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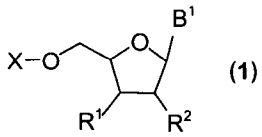
[Continued on next page]

(54) **Title:** USE OF PURINERGIC AND PYRIMIDINERGIC RECEPTOR AGONISTS FOR DENDRITIC CELLS BASED IMMUNOTHERAPIES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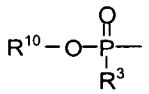
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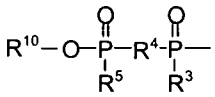
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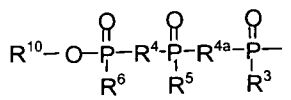
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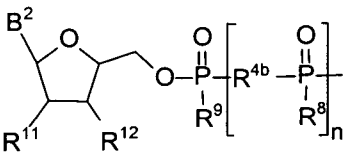
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(57) **Abstract:** The present invention relates to the use of at least one purinergic and pyrimidinergic receptor agonist of formula (I) for the preparation of a medicament for inducing immunotolerance to an antigen, wherein B^1 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, R^1 and R^2 represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (II), (III) or (IV), wherein R^{10} represents hydrogen or is of formula (VII), wherein B^2 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, wherein n is 0, 1, 2, 3 or 4, and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6, R^8, R^9 represent each independently OH or SH, and wherein R^{11} and R^{12} represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 is as defined above.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

USE OF PURINERGIC AND PYRIMIDINERGIC RECEPTOR AGONISTS FOR DENDRITIC CELLS BASED IMMUNOTHERAPIES

FIELD OF THE INVENTION

The invention relates to the field of immunology, more in particular to the field of immune therapy. In particular, the present invention relates to purinergic and pyrimidinergic receptor agonists and pharmaceutical compositions thereof useful in dendritic cells based immunotherapies.

BACKGROUND TO THE INVENTION

Dendritic cells (DCs) are antigen-presenting cells (APCs) playing a crucial role in the induction and regulation of immune responses. They are considered to be guardians of the immune system. They are in fact located almost everywhere, namely in the thymus, the systemic circulation and the secondary lymphoid organs and also in the peripheral tissues such as the skin and mucous membranes, whether they can be monostratal or of the malpighian type, i.e. comprising a multistratal epithelium, namely those of the vagina, the outer cervix, the vulva, the perianal region, the esophagus and the mouth. Although in very small numbers in the organism, dendritic cells are at the center of the triggering of specific immune responses, exerting control over the specificity, intensity and nature of the immune response, and are located at the interface of innate and acquired immunity. Apart from their function of "switching on" the immune response, dendritic cells also have a role to play in the induction of peripheral tolerance.

The expression in dendritic cells is induced directly by the biological activity of environmental substances and/or indirectly by the reactivity products (stress and inflammatory mediators, necrotic cells) generated in response to these substances by other cells within the APC tissue micro-environment.

In response to danger signals like proinflammatory cytokines such as TNF-α and IL-1β, pathogen-related molecules (LPS, double-stranded RNA, bacterial DNA), and T cell-derived signals such as CD40 ligand, immature dendritic cells undergo maturation. Maturation of dendritic cells induces the loss of endocytosis, the surface expression of stable MHC-peptide complexes and costimulatory molecules (CD80, CD86) and the production of cytokines like IL-12 (Banchereau J et al. Annu Rev Immunol. 2000;18:767-811). Maturation is also accompanied by a shift in the expression of chemokines and their receptors allowing dendritic cells migration to lymphoid organs. High secretion of IL-12 by mature dendritic cells induces differentiation of $CD4^+$ T cells into Th1 cells secreting IFN-γ whereas low IL-12 release combined with IL-10 production induces a Th2 response or a T regulatory response associated with induced tolerance (Banchereau J et al. Annu Rev Immunol. 2000;18:767-811; Akbari O et al. Nat Immunol. 2001;2:725-731) . DCs exposed to ATP show a reduced ability to attract Th1 lymphocytes and an increased capacity to promote a Th2 response (La Sala et al. Blood. 2002;99;5:1715-1722). This Th1/Th2 shift however can be linked to immediate hypersensitivity and anaphylactic reactions.

Increasing number of reports provide strong evidence that APCs, and particularly dendritic cells, are also involved in central and peripheral tolerance (Moser M. Immunity. 2003;19:5-8; Shortman K, Heath WR. Nat Immunol. 2001;2:988-989). This tolerogenicity seems to be mediated by both specialized regulatory dendritic cells and dendritic cells undergoing semi-maturation.

Approaches to treat autoimmune and allergic diseases have been traditionally focused on immunosuppressive agents that block the activation and expansion of T cells. In particular, WO 03/028712 discloses the use of purinergic and pyrimidinergic receptor agonists for inhibition of CD4+ T lymphocytes activation and as immunosuppressive agents. These therapies are effective but require continuous use because they do not redirect the immune system to a state of tolerance. This continuous treatment leads to long-term toxicity and suppresses protective immune responses against infection. Therapeutic vaccination constitutes an alternative approach, characterized by its specificity for one or several specific antigens. The objective of therapeutic vaccination is to obtain one of the following outcomes: specific T cell ablation, specific T cell anergy, induction of regulatory T cells, shift of specific T cell phenotype from Th1 to Th2 (in case of rheumatoid arthritis, diabetes or multiple sclerosis...) or from Th2 to Th1 (in case of asthma and other allergic diseases). US Pat. Application 2005/0053612 describes the use of purine receptor antagonists to diminish or decrease an undesirable immune response and describes the use of purine receptor agonists such as ATP and ATPγS to stimulate the immune system.

Several strategies have been attempted in order to obtain vaccination that leads to tolerance rather than the usual Th2 or Th1 response: such as administration in non-inflammatory manner, which is without any adjuvant, administration by a specific route, in particular oral administration and structural modifications of the antigen.

However, there remains a need for alternative or improved strategy to prevent or treat autoimmune and allergic diseases.

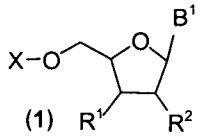
It is an object of the present invention to provide effective compositions for dendritic cells based immunotherapies and in particular for the induction of immunotolerance in the prevention or treatment of inflammatory, allergic, autoimmune diseases and graft/transplantation rejection. It is another object to provide with agents for inducing immune tolerance to an antigen. It is yet another object to provide agents for improving the effectiveness of tolerogenic vaccines.

SUMMARY OF THE INVENTION

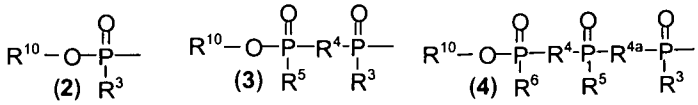
The present invention provides an alternative strategy which involves the administration of antigen(s) with a particular kind of adjuvant that orient the immune response towards tolerance. According to the present invention, nucleotides, in particular agonists of the $P2Y_{11}$ receptor, constitute such an adjuvant, via their specific action on dendritic cells.

Accordingly, in one aspect, the present invention relates to the use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof for the

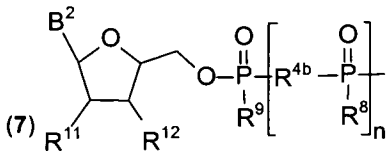
preparation of a medicament for inducing immunotolerance to an antigen whereby the individual is immunized with an antigen in combination with the agonist of formula (1)



wherein B^1 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, R^1 and R^2 represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



wherein R^{10} represents hydrogen or is of formula (7),



wherein B^2 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, wherein n is 0, 1, 2, 3 or 4, and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6, R^8, R^9 represent each independently OH or SH, and wherein R^{11} and R^{12} represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 is as defined above.

The antigen may be present in the body already, as is the case with some allergic diseases. In that case, stimulation and/or upregulation of the production of thrombospondin-1 in dendritic cells with the purinergic and pyrimidinergic receptor agonists of formula (1) alone will enhance the induction of tolerance. Therefore, in another aspect, the present invention relates to the use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) as defined above for the preparation of a medicament for inducing immunotolerance.

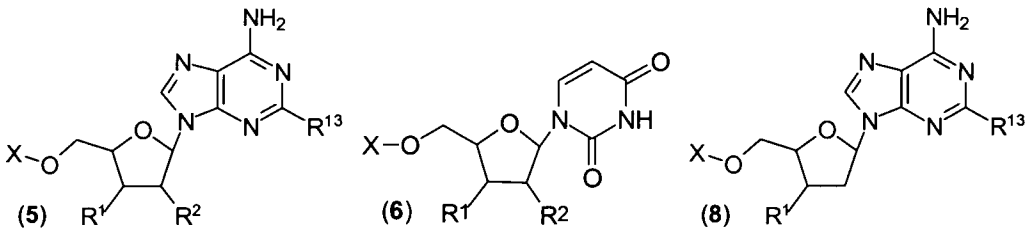
In a further aspect, the present invention relates to the use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) as defined above for the preparation of a medicament for inducing immunotolerance to an antigen in an individual whereby said agonist of formula (1) is administered to an individual which has previously been exposed to an antigen.

In a particular embodiment, said immunotolerance is being induced to prevent or treat autoimmune diseases, allergic diseases and/or transplant rejection and graft-versus-host disease.

In an embodiment, the purinergic and pyrimidinergic receptor agonists of formula (1) are used for the preparation of a tolerogenic vaccine for inducing immunotolerance to an antigen. Said vaccine suitable for immunotolerance therefore comprises at least one compound of formula (1) and

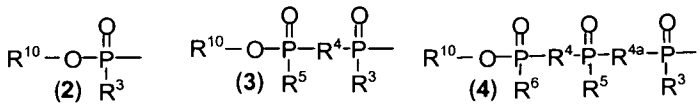
optionally at least one antigen. Preferably, said vaccine comprises at least one compound of formula (1) and at least one antigen. Preferably, said vaccine is administered only once, twice or thrice, preferably only once.

According to a particular embodiment, said purinergic or pyrimidinergic receptor agonist is of formula (5), (6) or (8) or a pharmaceutically acceptable salt thereof,



wherein R^1, R^2 and X have the same meaning as defined above and R^{13} is selected from hydrogen, thioalkyl, thioaryl or thioalkylaryl.

According to a particular embodiment, said purinergic or pyrimidinergic receptor agonist is of formula (5), (6) or (8) wherein R^{13} is selected from hydrogen, thiomethyl, thiobutyl, thiopentyl, thiophenyl or thiobenzyl, and R^1 and R^2 are each independently selected from OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



wherein R^{10} represents hydrogen and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6 represent each independently OH or SH.

Interesting purinergic receptor agonists of formula (1) are selected from the group comprising ATP, adenosine 5'-O-(3-thiotriphosphate) (ATPγS), 2'- and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP), 2-propylthio-β,γ-dichloromethylene-D-ATP, 2-methylthio-ATP (2-MeSATP), ADP, 2-methylthio-ADP (2-MeSADP), adenosine 5'-O-(2-thiodiphosphate) (ADPβS), P^1,P^3-di(adenosine-5')triphosphate (Ap_3A), P^1,P^4-di(adenosine-5')tetraphosphate (Ap_4A), P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A), P^1,P^6-di(adenosine-5')hexaphosphate (Ap_6A), a deoxyderivative thereof or a pharmaceutically acceptable salt thereof.

According to a particular embodiment, said purinergic or pyrimidinergic receptor agonist of formula (1) is selected from the group of compounds 1 to 332 as depicted in tables A, B or C. Preferably said agonist of formula (1) is a purinergic receptor agonist of formula (1) wherein B^1 is selected from the group comprising adenine guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl.

According to an embodiment, said antigen is selected from the group comprising antigens responsible of asthma, including bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, antigens responsible of allergic rhinitis, antigens responsible of

conjunctivitis, antigens responsible of keratitis, antigens responsible of keratoconjunctivitis, antigens responsible of uveitis, antigens responsible of psoriasis, antigens responsible of eczema, antigens responsible of atopic dermatitis, antigens responsible of contact dermatitis, antigens responsible of cutaneous T cell lymphoma (CTCL), antigens responsible of Sezary syndrome, antigens responsible of pemphigus vulgaris, antigens responsible of bullous pemphigoid, antigens responsible of pemphigus foliaceus, antigens responsible of dermatomyositis, antigens responsible of erythema nodosum, antigens responsible of scleroderma, antigens responsible of Bechet's disease, antigens responsible of sarcoidosis, antigens responsible of Sjögren's syndrome, antigens responsible of rheumatoid arthritis, antigens responsible of juvenile arthritis, antigens responsible of Reiter's syndrome, antigens responsible of lupus erythematosus, antigens responsible of polymyositis, antigens responsible of myocarditis, antigens responsible of primary biliary cirrhosis, antigens responsible of Crohn's disease, antigens responsible of ulcerative colitis, antigens responsible of multiple sclerosis or other demyelinating diseases, antigens responsible of idiopathic thrombocytopenic purpura, antigens responsible of Graves' disease or Hashimoto's disease, antigens responsible of Addison's disease, antigens responsible of insulin-dependent diabetes mellitus (type 1), or antigens responsible of transplant rejection or graft-versus-host disease.

In a particular embodiment, said antigen is selected from the group comprising antigens responsible of autoimmune disease selected from psoriasis, rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus (type 1), of antigens responsible of allergic disease selected from bronchial asthma and atopic dermatitis, or antigens responsible of transplant rejection and graft-versus-host disease.

In another aspect, the present invention relates to a pharmaceutical composition comprising at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined above, a pharmaceutically acceptable carrier and at least one antigen.

In particular, the present invention relates to tolerogenic vaccine comprising at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined above as an adjuvant for inducing immune tolerance to an antigen. In a particular embodiment, said tolerogenic vaccine further comprises an antigen.

In yet another aspect, the present invention relates to a method to stimulate the production of TSP-1 in dendritic cells comprising submitting said cell to at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined above.

In particular, the present invention relates to a method of inducing immunotolerance to an antigen comprising administering to an individual in need thereof, a therapeutically effective amount of a purinergic or pyrimidinergic receptor agonist of formula (1) as defined above. In a particular embodiment, said method comprises stimulating the production of TSP-1 by dendritic cells using at least one purinergic and pyrimidinergic receptor agonists of formula (1). Said administration may be by oral, and/or parenteral, and/or intranasal, and/or dermal administration.

