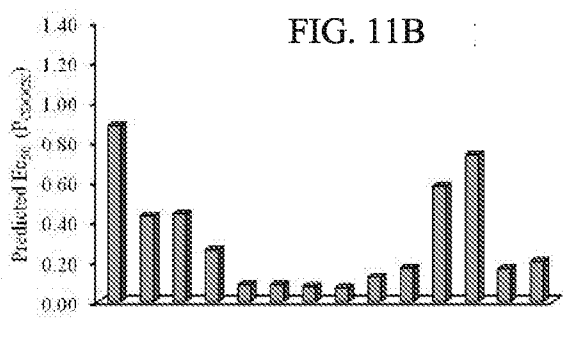
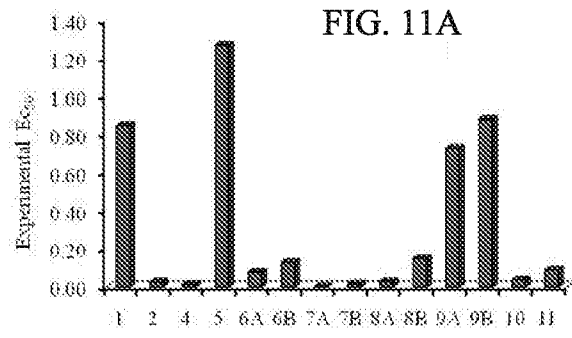