According to an embodiment, the method of the present invention comprises co-administering an antigen. In a particular embodiment, said co-administration is sequential. In another particular embodiment, said co-administration is simultaneous.

Compared to conventional antigen vaccination, these combination methods offer significant advantages, such as (i) better efficacy leading to stronger reduction of symptoms, (ii) reduction of the need for drugs, in particular glucocorticoids, (iii) prevention of the progression into more severe disease, (iv) faster onset of beneficial effects leading to shorter treatment period (v) use of lower amounts of antigen and (vi) less unwanted side effects.

In preferred embodiments, said purinergic or pyrimidinergic receptor agonist of formula (1) are used as adjuvant for inducing immune tolerance to antigens responsible of and/or triggering immune disease, wherein non limiting examples of said immune diseases triggered by said antigens include asthma including bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, allergic rhinitis, conjunctivitis, keratitis, keratoconjunctivitis, uveitis, psoriasis, eczema, atopic dermatitis, contact dermatitis, cutaneous T cell lymphoma (CTCL), Sezary syndrome, pemphigus vulgaris, bullous pemphigoid, pemphigus foliaceus, dermatomyositis, erythema nodosum, scleroderma, Bechet's disease, sarcoidosis, Sjögren's syndrome, rheumatoid arthritis, juvenile arthritis, Reiter's syndrome, lupus erythematosus, polymyositis, myocarditis, primary biliary cirrhosis, Crohn's disease, ulcerative colitis, multiple sclerosis and other demyelinating diseases, idiopathic thrombocytopenic purpura, Graves' disease and Hashimoto's disease, Addison's disease, insulin-dependent diabetes mellitus (type 1), transplant rejection or graft-versus-host disease.

Preferably said purinergic or pyrimidinergic receptor agonist of formula (1) are used as adjuvant for inducing immune tolerance fro the treatment of autoimmune disease including psoriasis, rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus (type 1), of allergic disease including bronchial asthma and atopic dermatitis, or of transplant rejection and graft-versus-host disease.

The present invention will be further disclosed in detail hereunder. Examples are given which will further support the description.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, purinergic or pyrimidinergic receptor agonist of formula (1) play a crucial role in up-regulating two targets thrombospondin-1 (TSP-1) and indoleamine 2,3-dioxygenase (IDO) involved in immunotolerance.

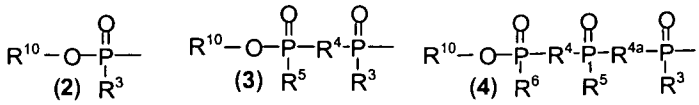
The present invention provides a method for preventing onset of an immune disease in an individual said method comprising administering to said individual an effective amount of an agent (compound of formula (1)) which selectively increases the levels of TSP-1 production by dendritic cells. Preferably, said compound of formula (1) is administered together with at least one antigen responsible of and/or triggering said immune disease. In particular the immune disease is selected

from autoimmune disease including psoriasis, rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus (type 1), allergic disease including bronchial asthma and atopic dermatitis, or transplant rejection and graft-versus-host disease. The present invention contemplates enhancing the degree of tolerogenicity in an individual, said method comprising administering to said individual a tolerogenic state-enhancing or maintaining effective amount of purinergic and pyrimidinergic receptor agonists of formula (1). More specifically, the present invention relates to adjuvant formulations for vaccines with enhanced tolerogenic activity said formulations comprising at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined herein and optionally at least one antigen.

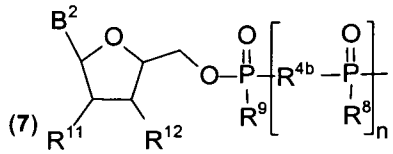
The present invention also relates to the use of purinergic and pyrimidinergic receptor agonists of formula (1) or a pharmaceutically acceptable salt thereof for the stimulation of TSP-1 production by dendritic cells,

wherein B^1 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, preferably wherein B^1 is selected from the group comprising adenine guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, more preferably wherein B^1 is adenine optionally substituted by thioalkyl, thioaryl, or thioalkylaryl,

R^1 and R^2 represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



wherein R^{10} represents hydrogen or is of formula (7),



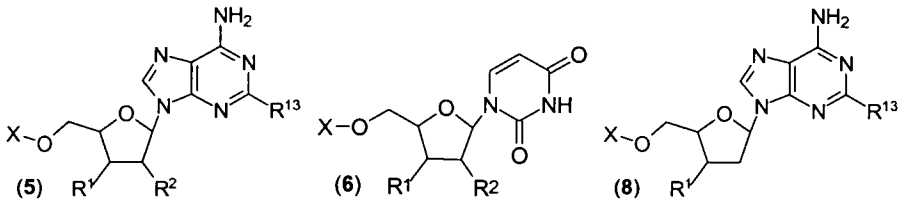
wherein B^2 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, wherein n is 0, 1, 2, 3 or 4, and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6, R^8, R^9 represent each independently OH or SH, and wherein R^{11} and R^{12} represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 is as defined above.

According to a preferred embodiment, B^1 in the above formula (1) is adenine optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, preferably thiomethyl, thio-n-butyl, thio-n pentyl, thiophenyl or thiobenzyl.

According to a particular embodiment, in the above formula (7), B^2 preferably is adenine, uracil, cytosine or guanine, preferably adenine, cytosine or guanine. In an interesting embodiment, B^2 is adenine in the formula (7) and n is 1, 2, 3 or 4.

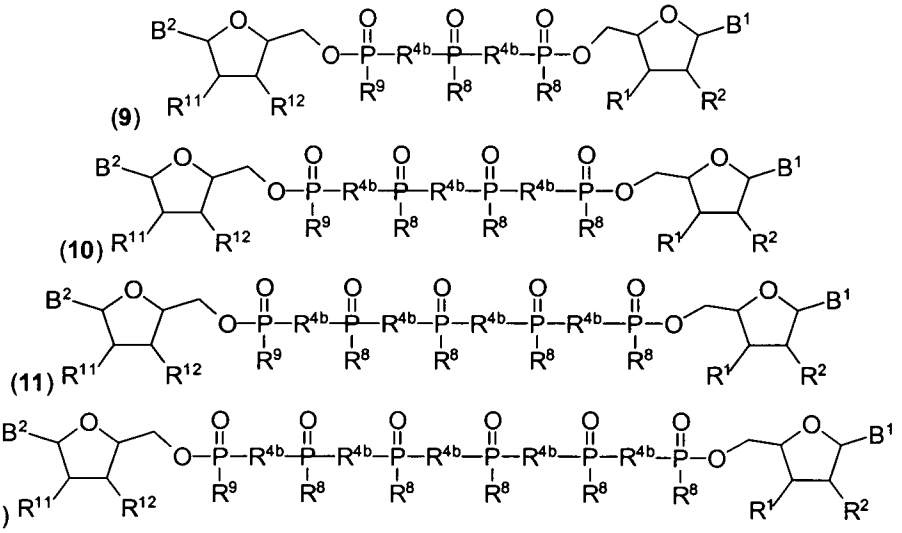
Although in the above formula (1), when X is of formula (2), (3) or (4) and R^{10} is of formula (7), the linkage between the nucleotides is from 5'→5' (e.g. P^1, P^3 – di(adenosine-5') triphosphate) , the present invention also relates to compounds wherein the linkage between the nucleotides is 2'→5' or 3'→5'. For instance, examples of such compounds which are commercially available are: adenylyl(2′→5′)cytidine, adenylyl(3′→5′)cytidine, adenylyl(2′→5′) uridine, adenylyl(3′→5′)uridine, adenylyl(3′→5′)adenosine, adenylyl(3′→5′)guanosine, cytidylyl (3'→5') cytidine, cytidylyl(2′→5′)adenosine, cytidylyl(2′→5′)guanosine, cytidylyl(3′→5′)cytidine, cytidylyl(3′→5′)guanosine, cytidylyl(3′→5′)uridine, guanylyl(2′→5′)adenosine, guanylyl(2′→5′) cytidine, guanylyl(3′→5′)adenosine, guanylyl(3′→5′)cytidine, guanylyl(3′→5′)uridine, uridylyl (2′→5′)uridine, uridylyl(3′→5′)adenosine, uridylyl (3′→5′)guanosine, uridylyl(3′→5′)uridine, 3-acetylpyridine adenine dinucleotide, and their salts.

According to a particular embodiment, said purinergic or pyrimidinergic receptor agonist is of formula (5), (6) or (8) or a pharmaceutically acceptable salt thereof,

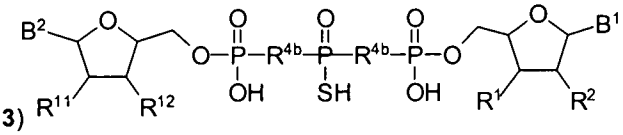


wherein R^1, R^2 and X have the same meaning as defined above and R^{13} is selected from hydrogen, thioalkyl, thioaryl or thioalkylaryl. Interesting compounds of the above formula are depicted in Table A.

According to a particular embodiment, said purinergic or pyrimidinergic receptor agonist is of formula (9), (10), (11) or (12) or a pharmaceutically acceptable salt thereof,



wherein B^1, B^2, R^1, R^2, R^{4b}, R^8, R^9, R^{11} and R^{12} have the same meaning as that defined above, and wherein in each molecule (9), (10), (11) or (12), each R^8 has independently from the other R^8 substituents an individual meaning and can change in each formula, for instance a purinergic or pyrimidinergic receptor agonist of formula (13),



(13)

Interesting compounds are those compounds of formula (9), (10), (11) or (12), wherein B^1 and B^2 are adenine. Non-limiting examples of such compounds are depicted in Table B.

Other interesting compounds are those compounds of formula (9), (10), (11) or (12), wherein B^1 and B^2 are different. Non-limiting examples of such compounds are depicted in Table C.

Interesting purinergic receptor agonists of formula (1) are selected from the following groups (i) to (iii) comprising: (i) ATP and derivatives, (ATPγS, BzATP, 2-MeSATP, deoxyderivatives thereof); (ii) ADP and derivatives (ADPβS, 2-MeSADP); (iii) dinucleotides.

More preferably said purinergic receptor agonists of formula (1) are adenosine 5'-triphosphate (ATP), adenosine 5'-O-(3-thiotriphosphate) (ATPγS), 2'- and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP), 2-propylthio-βγ-dichloromethylene-D-ATPadenosine 5'-diphosphate (ADP), 2-methylthio-ADP (2-MeSADP), adenosine 5'-O-(2-thiodiphosphate) (ADPβS), P^1,P^3-di(adenosine-5')triphosphate (Ap_3A), P^1,P^4-di(adenosine-5')tetraphosphate (Ap_4A), P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A), P^1,P^6-di(adenosine-5')hexaphosphate (Ap_6A), deoxyderivative thereof or a pharmaceutically acceptable salt of any of the aforementioned nucleotide derivatives.

It should be noted that when the compound "2'- and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP)" is mentioned, a mixture of 2'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate can be meant. The compound BzATP is a mixture of positional isomers 2'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate.

The cited nucleotides are potent agonists of the metabotropic $P2Y_{11}$ receptors, which are parts of the superfamily of G-protein-coupled receptors. The purinergic and pyrimidinergic receptor agonists useful according to the present invention may have asymmetric centers, occur as racemates, racemic mixtures, and as individual diastereoisomers, with all possible stereochemical isomers including optical isomers, being included in the present invention.

The present invention when referring to purinergic or pyrimidinergic receptor agonist, as cited herein, also includes within its scope not only the specific compound(s) listed or described, but also alternative forms of the compound such as pharmaceutically acceptable salts, solvates, hydrates, and the like. The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds as formed, e.g., from non-toxic inorganic or organic

acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

As used in the present specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to an "agonist" includes a single agonist, as well as two or more agonist.

The term “alkyl” as used herein refers to saturated monovalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof and contains 1-20 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-8 carbon atoms, still more preferably 1-6 carbon atoms, yet more preferably 1-4 carbon atoms. Alkyl radicals of interest are methyl, ethyl, propyl, isopropyl, *n*-butyl, isobutyl, pentyl, isoamyl, hexyl, cyclohexyl.

The term “aryl” as used herein, includes a monovalent organic radical derived from an aromatic hydrocarbon by removal of one hydrogen, such as phenyl.

The term “alkylene” as used herein refers to saturated bivalent hydrocarbon radicals having straight, branched or cyclic moieties or combinations thereof and contains 1-20 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-8 carbon atoms, still more preferably 1-6 carbon atoms, yet more preferably 1-4 carbon atoms. Examples of alkylene radicals are methylene, ethylene, and propylene.

The term “halo” or “halogen” as used herein is generic for fluoro, chloro, bromo or iodo. The terms “monohaloalkylene” and “dihaloalkylene” as used herein refers to alkylene radical as defined above substituted by respectively one and two halogen radicals.

The term “thioalkyl” as used herein refers as used herein refers to a radical R’”S-, wherein R’” is an alkyl radical as defined herein. Examples of thioalkyl include, but are not limited to thiomethyl, thioethyl, thiobutyl, thio-n-pentyl and the like.

The term “thioaryl” as used herein refers as used herein refers to a radical R’S-, wherein R’ is an aryl radical as defined herein. Examples of thioaryl include, but are not limited to thiophenyl and the like.

The term “thioalkylaryl” as used herein refers as used herein refers to a radical R’R’’S-, wherein R’ is an aryl radical as defined herein, and wherein R’’ is an alkylene radical as defined herein. Examples of thioalkylenearyl include, but are not limited to thiobenzyl and the like.

The present invention provides methods of inducing immunotolerance for the prevention or treatment of inflammatory, allergic, autoimmune diseases and graft/transplantation rejection.

The term "immunotolerance" as used herein refers to immune unresponsiveness to an antigen implicated in causing disease.

The methods generally comprise administering to an individual in need thereof, a therapeutically effective amount of at least one purinergic and pyrimidinergic receptor agonists of formula (1) with or without administering an antigen. Preferably said compound is administered only once, twice or thrice, preferably only once. Said purinergic and pyrimidinergic receptor agonists of formula (1) stimulate TSP-1 secretion in dendritic cells thereby inducing immunotolerance. Preferably, the method comprises administering to an individual in need thereof, a therapeutically effective amount of at least one compound of formula (1) together with at least one antigen. Therefore, the present invention also teaches a method to induce and/or increase tolerance to an antigen in an individual, comprising stimulation and/or upregulation of the production of thrombospondin-1 in dendritic cells in the presence of an antigen.

Of course, said antigen may be present in the body already, as is the case with some allergic diseases. In that case, stimulation and/or upregulation of the production of thrombospondin-1 in dendritic cells with the purinergic and pyrimidinergic receptor agonists of formula (1) alone will enhance the induction of tolerance. As a consequence of said effect on the dendritic cells, said purinergic and pyrimidinergic receptor agonists of formula (1) are useful as inducers of immunotolerance.

Of course, said antigen may be present in the patient surroundings, i.e. may be exogenous, such as pollen and the like. In that case, stimulation and/or upregulation of the production of thrombospondin-1 in dendritic cells with the purinergic and pyrimidinergic receptor agonists of formula (1) in the presence of said exogenous antigen will enhance the induction of tolerance.

The "therapeutically effective amount" of said above-described purinergic or pyrimidinergic receptor agonist relates to the amount or quantity of said agonist required to achieve the desired therapeutic and/or prophylactic effect, for example the dosage of said agonist which results in inducing immune tolerance in the individual. Effective amounts may be measured from ng/kg body weight to g/kg body weight per minute, hour, day, week or month.

The term "individual" as used herein includes inter alia a subject, patient, target, host or recipient regardless of whether the individual is a human or non-human animal including avian species. The term "individual", therefore, includes a human, non-human primate (e.g. gorilla, marmoset, African Green Monkey), livestock animal (e.g. sheep, cow, pig, horse, donkey, goat), laboratory test animal (e.g. rat, mouse, rabbit, guinea pig, hamster), companion animal (e.g. dog, cat), captive wild animal (e.g. fox, deer, game animals) and avian species including poultry birds (e.g. chickens, ducks, geese, turkeys).

The preferred individual, however, is a human. However, insofar as the present invention extends to an animal model, the individual may be a mouse, rat, pig, sheep, non-human primate or other non-human animal.

In an embodiment, the present invention provides a method of inducing immunotolerance comprising administering to an individual, in need thereof, a therapeutically effective amount of a purinergic or pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof. The present invention also relates to the use of a purinergic or pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for inducing tolerance to antigens, optionally in the presence of said antigens.

Above-mentioned agonists of formula (1) are useful as vaccine adjuvants and promote the development of tolerance to antigens, through stimulation of TSP-1 secretion and potentiation of IDO expression in response to interferon-gamma. Said agonist of formula (1) may be given orally, or by inhalation, parenterally or via the skin or a mucosal surface with the purpose to induce tolerance to an antigen.

In another embodiment, said agonist of formula (1) may be combined with said antigens before administration to an individual. Therefore, the present invention also discloses a method to induce immunotolerance to an antigen, as mentioned above wherein said agonist of formula (1) is combined with said antigen before administration to an individual. And in another embodiment, the present invention also discloses a method to induce immunotolerance to an antigen, comprising providing the agonist of formula (1) and further administering the antigen.

In a further aspect, the present invention relates to a pharmaceutical composition suitable for inducing immunotolerance to antigen responsible of immune diseases comprising a pharmaceutically acceptable carrier and a purinergic or pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof, and at least one antigen.

Administration of above-mentioned pharmaceutical compositions increases the induction of tolerance to antigens and may diminish disease symptoms in individuals suffering from hypersensitivity to various antigens.

The invention further relates to the use of a purine or pyrimidine analogue or derivative of formula (1) or a pharmaceutically acceptable salt of any of the aforementioned nucleotide derivatives, as an immunotolerance inducer.

The present invention provides a specific immunotolerance with respect to a particular antigen, leaving the general competence of the immune system intact. In contrast with immunosuppression, immunotolerance and therapeutic vaccination have an improved risk/benefit ratio as well as cost-benefit ratio. Indeed, only a limited number of treatment courses is needed, whereas immunosuppression should be continuous. In addition, vaccination according to the invention, i.e. with at least one compound of formula (1) alone or with at least one antigen, should be specific for said one (or a few) antigen(s) and does not decrease in a non-specific way immune responses against pathogens. It is therefore expected that the side effects and the costs of immunotolerance-based strategies would be much less than for immunosuppression-based strategies.

The amount and nature of the material used for administration of the agonist of formula (1) and the antigen to confer the desired specific immunotolerance depends on the type of antigen. The agonist of formula (1) may be administered simultaneously with the antigen or sequentially.

As used herein "simultaneously" when referenced to administration of antigen and the agonist of formula (1) refers to injection or administration of one within approximately 24-48 hours of the other. Either may be administered first. It is preferable, however, that the administration of the agonist of formula (1) be carried out substantially contemporaneously with or within 48 hours prior to injection or other administration of the antigen.

The term "antigen" as used herein refers to the immunogenic substance of interest which when exposed to an individual induces an immune response in that individual. Said antigens can be exogenous antigens which are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. These antigens can be endogenous antigens that have been generated within the cell, as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. Said antigens can be autoantigens which are usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of subjects suffering from a specific autoimmune disease.

Thus, specific antigens include allergens, nucleic acid, globular proteins, carbohydrate, glycoproteins such as immunoglobulins, materials carried on particles such as pollen proteins, polypeptides intended for therapeutic use such as interferon, interleukin-2, or tumor necrosis factor, hormone replacements, such as leutinizing hormone or its analogs or antagonists, and the like. Synthetic peptide analogs of protein therapeutic agents which are used for receptor blockade are another important class of antigen. Still another important subclass is that of allo-antigens, i.e., those which are products of the major histocompatibility complex. It is these allo-antigens which are presumably responsible for rejection of foreign tissue in tissue transplants or skin grafts.

For antigens, such as drugs, the antigenic component may be conveniently administered intravenously in suitable excipients. Alternatively, for environmental antigen, exposure may mimic "natural" exposure and employ aerosols or oral compositions and quantities approximating the estimated degree of exposure are appropriate. For example, one might use a "field of flowers" approach in inducing an immunologic tolerance to naturally occurring pollen. That is, at the beginning of the acacia blooming season, for example, the individual is placed in proximity to the offending blooms while simultaneously being administered protective purine or pyrimidine analogue or derivative of formula (1) or within the recovery period after such administration. Gradations between these natural exposures and the highly artificial method of isolating the specific antigen and injection with or just after the agonist of formula (1) may also be used. The formulation of appropriate pharmaceutical compositions to administer the antigen is well known to those in the art.

If the antigen is a therapeutic agent, amounts of antigen approximating those intended to be administered and a similar route of administration may be used. In general, this protection affords a simpler problem as generally speaking the antigen is already available in a suitably formulated

pharmaceutical composition and the levels of exposure expected and expected routes of administration are already known.

For induction of immunotolerance in the case of transplanted tissue, specifically, with respect to allografts, a variety of modes of administration are feasible. Allografts are multiplicities of cells or associated cells that define a tissue or organ from an individual of the same species as that of the intended recipient, but which derive from an individual genetically dissimilar from the recipient. These cell multiplicities themselves, include vascularized organs such as heart, kidney, liver, lungs, etc. Endocrine glands (pituitary, thyroid, adrenal, parathyroid, and pancreas) or skin grafts may not contain the major histocompatibility antigens which are responsible for triggering the rejection of the transplant. These antigens are carried by passenger cells such as leukocytes which are included in the transplanted cells as impurities.

Two general approaches may be used: the transplant materials can themselves be used as a source of antigen, or the particular histocompatibility antigens may be obtained separately and administered alone or as cells bearing the histocompatibility antigens of the transplant donor, i.e., peripheral blood lymphocytes. A major antigen responsible for tissue rejection carried of such passenger leukocytes is the murine Ia-equivalent antigen which in humans is designated HLA-DR (MHC class II antigens). Human HLA-DR antigens have been subclassified and if the donor has been typed, suitable antigen, e.g., peripheral blood lymphocytes, associated with the donor tissue is conveniently obtainable and injected prior to transplant along with or within the recovery period of administering the agonist of formula (1). The perhaps more crude, but effective, manner of administration is simultaneous injection of the agonist of formula (1) along with or just before the transplant itself or a pre-transplant simultaneous injection of a portion of the tissue suitably finely divided and formulated along with the agonist of formula (1).

The agonist of formula (1) may be incorporated in the pharmaceutical composition with a suitable diluent. Said diluent may be any fluid acceptable for intravenous or parenteral inoculation. In one embodiment the suitable diluent may comprise water and/or oil and/or a fatty substance. In an embodiment, said agonists are further combined with at least one antigen. Therefore, the present invention teaches a pharmaceutical composition comprising at least one agonists of formula (1) and at least one antigen, which may further comprises a suitable diluent. Said agonists of formula (1) may be administrated to an individual in need of such treatment before the administration of the antigens. The agonists of formula (1) may be administered via another route than said antigens. For example, the agonists of formula (1) may be provided orally, or topically, followed by topical administration of the antigens. Said topical administration comprises administration on the skin, and/or on the mucosa of the airways and/or of the oro-nasal cavity, and/or of the gastro-intestinal mucosa.

The pharmaceutical composition of the present invention may further include thickeners, buffers, preservatives, surface active agents, liposomes, or lipid formulations, and the like. Pharmaceutically acceptable carriers may include sterile aqueous or non-aqueous solutions,

suspensions, and emulsions suitable for ingestion, inhalation, intranasal administration, ocular application, skin application or administration as a suppository to the rectum or vagina. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and certain organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.

The pharmaceutical composition may be administered to an individual in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topical including on the skin, ophthalmic, vaginal, rectal, intranasal, oral, by inhalation, or parenteral, for example by intravenous drip, subcutaneous, intratumor, intraperitoneal, intralymphatic or intramuscular injection.

The pharmaceutical composition according to the invention may suitably be provided in the form of a spray, an aerosol, tablets (entero-or not-enterocoated), capsule (hard or soft, entero-or not-enterocoated, controlled ileal release or not), a suspension, a dispersion, granules, a powder, a solution, an emulsion, chewable tablets, tablets for dissolution, drops, a gel, a paste, a syrup, a cream, a lozenge (powder, granulate, tablets), a lotion, a foam, an instillation fluid, a gas, a vapor, an ointment, a patch, a stick, implants (ear, eye, skin, nose, rectal, or vaginal), vagitories, suppositories, enema, foam, or uteritories suitable for administration via the oral, nasal, vaginal, sublingual, ocular, rectal, urinary, intramammary, pulmonary, otolar, or buccal route.

As seen from the previous paragraphs, each specific antigen offers alternative routes for administration appropriate to its nature. All offer the possibility of obtaining purified antigen and utilizing intravenous administration. In certain instances, it is also possible to inject a composition containing a mixture of the specific antigen along with the agonist of formula (1) in a suitable pharmaceutical composition.

In summary, for antigens in general, while intravenous administration is most convenient, other forms of administration are useful as well. Other routes of parenteral administration include subcutaneous, intraperitoneal, or intramuscular injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like. For substances intended to stimulate the immune system, such as the specific antigen administered in the method of the invention, an adjuvant, such as complete Freund's adjuvant is generally used.

An additional approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained.

Systemic administration may be effected via suppository. For such formulations, traditional binders and carriers include, e.g., polyalkalene glycols or triglycerides. Such suppositories may be formed

from mixtures containing the agonist of formula (1) or antigen in the range of 0.5%-10%; preferably 1%-2%.

For aerosol administration, the antigen is preferably supplied in finely divided form along with a surfactant and a propellant. Typical percentages of active ingredients are 0.01% to 20% by weight, preferably 0.4% to 1.0%. Surfactants must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids, such as caproic or octanoic acids, polyols such as mannitol or sorbitol, or their anhydrides or esters and their polyoxyethylene and polyoxypropylene derivatives. Preferred surface-active agents include the oleates of sorbitan, e.g., those sold under the trademarks "Arlacel C", "Span 80", and "Span 85". The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25%-5%. The balance of the composition is ordinarily propellant; for example the lower alkanes, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes, such as are sold under the trademark "Freon". In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided active ingredient and surfactant. The ingredients are thus maintained at an elevated pressure until released by action of the valve.

Solid compositions of antigen or agonist of formula (1) may be used if administered orally or if reconstituted for administration. Conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. The corresponding liquid pharmaceutically administrable compositions can be prepared by dissolving, dispersing, etc, the antigen or agonist of formula (1) above and optional pharmaceutical adjuvants in a carrier as described above.

Actual methods of preparing the above dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, latest edition. The composition or formulation to be administered will, in any event, contain a quantity of the agonist of formula (1) or antigen in an amount effective to obtain the desired specific immunotolerance.

It is convenient to package the specific antigen and the agonist of formula (1) into kits for administration of the treatment. Thus, for example, kits for the treatment of allergy would comprise containers with, preferably, unit dosage levels of allergen such as ragweed protein, milk protein, or other allergenic material in suitable excipients including most preferably adjuvant preparations such as Freund's complete adjuvant. An additional container would supply, e.g., the agonist of formula (1) in form for administration such as suspension in physiological saline or other pharmaceutical excipient or in solid form to be reconstituted. For certain antigen/ agonist of formula (1) combinations, these components may be supplied as a mixture. Additional components of such kits might be sterile disposable syringes. All materials may be packaged in convenient containers for administration as is known in the art, along with instructions for their use.