FIG. 10D





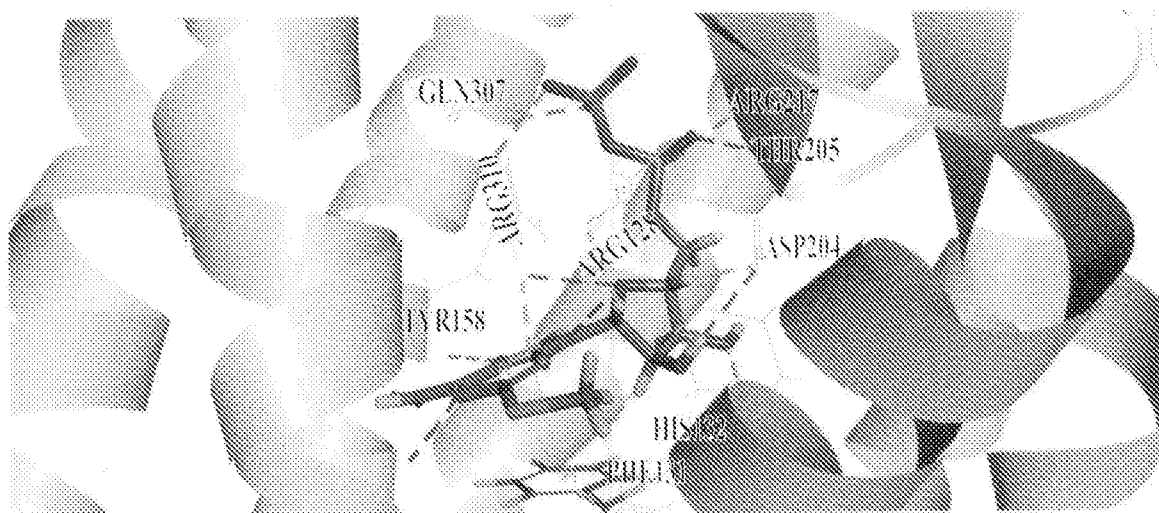


FIG. 12A

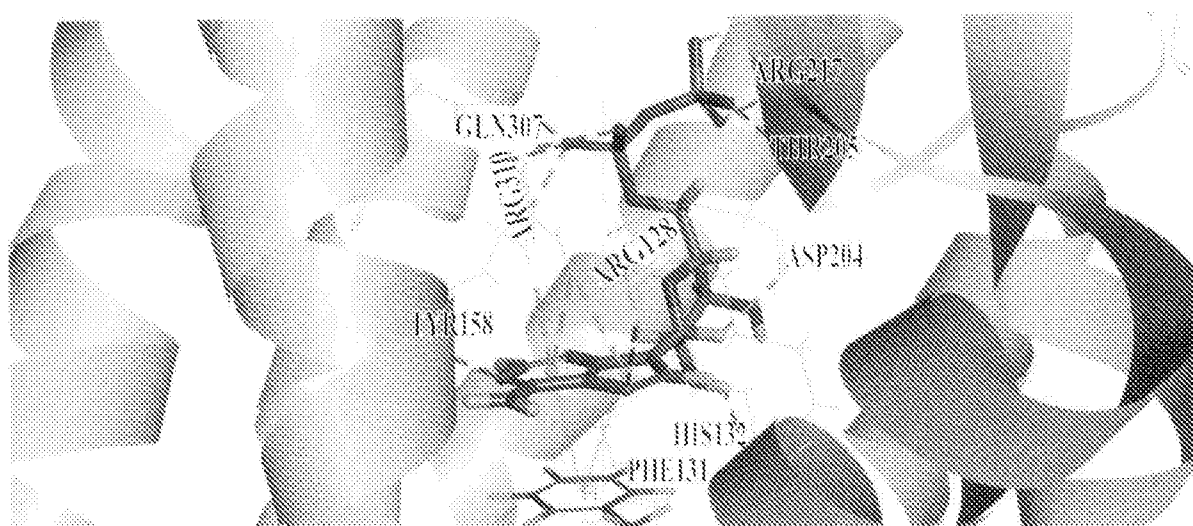


FIG. 12B

13/16

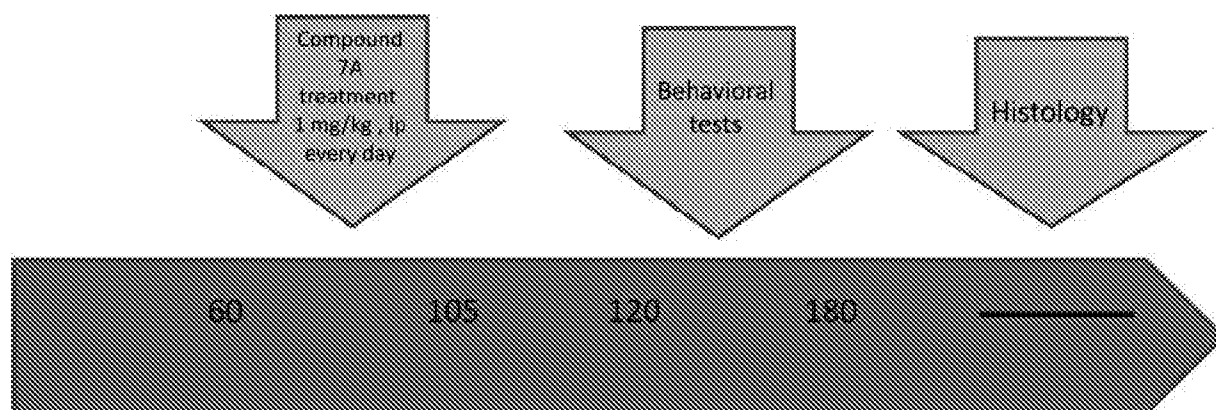


FIG. 13

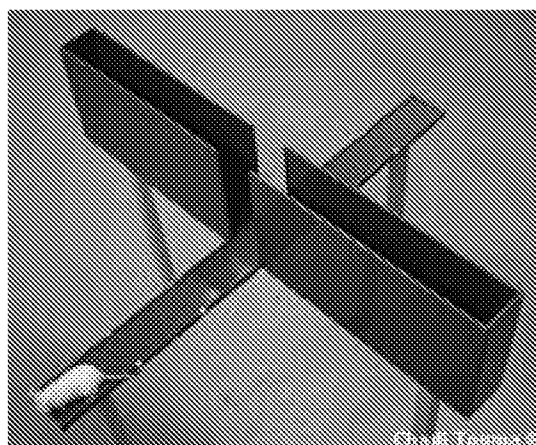


FIG. 14A

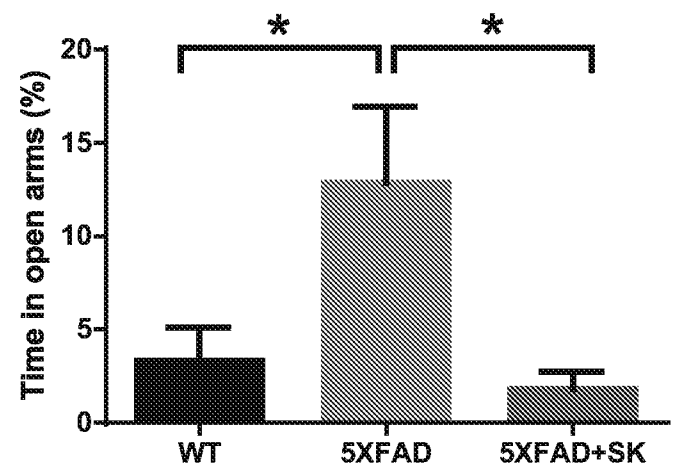


FIG. 14B

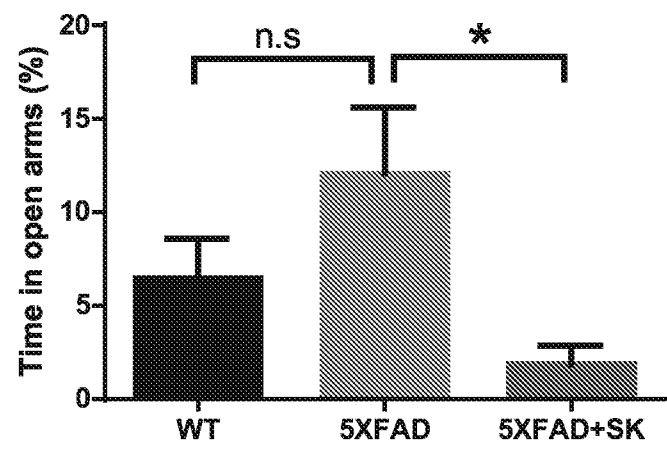


FIG. 14C

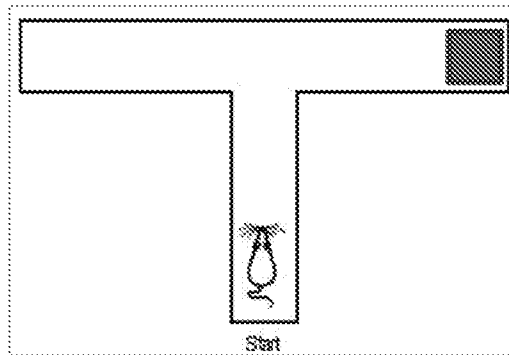


FIG. 15A

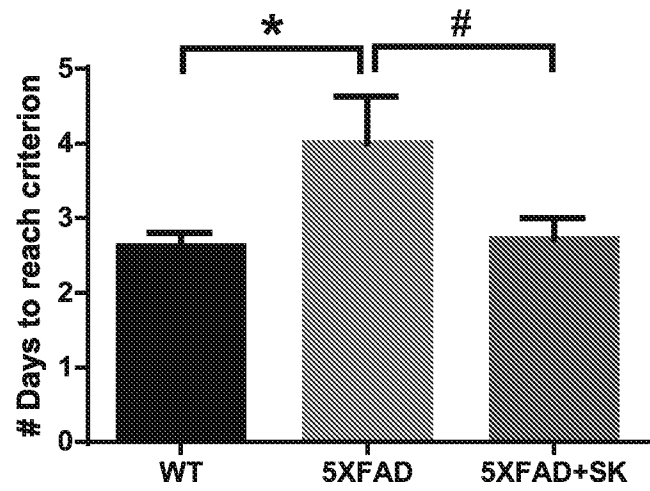


FIG. 15B