Specifically, the present invention relates to a method of inducing immunotolerance to an antigen comprising administering to an individual, in need thereof, a therapeutically effective amount of a purinergic or pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof alone or with at least one antigen. Preferably said immunotolerance is being induced to antigens responsible of asthma including bronchial asthma, allergic asthma, intrinsic asthma and dust asthma. Preferably said immunotolerance is being induced to antigens responsible of allergic rhinitis. Preferably said immunotolerance is being induced to antigens responsible of conjunctivitis. Preferably said immunotolerance is being induced to antigens responsible of keratitis. Preferably said immunotolerance is being induced to antigens responsible of keratoconjunctivitis. Preferably said immunotolerance is being induced to antigens responsible of uveitis. Preferably said immunotolerance is being induced to antigens responsible of psoriasis. Preferably said immunotolerance is being induced to antigens responsible of eczema. Preferably said immunotolerance is being induced to antigens responsible of atopic dermatitis. Preferably said immunotolerance is being induced to antigens responsible of contact dermatitis. Preferably said immunotolerance is being induced to antigens responsible of cutaneous T cell lymphoma (CTCL). Preferably said immunotolerance is being induced to antigens responsible of Sezary syndrome. Preferably said immunotolerance is being induced to antigens responsible of pemphigus vulgaris. Preferably said immunotolerance is being induced to antigens responsible of bullous pemphigoid. Preferably said immunotolerance is being induced to antigens responsible of pemphigus foliaceus. Preferably said immunotolerance is being induced to antigens responsible of dermatomyositis. Preferably said immunotolerance is being induced to antigens responsible of erythema nodosum. Preferably said immunotolerance is being induced to antigens responsible of scleroderma. Preferably said immunotolerance is being induced to antigens responsible of Bechet's disease. Preferably said immunotolerance is being induced to antigens responsible of sarcoidosis. Preferably said immunotolerance is being induced to antigens responsible of Sjögren's syndrome. Preferably said immunotolerance is being induced to antigens responsible of rheumatoid arthritis. Preferably said immunotolerance is being induced to antigens responsible of juvenile arthritis. Preferably said immunotolerance is being induced to antigens responsible of Reiter's syndrome. Preferably said immunotolerance is being induced to antigens responsible of lupus erythematosus. Preferably said immunotolerance is being induced to antigens responsible of polymyositis. Preferably said immunotolerance is being induced to antigens responsible of myocarditis. Preferably said immunotolerance is being induced to antigens responsible of primary biliary cirrhosis. Preferably said immunotolerance is being induced to antigens responsible of Crohn's disease. Preferably said immunotolerance is being induced to antigens responsible of ulcerative colitis. Preferably said immunotolerance is being induced to antigens responsible of multiple sclerosis and other demyelinating diseases. Preferably said immunotolerance is being induced to antigens responsible of idiopathic thrombocytopenic purpura. Preferably said immunotolerance is being induced to antigens responsible of Graves' disease and Hashimoto's disease. Preferably said immunotolerance is being induced to antigens responsible of Addison's disease. Preferably said immunotolerance is being induced to antigens responsible of insulin-dependent diabetes

mellitus (type 1). Preferably said immunotolerance is being induced to antigens responsible of transplant rejection or graft-versus-host disease.

In a particular embodiment of the present invention, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used with at least one antigen responsible of and/or triggering an autoimmune disease, for the preparation of a medicament for treating an autoimmune disease. Preferably, said autoimmune disease is selected from the group comprising acute disseminated encephalomyelitis (ADEM), Addison's disease, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hepatitis, coeliac disease, Crohn's disease, diabetes mellitus (type 1), Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS) (also known as acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculoneuritis, acute idiopathic polyneuritis and Landry's ascending paralysis), Hashimoto's disease, idiopathic thrombocytopenic purpura, lupus erythematosus, multiple sclerosis, myasthenia gravis, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis, pemphigus, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, Reiter's syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), warm autoimmune hemolytic anemia, and Wegener's granulomatosis. More preferably, said autoimmune disease is selected from diabetes mellitus (type 1), multiple sclerosis or rheumatoid arthritis.

In a particular embodiment, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used alone or with at least one antigen responsible of and/or triggering an autoimmune disease, for the preparation of a medicament for preventing said autoimmune disease, preferably in a subject at risk of developing said disease. Preferably, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used with at least one antigen responsible of and/or triggering and autoimmune disease, for the preparation of a medicament for preventing said autoimmune disease, preferably in a subject at risk of developing said disease.

As used herein “an individual at risk of developing an autoimmune disease” includes but is not limited to individuals having a history of autoimmune disease or individuals genetically susceptible to the development of autoimmune diseases (with genetic predisposition) or individuals selected on the basis of medical investigation results or relevant biomarkers.

The present compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering autoimmune disease to prepare vaccine to prevent autoimmune disease, wherein said autoimmune disease is selected from the group comprising acute disseminated encephalomyelitis (ADEM), Addison's disease, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hepatitis, coeliac disease, Crohn's disease, diabetes mellitus (type 1), Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS) (or acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculoneuritis, acute idiopathic polyneuritis and Landry's ascending paralysis), Hashimoto's disease, idiopathic thrombocytopenic purpura, lupus erythematosus, multiple sclerosis , myasthenia gravis, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis, pemphigus, primary biliary cirrhosis, psoriasis, rheumatoid

arthritis, Reiter's syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), warm autoimmune hemolytic anemia , and Wegener's granulomatosis.

In particular, the present invention relates to the use of at least one compound of formula (1) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating an autoimmune disease wherein said compound is administered only once. In particular, the present invention relates to the use of at least one compound of formula (1) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating an autoimmune disease wherein said compound is administered for a limited period of time in the early phase of the disease.

In particular, the present invention relates to the use of at least one compound of formula (1) or a pharmaceutically acceptable salt thereof alone or with at least one antigen responsible of and/or triggering autoimmune disease, for the preparation of a medicament for preventing or delaying relapsing of a disease of wherein said compound is administered during the relapsing phase of said disease.

In one example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering multiple sclerosis, for the preparation of a medicament for treating a subject at risk for multiple sclerosis (MS), e.g., at risk of progressive MS or relapsing MS.

In another example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering multiple sclerosis, for the preparation of a medicament for preventing or delaying (e.g., for at least one year, 2 years, 3 years, 4 years, 5 years, 10 years or more) the onset of clinical manifestations of MS (e.g., relapsing remitting MS) or for minimizing the severity of a subsequent (e.g., a second) clinical manifestation. In one embodiment, the subject has had fewer than two clinical episodes of focal neurologic deficit.

In one embodiment, the subject has experienced one clinical episode of focal neurologic deficit. The neurologic deficit can be evidenced by, e.g., one or more symptoms, such as weakness of one or more extremities, paralysis of one or more extremities, tremor of one or more extremities, uncontrollable muscle spasticity, sensory loss or abnormality, decreased coordination, loss of balance, loss of ability to think abstractly, loss of ability to generalize, difficulty speaking, and difficulty understanding speech.

In one embodiment, selection of a subject as being at risk for MS is done on the basis of one or more of: (a) cranial scan having evidence of myelin sheath damage, (b) presence of serum antibodies against one or both of MOG and MBP, (c) presence of increased levels of CSF IgG, (d) presence of elevated levels of MBP, and (e) occurrence of one clinical episode of focal neurologic deficit.

As used herein a "neurologic deficit" is a decrease in a function of the central nervous system. Examples include inability to speak, decreased sensation, loss of balance, weakness, cognitive dysfunction, visual changes, abnormal reflexes, and problems walking. A "focal neurologic deficit"

affects either a specific location (such as the left face, right face, left arm, right arm) or a specific function (for example, speech may be affected, but not the ability to write). When referring to a neurologic deficit, the term "clinical episode" means a neurologic deficit that lasts for hours, days or weeks (but from which partial or complete recovery can take place) and that is directly observable by outward physical signs of a patient, as distinguished from being observable only through a laboratory test or imaging of internal body tissues. A clinical neurologic deficit is typically determined by a medical history and/or a physical neurological exam.

A compound of the invention can be administered to a patient with a progressive course of MS to retard or prevent the progression of neurological impairment. A compound of the invention can also be administered to a subject with relapsing-remitting, secondary progressive or progressive-relapsing MS to inhibit relapse (e. g., an acute attack). For example, a compound of the invention can be administered to a subject with relapsing-remitting MS during the remitting phase of the disease to prevent or delay relapse. In a particular embodiment, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering multiple sclerosis, for the preparation of a medicament for preventing or delaying relapses in an individual having multiple sclerosis wherein said compound is administered during the remitting phase of said multiple sclerosis.

As used herein "relapses" involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also not getting any worse followed by a recovery (remitting) period. Recovery usually begins within a few weeks.

In another particular embodiment of the present invention, at least one compound of formula (1) is used in combination with at least one antigen responsible of and/or triggering an allergic disease for the preparation of a medicament for treating allergy to said antigen. The antigen to be used in combination with the compound of formula (1) can be any naturally occurring protein that has been reported to induce allergic, i.e. IgE mediated, reactions upon their repeated exposure to an individual. Suitable antigens include pollen allergens (tree-, herb, weed-, and grass pollen allergens), insect allergens (inhalant, saliva and venom allergens, e.g. mite allergens, cockroach and midges allergens, hymenopthera venom allergens), animal hair and dandruff allergens (from e.g. dog, cat, horse, rat, mouse etc.), and food allergens. Suitable pollen allergens comprises pollen allergens from trees, grasses and herbs are such originating from the taxonomic orders of Fagales, Oleales, Pinales and platanaceae including *inter alia* (i.a.) birch (Betula), alder (Alnus), hazel (Corylus), hornbeam (Carpinus) and olive (Olea), cedar (Cryptomeria and Juniperus), Plane tree (Platanus), the order of Poales including i.a. grasses of the genera Lolium, Phleum, Poa, Cynodon, Dactylis, Holcus, Phalaris, Secale, and Sorghum, the orders of Asterales and Urticales including i.a. herbs of the genera Ambrosia, Artemisia, and Parietaria. Other suitable allergens are

those from house dust mites of the genus Dermatophagoides and Euroglyphus, storage mite e.g. Lepidoglyphys, Glycyphagus and Tyrophagus, those from cockroaches, midges and fleas e.g. Blatella, Periplaneta, Chironomus and Ctenocepphalides, and those from mammals such as cat, dog and horse, venom allergens including such originating from stinging or biting insects such as those from the taxonomic order of Hymenoptera including bees (superfamily Apidae), wasps (superfamily Vespidea), and ants (superfamily Formicoidae), and allergens from fungi are i.a. such originating from the genera Alternaria and Cladosporium. Preferably, the antigen is selected from the group consisting of a tree pollen allergen, a grass pollen allergen, a dust mite allergen, a herb allergen and an animal allergen. Preferably, the antigen is selected from the group consisting of a grass pollen allergen, a dust mite allergen, a ragweed allergen, a cedar pollen, a cat allergen and a birch allergen.

In a particular embodiment, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used alone or with at least one antigen responsible of and/or triggering an allergic disease, for the preparation of a medicament for preventing said allergic disease, preferably in a subject at risk of developing allergy.

In particular, the present invention relates to the use of at least one compound of formula (1) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating an allergy wherein said compound is administered only once, twice or thrice, preferably only once.

For example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering atopy, for the preparation of a medicament for preventing atopic syndrome (atopy) in a subject at risk of developing said syndrome. For example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering atopic dermatitis, allergic conjunctivitis, allergic rhinitis and/or asthma, for the preparation of a medicament for preventing atopic dermatitis, allergic conjunctivitis, allergic rhinitis and/or asthma in individuals at risk of developing at least one of said disease.

In another example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering allergic conjunctivitis, allergic rhinitis and/or asthma, for the preparation of a medicament for preventing allergic conjunctivitis, allergic rhinitis and/or asthma in individuals having atopic dermatitis.

In another example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering asthma, for the preparation of a medicament for preventing or delaying asthma in individuals having atopic dermatitis or allergic rhinitis.

In yet another example, at least one compound of formula (1) can be used alone or with at least one antigen responsible of and/or triggering diabetes (type 1), for the preparation of a medicament for preventing diabetes in individuals at risk of developing diabetes.

In another particular embodiment of the present invention, at least one compound of formula (1) is used in combination with at least one antigen responsible of and/or triggering a graft/transplantation rejection, for the preparation of a medicament for treating graft/transplantation rejection. As defined above for transplant rejection, suitable source of antigen can be the transplant materials can themselves, or the particular histocompatibility antigens may be obtained separately and administered alone or as cells bearing the histocompatibility antigens of the transplant donor, i.e., peripheral blood lymphocytes.

In a particular embodiment, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used alone or with at least one antigen responsible of and/or triggering a graft/transplantation rejection, for the preparation of a medicament for preventing graft/transplantation rejection, preferably in a subject at risk of developing said rejection. Preferably, at least one compound of formula (1) or a pharmaceutically acceptable salt thereof is used with at least one antigen responsible of and/or triggering graft/transplantation rejection, for the preparation of a medicament for preventing graft/transplantation rejection, preferably in a subject at risk of developing said rejection.

Non limiting examples of purinergic and pyrimidinergic receptor agonists of formula (1) useful according to the invention are listed Tables A, B and C. The abbreviation "Cpd" refers to "Compound".

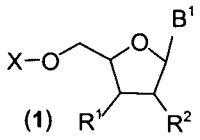
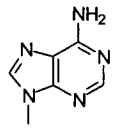
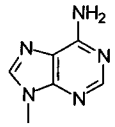
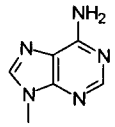
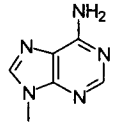
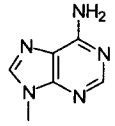
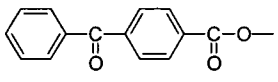
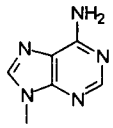
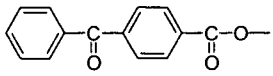
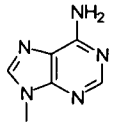
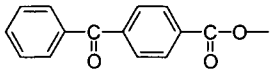
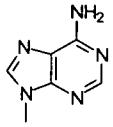
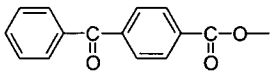
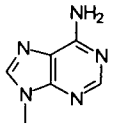
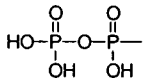
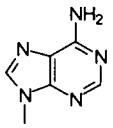
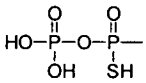
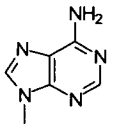
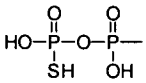
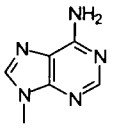
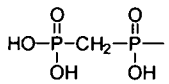
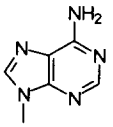
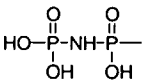
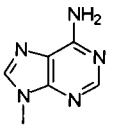
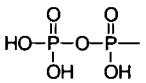
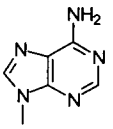
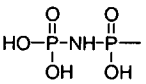
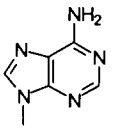
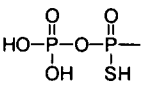
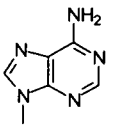
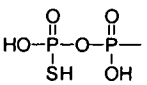
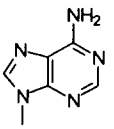
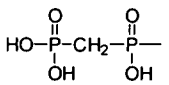
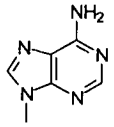
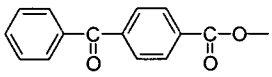
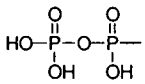
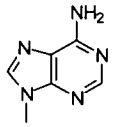
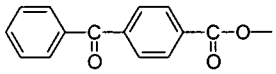
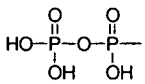
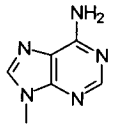
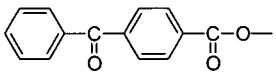
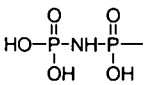
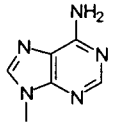
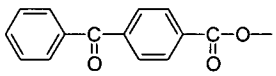
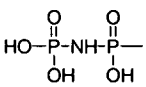
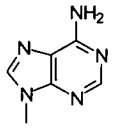
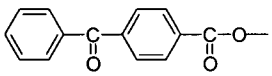
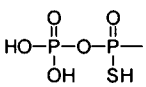
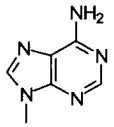
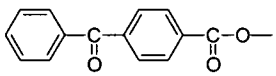
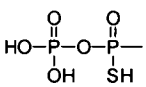
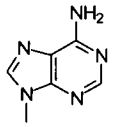
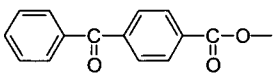
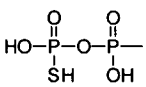
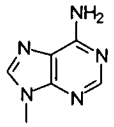
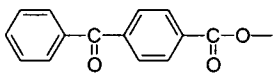
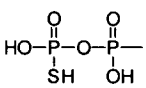
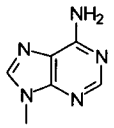
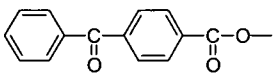
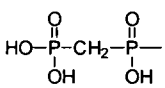
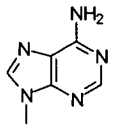
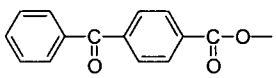
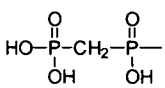
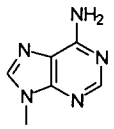
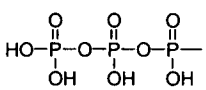
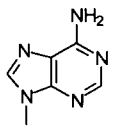
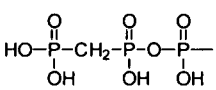
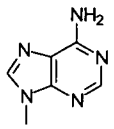
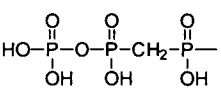


Table A

Cpd	B^1	R^1	R^2	X
1		OH	OH	
2		OH	OH	
3		OH	OCH_3	
4		OH	OCH_3	
5			OH	

Cpd	B^1	R^1	R^2	X
6		OH		
7			OH	
8		OH		
9		OH	OH	
10		OH	OH	
11		OH	OH	
12		OH	OH	
13		OH	OH	
14		OH	OCH_3	
15		OH	OCH_3	
16		OH	OCH_3	
17		OH	OCH_3	
18		OH	OCH_3	

Cpd	B^1	R^1	R^2	X
19			OH	
20		OH		
21			OH	
22		OH		
23			OH	
24		OH		
25			OH	
26		OH		
27			OH	
28		OH		
29		OH	OH	
30		OH	OH	
31		OH	OH	

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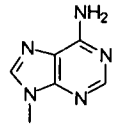
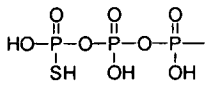
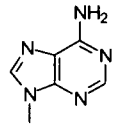
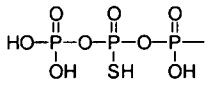
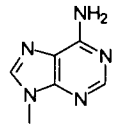
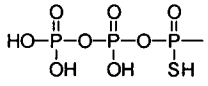
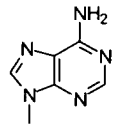
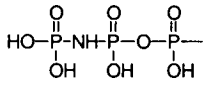
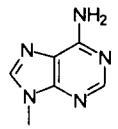
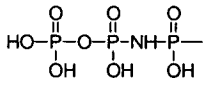
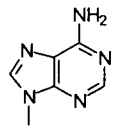
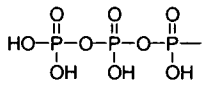
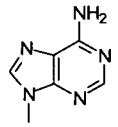
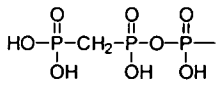
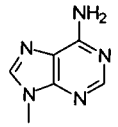
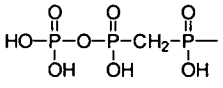
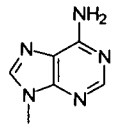
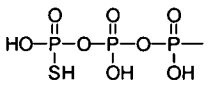
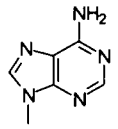
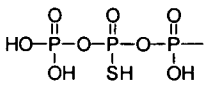
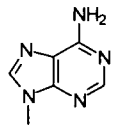
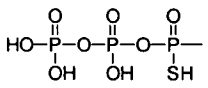
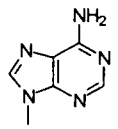
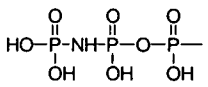
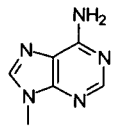
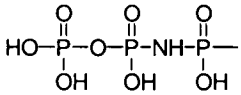
32		OH	OH	
33		OH	OH	
34		OH	OH	
35		OH	OH	
36		OH	OH	
37		OH	OCH_3	
38		OH	OCH_3	
39		OH	OCH_3	
40		OH	OCH_3	
41		OH	OCH_3	
42		OH	OCH_3	
43		OH	OCH_3	
44		OH	OCH_3	