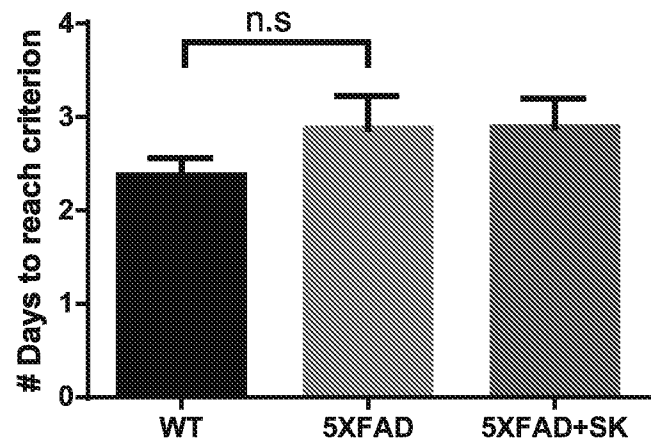


FIG. 15C

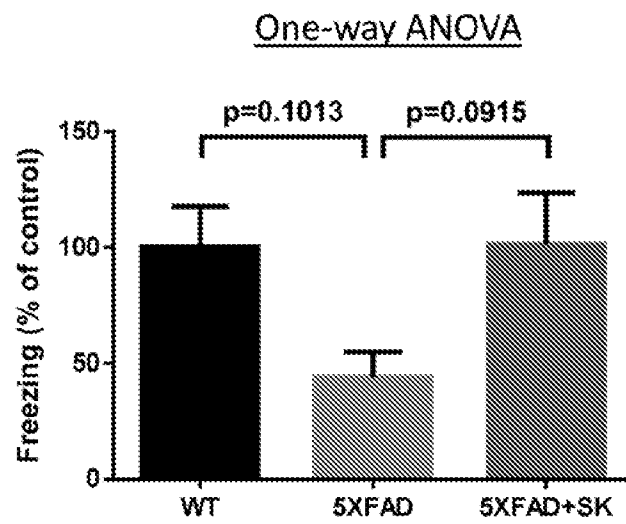


FIG. 16A

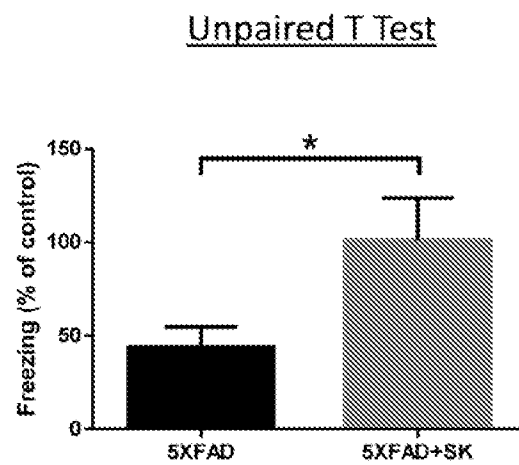


FIG. 16B

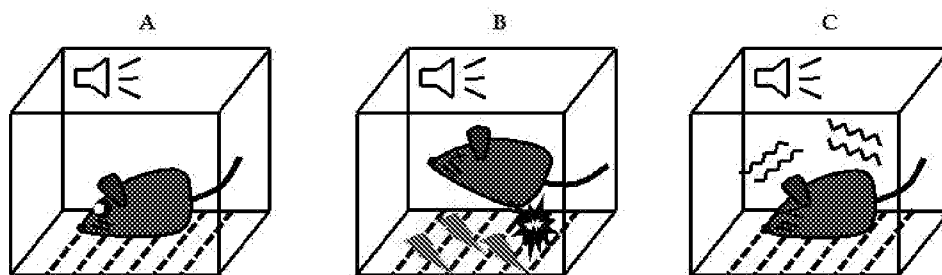


FIG. 16C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2016/050288

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2016.01) C07D 405/14, C07D 473/24, A61K 31/66, C07D 473/16, C07D 473/34, C07D 473/40, A61K 31/52, A61P 25/28, A61P 39/00, A61P 39/04, C07F 9/02, C07H 19/167, C07H 19/20, C07H 21/02, A61K 31/707600

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

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

Databases consulted: THOMSON INNOVATION, Google Patents, CAPLUS, MARPAT, REGISTRY, Google Scholar

Search terms used: ATP, ADP, AMP, analog, thiolated, phosphorothioate, thiophosphate, P2Y1, purinergic receptors, antioxidant, oxidative stress, neuroprotectant, neurodegeneration, Alzheimer's