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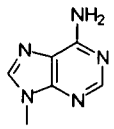
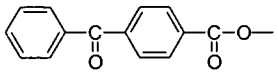
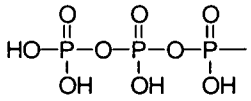
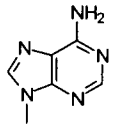
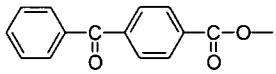
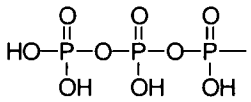
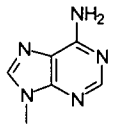
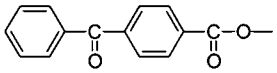
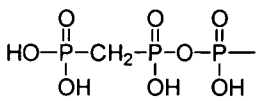
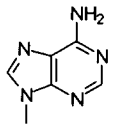
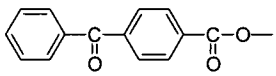
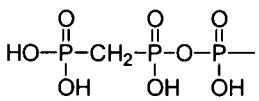
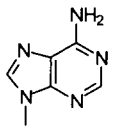
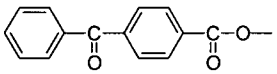
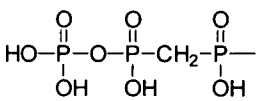
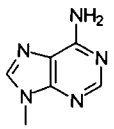
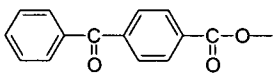
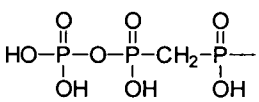
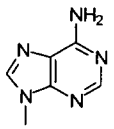
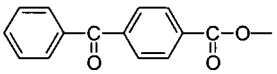
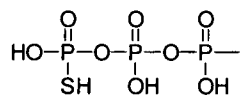
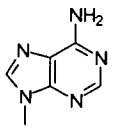
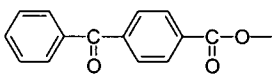
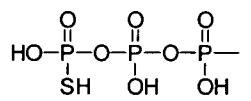
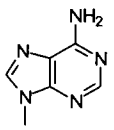
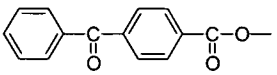
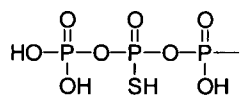
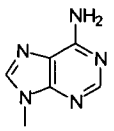
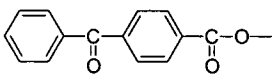
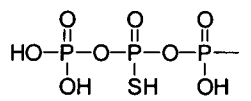
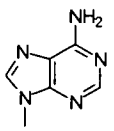
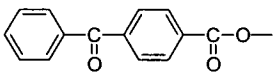
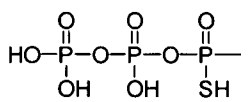
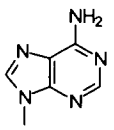
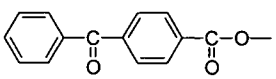
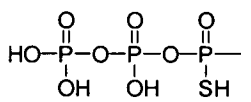
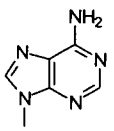
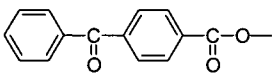
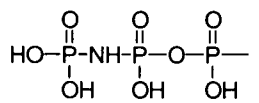
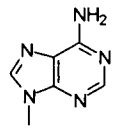
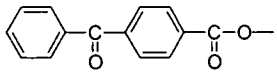
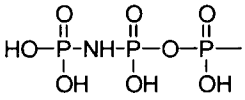
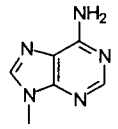
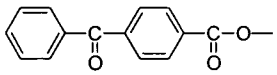
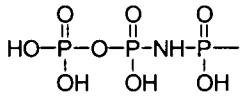
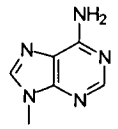
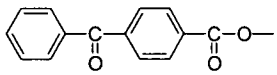
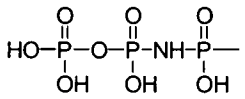
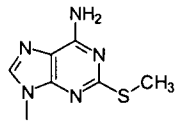
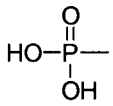
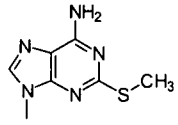
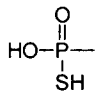
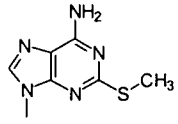
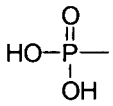
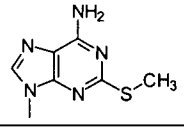
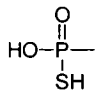
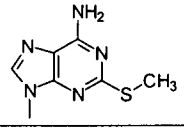
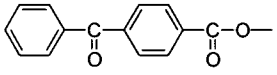
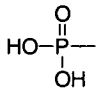
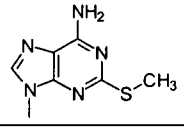
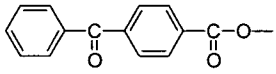
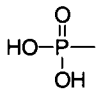
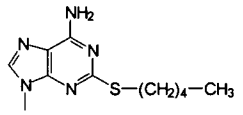
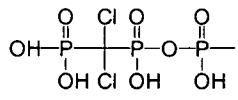
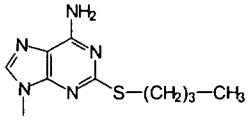
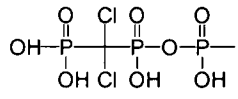
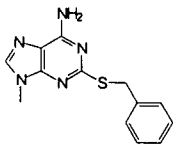
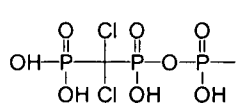
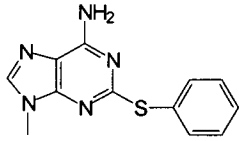
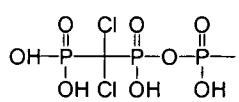
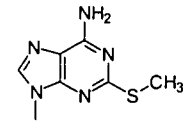
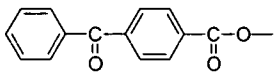
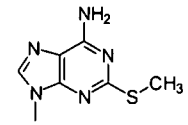
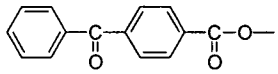
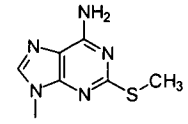
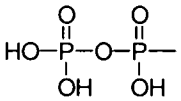
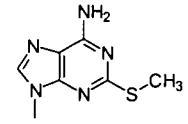
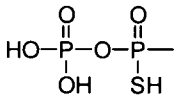
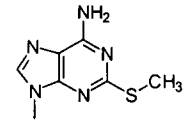
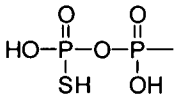
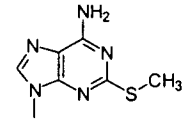
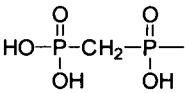
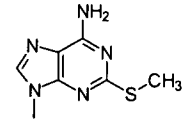
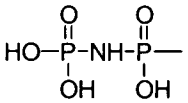
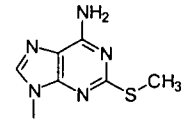
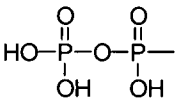
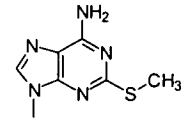
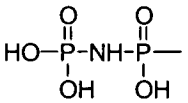
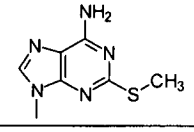
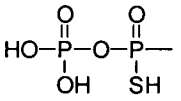
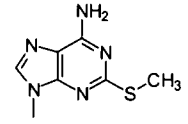
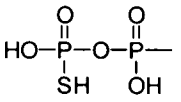
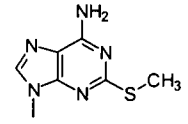
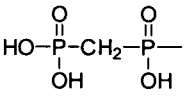
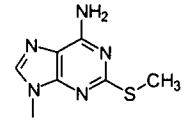
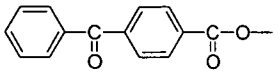
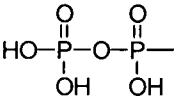
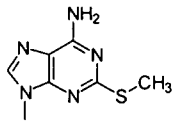
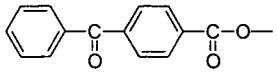
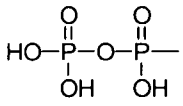
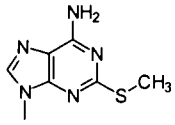
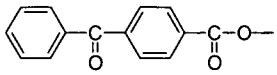
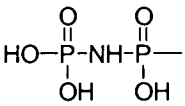
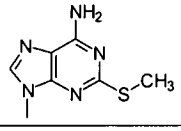
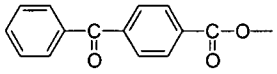
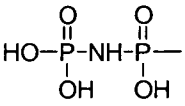
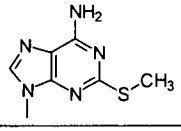
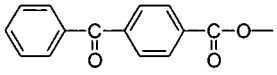
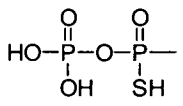
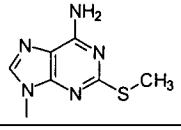
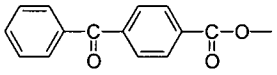
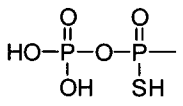
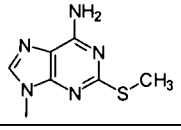
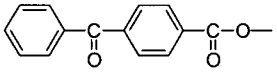
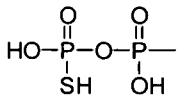
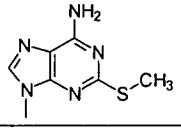
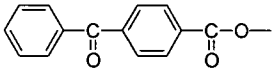
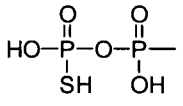
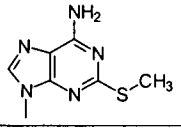
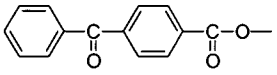
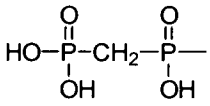
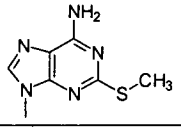
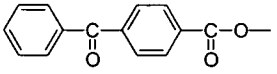
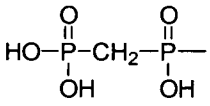
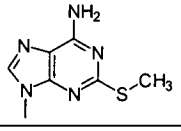
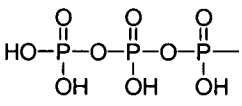
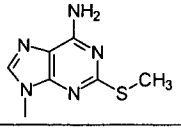
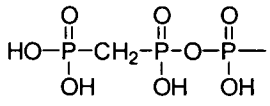
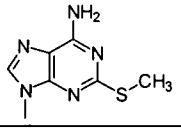
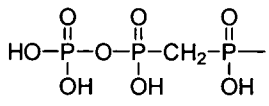
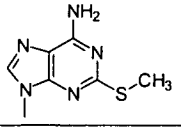
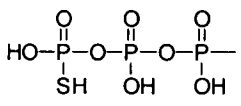
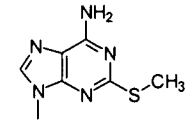
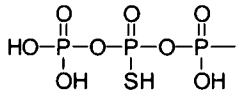
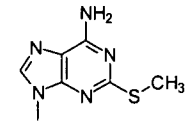
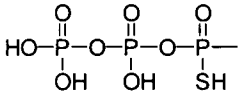
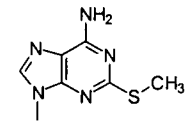
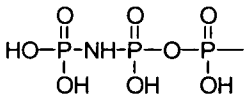
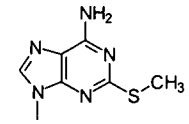
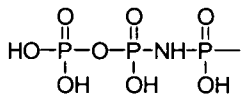
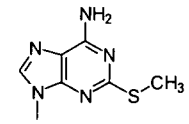
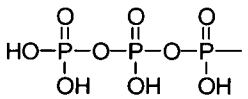
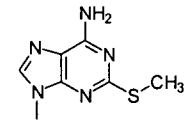
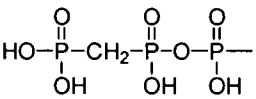
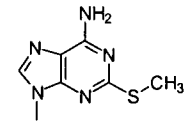
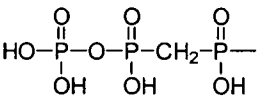
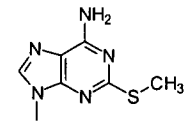
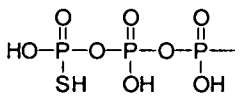
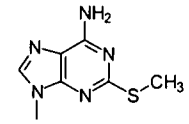
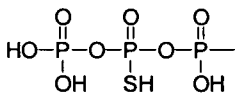
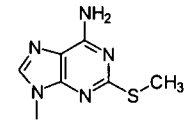
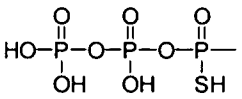
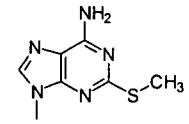
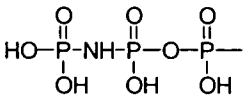
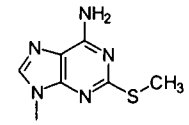
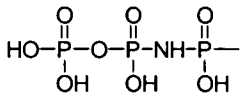
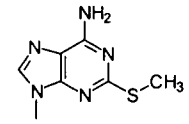
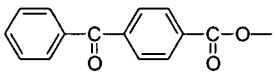
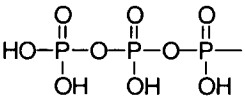
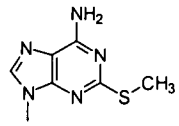
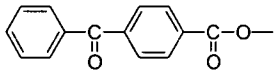
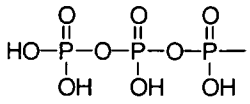
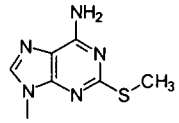
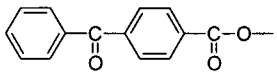
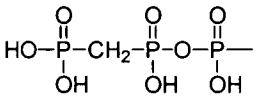
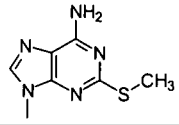
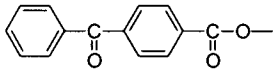
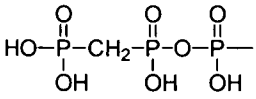
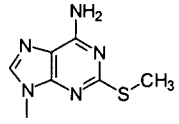
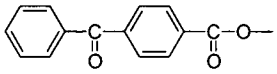
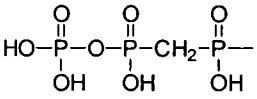
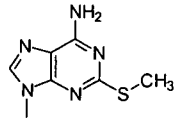
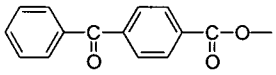
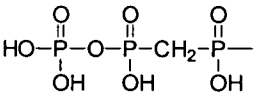
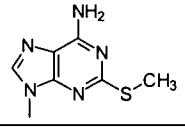
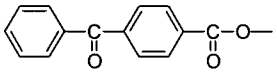
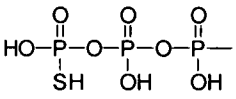
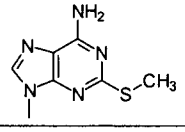
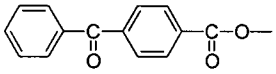
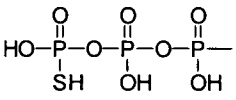
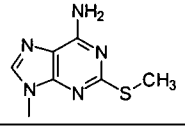
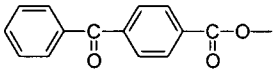
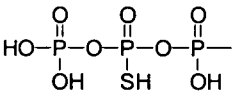
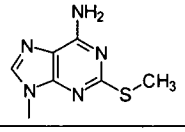
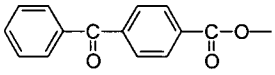
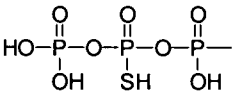
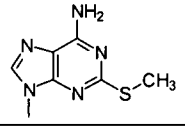
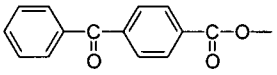
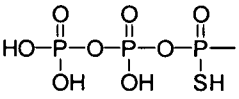
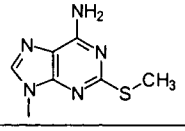
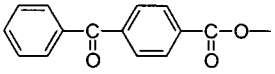
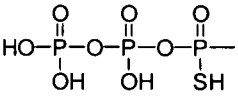
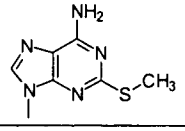
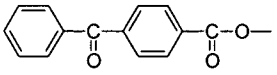
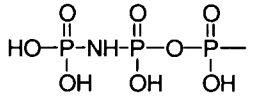
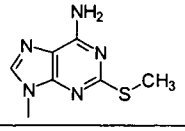
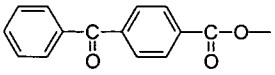
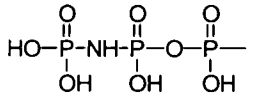
45			OH	
46		OH		
47			OH	
48		OH		
49			OH	
50		OH		
51			OH	
52		OH		
53			OH	
54		OH		
55			OH	
56		OH		
57			OH	

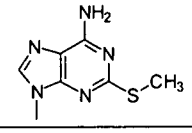
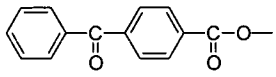
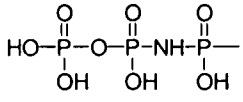
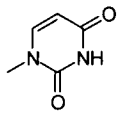
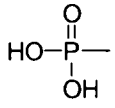
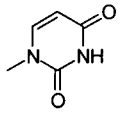
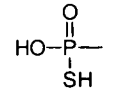
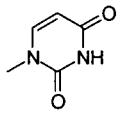
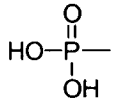
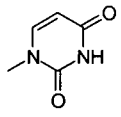
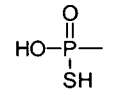
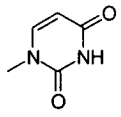
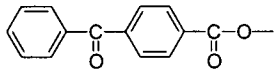
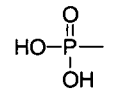
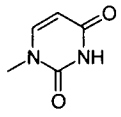
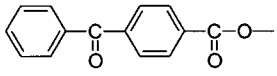
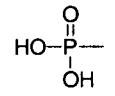
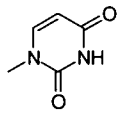
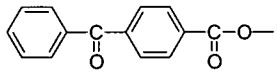
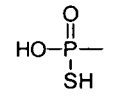
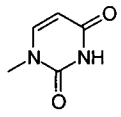
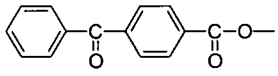
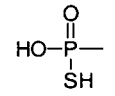
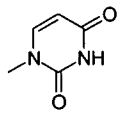
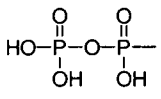
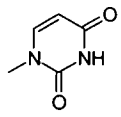
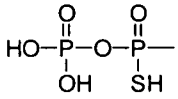
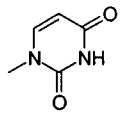
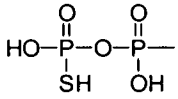
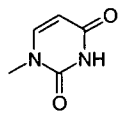
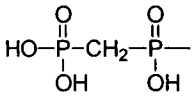
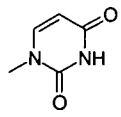
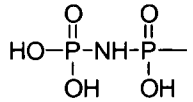
Cpd	B^1	R^1	R^2	X
58		OH		
59			OH	
60		OH		
61		OH	OH	
62		OH	OH	
63		OH	OCH_3	
64		OH	OCH_3	
65			OH	
66		OH		
67		OH	OH	
68		OH	OH	
69		OH	OH	
70		OH	OH	

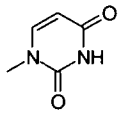
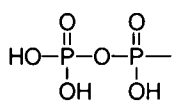
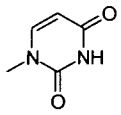
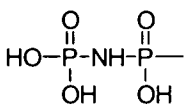
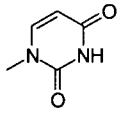
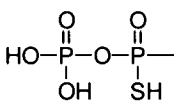
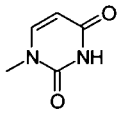
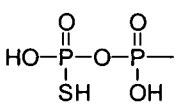
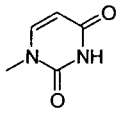
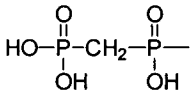
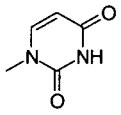
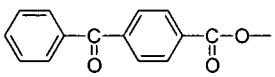
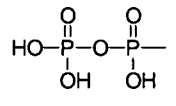
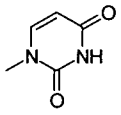
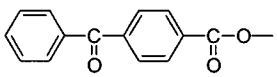
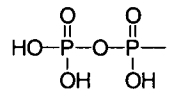
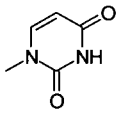
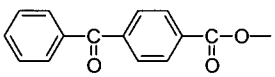
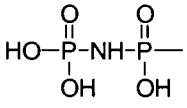
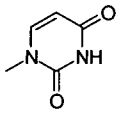
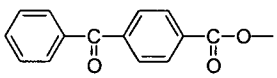
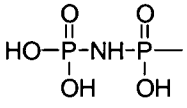
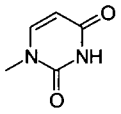
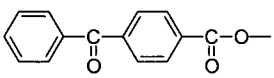
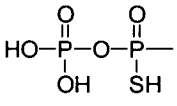
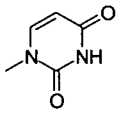
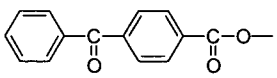
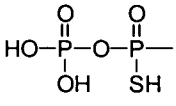
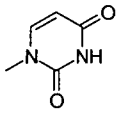
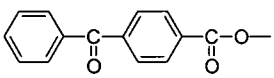
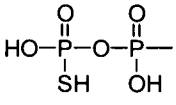
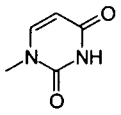
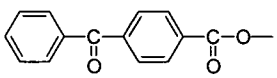
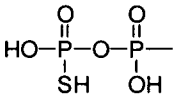
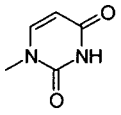
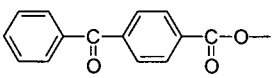
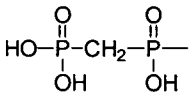
Cpd	B^1	R^1	R^2	X
71			OH	
72		OH		
73		OH	OH	
74		OH	OH	
75		OH	OH	
76		OH	OH	
77		OH	OH	
78		OH	OCH_3	
79		OH	OCH_3	
80		OH	OCH_3	
81		OH	OCH_3	
82		OH	OCH_3	
83			OH	