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Major, D. T. et al. Molecular Recognition in Purinergic Receptors. 2. Diastereoselectivity of the h-P2Y1-Receptor. J. Med. Chem. (03 Aug 2004), Vol. 47, No. 18, pages: 4405-4416, 03 Aug 2004 (2004/08/03) Especially pages 4405, 4406, 4409 and 4413 (compounds 9 and 10, their h-P2Y1-R activity and salts thereof).	1-3,5-11,14,16-18
Y	Whole document.	1-22
X	Fischer, B. et al. 2-Thioether 5'-O-(1-Thiotriphosphate)adenosine Derivatives as New Insulin Secretagogues Acting through P2Y-Receptors. J. Med. Chem. (17 Aug 1999), Vol. 42 No. 18, pp. 3636-3646 17 Aug 1999 (1999/08/17) Page 3637 scheme I, page 3641 Table I, page 3643 and page 3644 (compounds 4a, 5aA and 5aB and salts thereof).	1-8,10,11,14-18
Y	Whole document.	1-22

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search

25 May 2016

Date of mailing of the international search report

31 May 2016

Name and mailing address of the ISA:

Israel Patent Office

Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel

Facsimile No. 972-2-5651616

Authorized officer

Pinchover Miriam

Telephone No. 972-2-5651642

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2016/050288

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Cusack, N. J. and Hourani, S.M.O. Effects of RP and SP Diastereoisomers of Adenosine 5'-O-(1-Thiodiphosphate) on Human Platelets. Br. J. Pharmac. (1981), Vol. 73, No. 2, pages: 409-412. Retrieved from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5381.1981.tb10437.x/pdf>. 31 Dec 1981 (1981/12/31) Pages 409-411 (see compounds (R)-ADP-alpha-S and (S)-ADP-alpha-S).	12,16
X	Haas, M. et al. Sp-2-propylthio-ATP-alpha-B and Sp-2-propylthio-ATP-alpha-B,beta-gamma-dichloromethylene are novel potent and specific agonists of the human P2Y11 receptor. Biochem. Pharmac. (25 June 2013), Vol. 86, No. 5, pages: 645-655. 25 Jun 2013 (2013/06/25) See compounds 1A, 1B, 3A and 3B.	1-3,5-8,10,11,16
X	Gough, G. R. et al. New inhibitors of platelet aggregation. 5'-Phosphate, 5'-phosphorothioate, and 5'-O-sulfamoyl derivatives of 2-substituted adenosine analogs. J. Med. Chem. (1978), Vol. 21, No. 6, pp. 520-525. 31 Dec 1978 (1978/12/31) Page 521 (compounds 4b, 4e and 4f, and their ammonium salts).	1-9,11,14
X	WO 2007/020018 A1 UNIVERSITE LIBRE DE BRUXELLES [BE] 22 Feb 2007 (2007/02/22) See Table A, compounds 74, 75, 80, 81, 96-98, 104-106.	1-9,11,12,16
X	WO 2006/038865 A1 BETAGENON AB [SE] 13 Apr 2006 (2006/04/13) See Examples (such as the 13th-16th compounds on page 20) and their intermediate compounds (such as the intermediate of the 16th compound on page 20, which was also published as CAS Registry Number 882448-73-9; STN Entry Date 01 May 2006; CA Index Name: 5'-Adenylic acid, 2-chloro-, monoanhydride with phosphorothioic acid)	1-9,11,12,16
X	WO 2010/059215 A1 GLSYNTHESIS INC [US] 27 May 2010 (2010/05/27) Table 1, compounds 10, 17, 37, 39-46 and especially 57-59.	1,3-11,14-16
X	Basu, S. et al. Analysis of catalytic RNA structure and function by nucleotide analog interference mapping. Ribozymes: Methods in Molecular Biology (2012), Vol. 848, pages: 275-296. 31 Dec 2012 (2012/12/31) Page 288, compound 2FdA-alpha-S.	1-6,11
X	Suydam, I. T. and Strobel, S. A. Fluorine Substituted Adenosines As Probes of Nucleobase Protonation in Functional RNAs. J. Am. Chem. Soc. (15 Oct 2008), Vol. 130, No. 41, pages 13639-13648. Retrieved from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2633102/pdf/nihms-86346.pdf>. 15 Oct 2008 (2008/10/15) Compound 7E.	1-3,5,6,11
X	CAS Registry Number 882449-67-4; STN Entry Date 01 May 2006; CA Index Name: Adenosine, 2-methyl-, 5'-(dihydrogen phosphorothioate). 01 May 2006 (2006/05/01)	1-6,11
X	CAS Registry Number 882449-57-2; STN Entry Date 01 May 2006; CA Index Name: Adenosine, 2-methoxy-, 5'-(dihydrogen phosphorothioate). 01 May 2006 (2006/05/01)	1-6,11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2016/050288