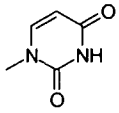
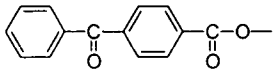
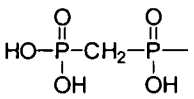
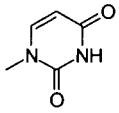
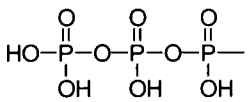
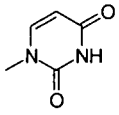
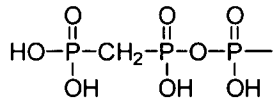
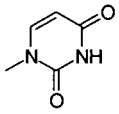
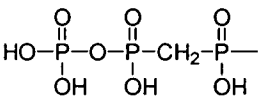
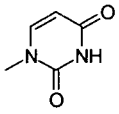
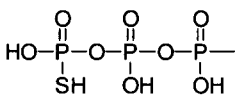
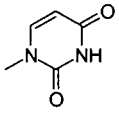
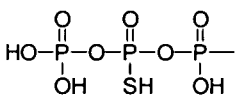
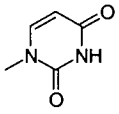
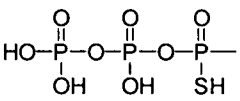
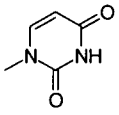
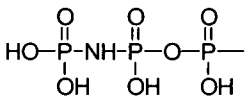
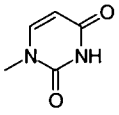
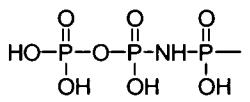
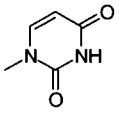
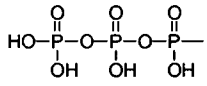
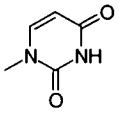
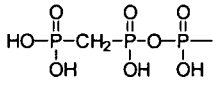
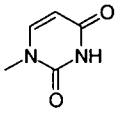
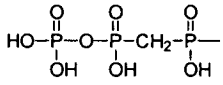
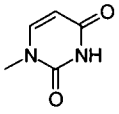
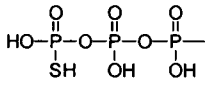
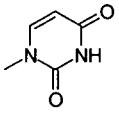
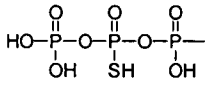
Cpd	B^1	R^1	R^2	X
84		OH		
85			OH	
86		OH		
87			OH	
88		OH		
89			OH	
90		OH		
91			OH	
92		OH		
93		OH	OH	
94		OH	OH	
95		OH	OH	
96		OH	OH	

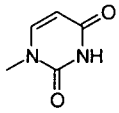
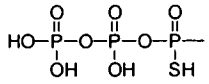
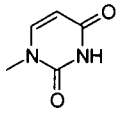
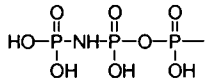
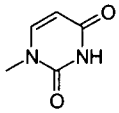
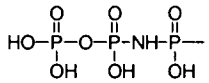
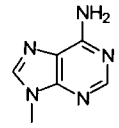
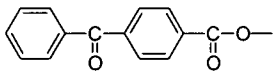
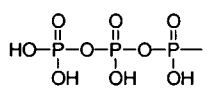
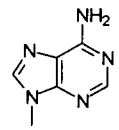
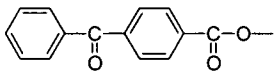
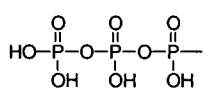
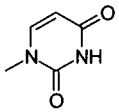
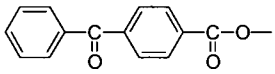
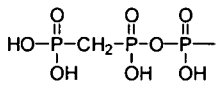
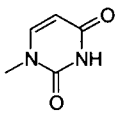
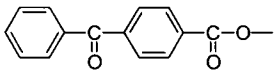
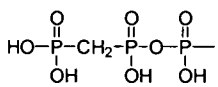
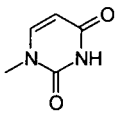
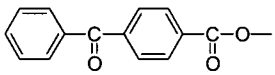
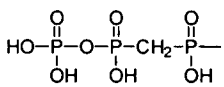
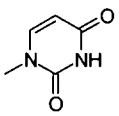
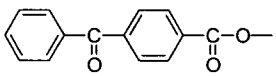
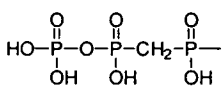
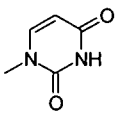
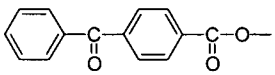
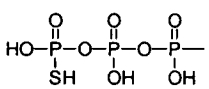
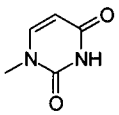
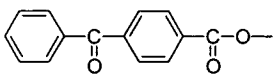
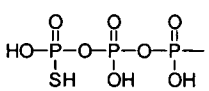
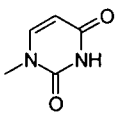
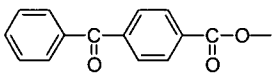
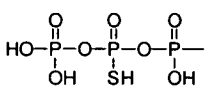
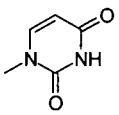
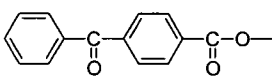
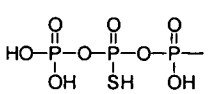
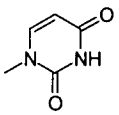
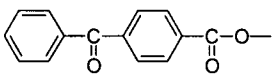
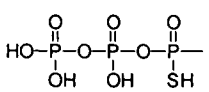
Cpd	B^1	R^1	R^2	X
97		OH	OH	
98		OH	OH	
99		OH	OH	
100		OH	OH	
101		OH	OCH_3	
102		OH	OCH_3	
103		OH	OCH_3	
104		OH	OCH_3	
105		OH	OCH_3	
106		OH	OCH_3	
107		OH	OCH_3	
108		OH	OCH_3	
109			OH	

Cpd	B^1	R^1	R^2	X
110		OH		
111			OH	
112		OH		
113			OH	
114		OH		
115			OH	
116		OH		
117			OH	
118		OH		
119			OH	
120		OH		
121			OH	
122		OH		

Cpd	B^1	R^1	R^2	X
123			OH	
124		OH	OH	
125		OH	OH	
126		OH	OCH_3	
127		OH	OCH_3	
128			OH	
129		OH		
130			OH	
131		OH		
132		OH	OH	
133		OH	OH	
134		OH	OH	
135		OH	OH	
136		OH	OH	

Cpd	B^1	R^1	R^2	X
137		OH	OCH_3	
138		OH	OCH_3	
139		OH	OCH_3	
140		OH	OCH_3	
141		OH	OCH_3	
142			OH	
143		OH		
144			OH	
145		OH		
146			OH	
147		OH		
148			OH	
149		OH		
150			OH	

Cpd	B^1	R^1	R^2	X
151		OH		
152		OH	OH	
153		OH	OH	
154		OH	OH	
155		OH	OH	
156		OH	OH	
157		OH	OH	
158		OH	OH	
159		OH	OH	
160		OH	OCH_3	
161		OH	OCH_3	
162		OH	OCH_3	
163		OH	OCH_3	
164		OH	OCH_3	

Cpd	B^1	R^1	R^2	X
165		OH	OCH_3	
166		OH	OCH_3	
167		OH	OCH_3	
168			OH	
169		OH		
170			OH	
171		OH		
172			OH	
173		OH		
174			OH	
175		OH		
176			OH	
177		OH		
178			OH	

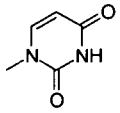
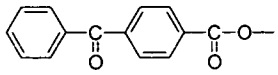
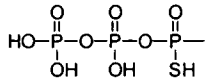
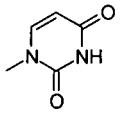
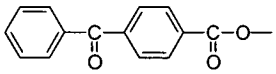
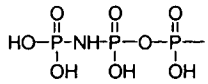
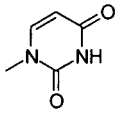
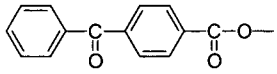
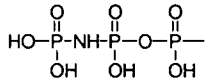
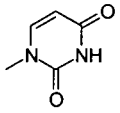
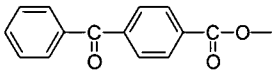
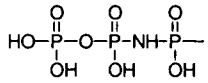
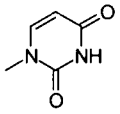
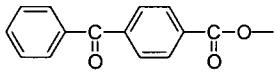
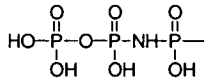
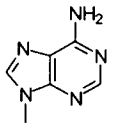
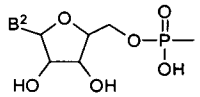
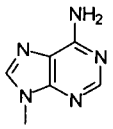
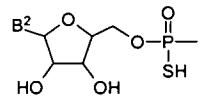
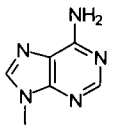
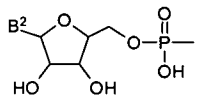
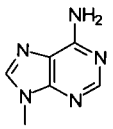
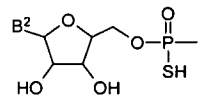
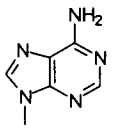
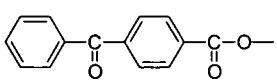
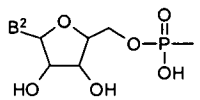
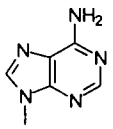
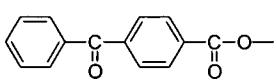
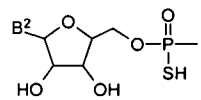
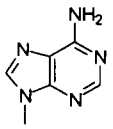
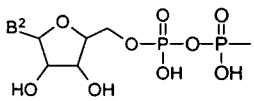
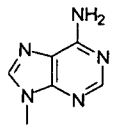
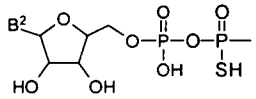
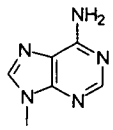
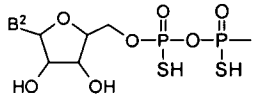
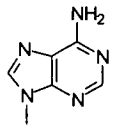
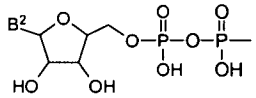
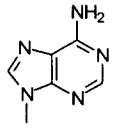
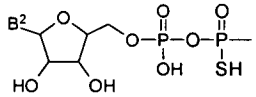
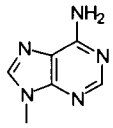
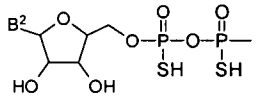
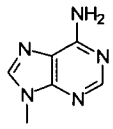
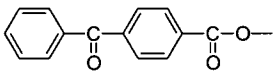
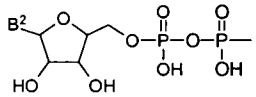
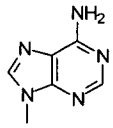
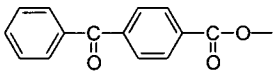
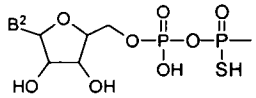
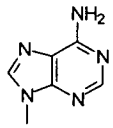
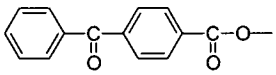
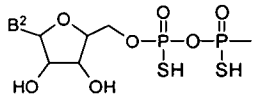
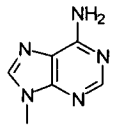
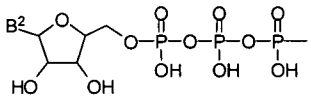
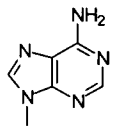
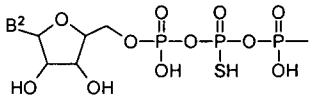
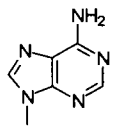
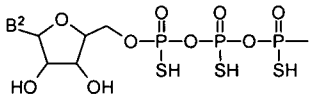
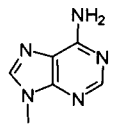
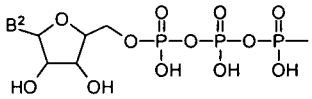
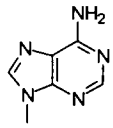
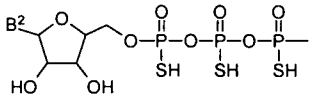
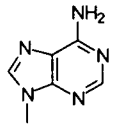
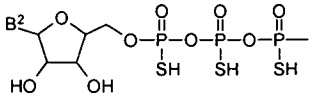
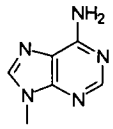
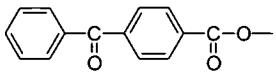
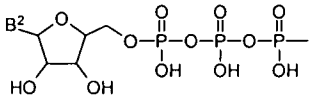
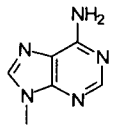
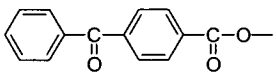
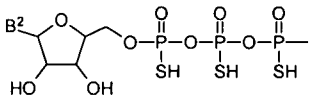
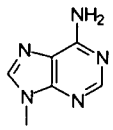
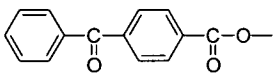
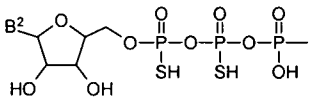
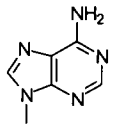
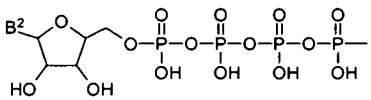
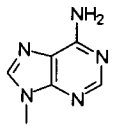
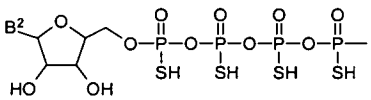
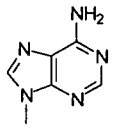
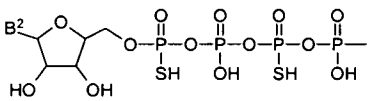
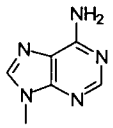
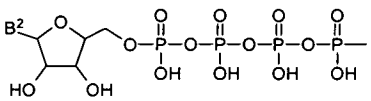
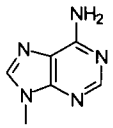
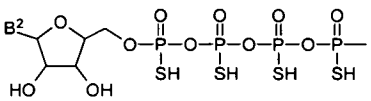
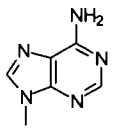
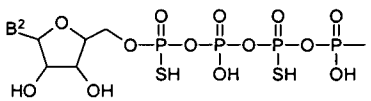
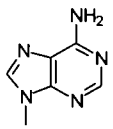
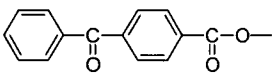
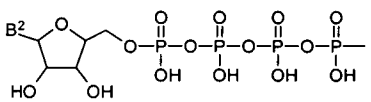
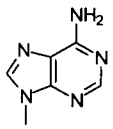
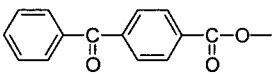
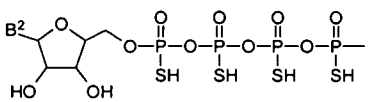
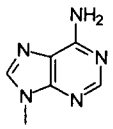
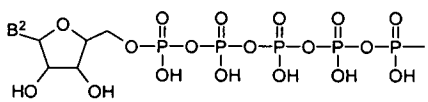
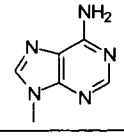
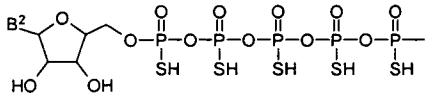
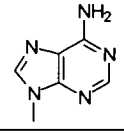
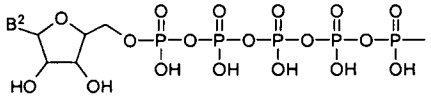
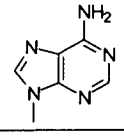
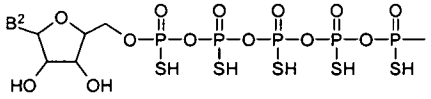
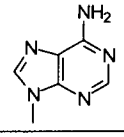
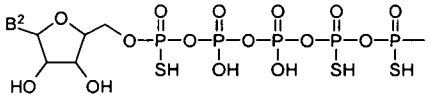
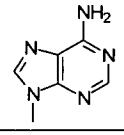
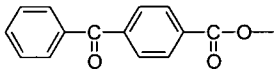
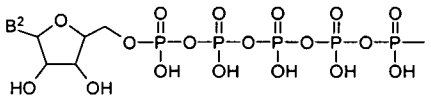
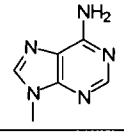
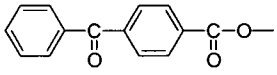
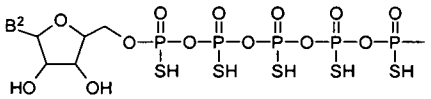
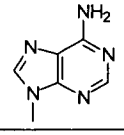
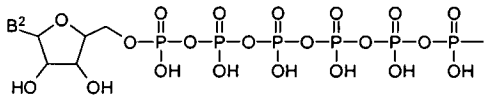
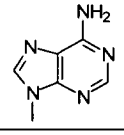
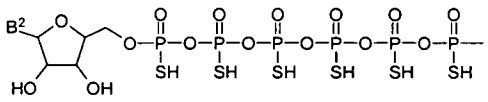
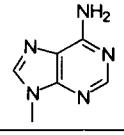
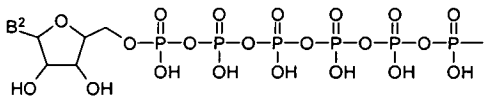
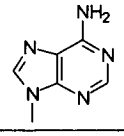
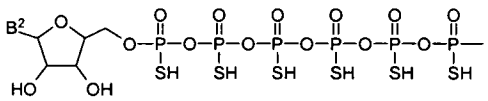
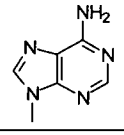
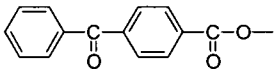
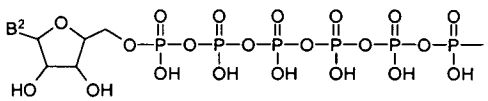
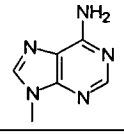
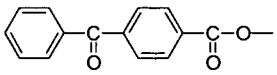
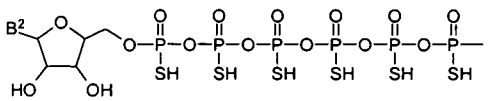
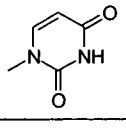
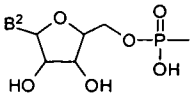
Cpd	B^1	R^1	R^2	X
179		OH		
180			OH	
181		OH		
182			OH	
183		OH		