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAS Registry Number 882448-92-2; STN Entry Date Entered STN: 01 May 2006; CA Index Name: 5'-Adenylic acid, 2-methyl-, monoanhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-4,11
X	CAS Registry Number 882448-82-0 ; STN Entry Date 01 May 2006; CA Index Name: 5'-Adenylic acid, 2-methoxy-, monoanhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-4,11
X	CAS Registry Number 882448-41-1; STN Entry Date Entered STN: 01 May 2006; CA Index Name: 5'-Adenylic acid, 2-(hexylthio)-, monoanhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-4,7,8,11
X	CAS Registry Number 882448-11-5; STN Entry Date Entered STN: 01 May 2006; CA Index Name: Adenosine 5'-(trihydrogen diphosphate), 2-methyl-, P'-anhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-3,11
X	CAS Registry Number 882448-02-4; STN Entry Date Entered STN: 01 May 2006; CA Index Name: Adenosine 5'-(trihydrogen diphosphate), 2-methoxy-, P'-anhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-3,11
X	CAS Registry Number 882447-93-0; STN Entry Date Entered STN: 01 May 2006; CA Index Name: Adenosine 5'-(trihydrogen diphosphate), 2-chloro-, P'-anhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-3,11
X	CAS Registry Number 882447-66-7; STN Entry Date Entered STN: 01 May 2006; CA Index Name: Adenosine 5'-(trihydrogen diphosphate), 2-(hexylthio)-, P'-anhydride with phosphorothioic acid. 01 May 2006 (2006/05/01)	1-3,7,8,11
X	CAS Registry Number 1227194-35-5 ; STN Entry Date 08 Jun 2010. 08 Jun 2010 (2010/06/08)	1-6,11
X	CAS Registry Number 1227194-22-0; STN Entry Date 08 Jun 2010. 08 Jun 2010 (2010/06/08)	1-8,11
X	CAS Registry Number 1227194-08-2; STN Entry Date 08 Jun 2010. 08 Jun 2010 (2010/06/08)	1-8,11
X	Ecke D. et al. Opposite diastereoselective activation of P2Y1 and P2Y11 nucleotide receptors by adenosine 5'-O-(alpha-boranotriphosphate) analogues. British Journal of pharmacology (published online: 04 Sep 2006), Vol. 149, pages: 416-423. 04 Sep 2006 (2006/09/04) Figure 1(a) and Table 1.	1-3,5-11,16-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2016/050288

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Whole document	1-22
Y	Fujita, T. et al. P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures. <i>Glia</i> (published online: 28 AUG 2008), Vol. 57, No. 3, pages: 244-257. 28 Aug 2008 (2008/08/28) Whole document.	1-22
P,X	Azran, S. et al. Identification of Highly Promising Antioxidants/Neuroprotectants Based on Nucleoside 5'-Phosphorothioate Scaffold. Synthesis, Activity, and Mechanisms of Action. <i>J. Med. Chem.</i> (08 Oct 2015), Vol. 58, No. 21, pages: 8427-8443. 08 Oct 2015 (2015/10/08) Whole document	1,3-6,11,16-21
P,Y	Whole document.	1,3-11,14-22
P,X	Yanachkov, I. B. et al. New highly active antiplatelet agents with dual specificity for platelet P2Y1 and P2Y12 adenosine diphosphate receptors. <i>European Journal of Medicinal Chemistry</i> (1 Jan 2016; published online: 9 Nov 2015), Vol. 107, pages: 204-218. 09 Nov 2015 (2015/11/09)	1,3-11,14-19
P,Y		1,3-11,14-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/IL2016/050288

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A. CLASSIFICATION OF SUBJECT MATTER:

IPC (2016.01) C07D 405/14, C07D 473/24, C07D 473/34, C07D 473/40, C07D 473/16, A61K 31/66, A61K 31/52, A61P 25/28, A61P 39/00, A61P 39/04, C07H 19/167, C07H 19/20, C07H 21/02, C07F 9/02, A61K 31/707600