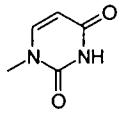
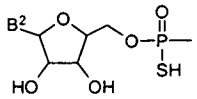
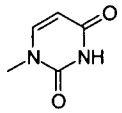
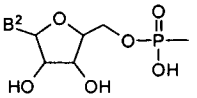
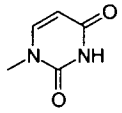
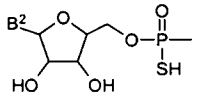
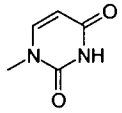
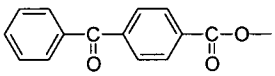
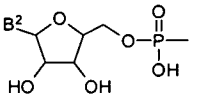
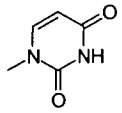
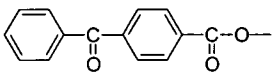
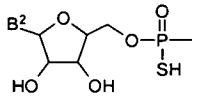
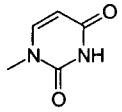
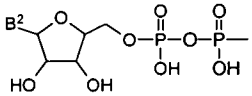
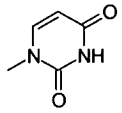
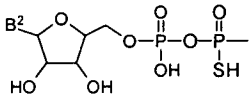
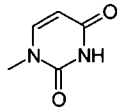
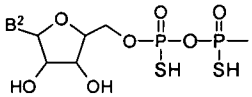
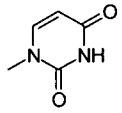
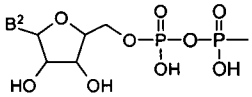
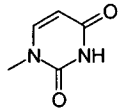
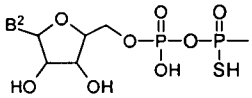
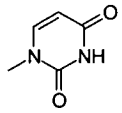
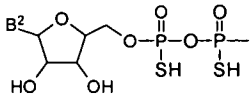
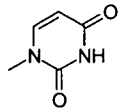
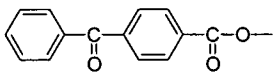
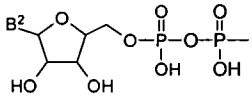
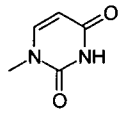
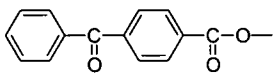
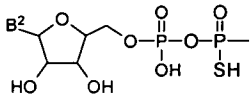
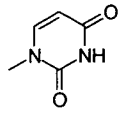
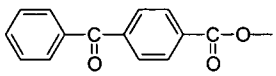
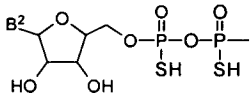
Table B

Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
184		OH	OH	
185		OH	OH	
186		OH	OCH_3	
187		OH	OCH_3	
188			OH	
189			OH	
190		OH	OH	

Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
191		OH	OH	
192		OH	OH	
193		OH	OCH_3	
194		OH	OCH_3	
195		OH	OCH_3	
196			OH	
197			OH	
198			OH	
199		OH	OH	
200		OH	OH	
201		OH	OH	
202		OH	OCH_3	
203		OH	OCH_3	

Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
204		OH	OCH_3	
205			OH	
206			OH	
207			OH	
208		OH	OH	
209		OH	OH	
210		OH	OH	
211		OH	OCH_3	
212		OH	OCH_3	
213		OH	OCH_3	
214			OH	
215			OH	
216		OH	OH	

Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
217		OH	OH	
218		OH	OCH_3	
219		OH	OCH_3	
220		OH	OCH3	
221			OH	
222			OH	
223		OH	OH	
224		OH	OH	
225		OH	OCH_3	
226		OH	OCH_3	
227			OH	
228			OH	
229		OH	OH	

Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
230		OH	OH	
231		OH	OCH_3	
232		OH	OCH_3	
233			OH	
234			OH	
235		OH	OH	
236		OH	OH	
237		OH	OH	
238		OH	OCH_3	
239		OH	OCH_3	
240		OH	OCH_3	
241			OH	
242			OH	
243			OH	

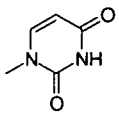
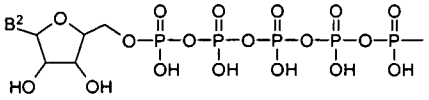
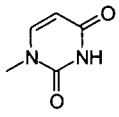
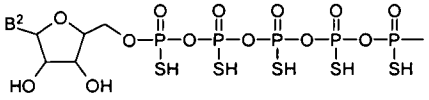
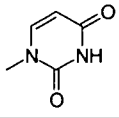
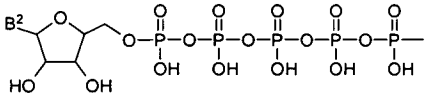
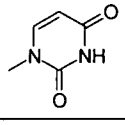
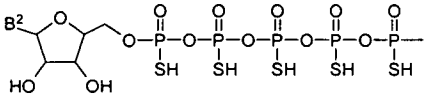
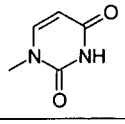
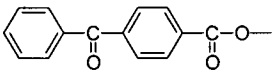
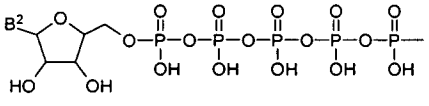
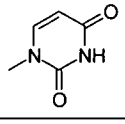
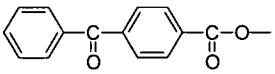
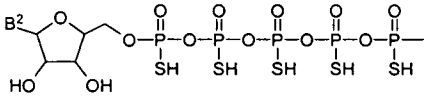
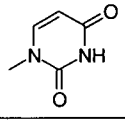
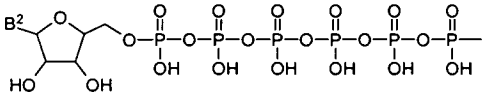
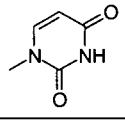
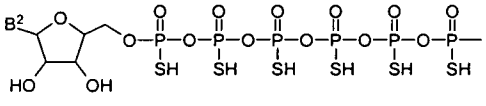
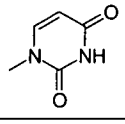
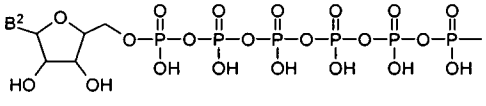
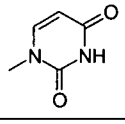
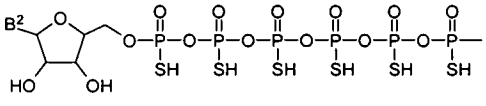
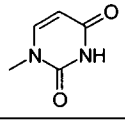
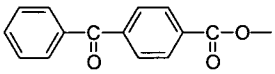
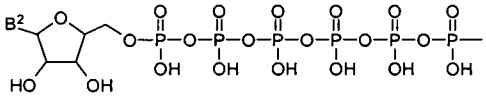
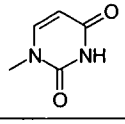
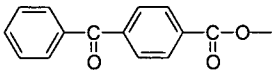
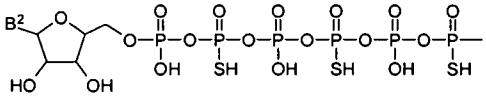
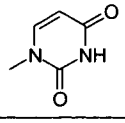
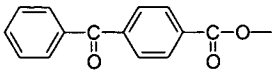
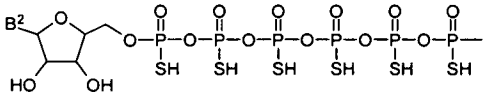
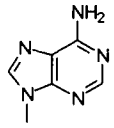
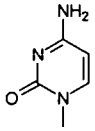
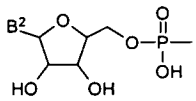
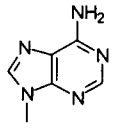
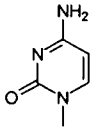
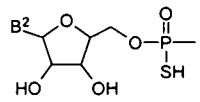
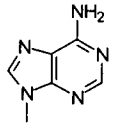
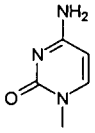
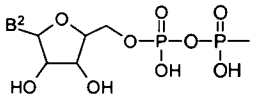
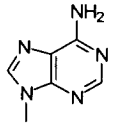
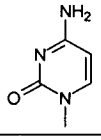
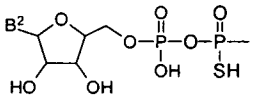
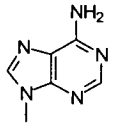
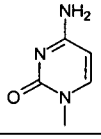
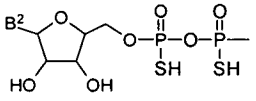
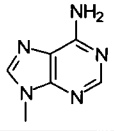
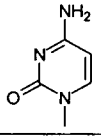
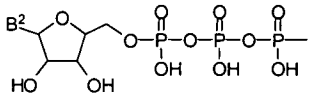
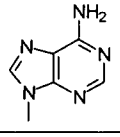
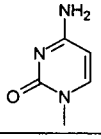
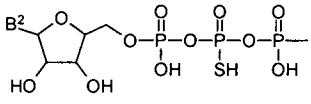
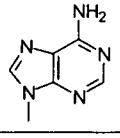
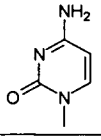
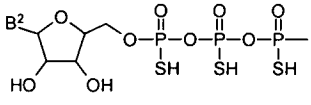
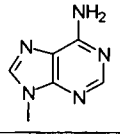
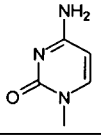
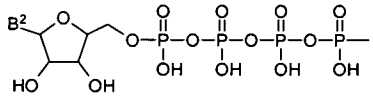
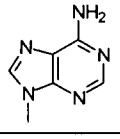
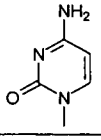
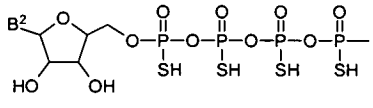
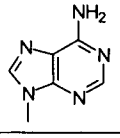
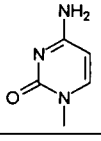
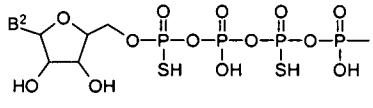
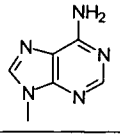
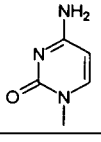
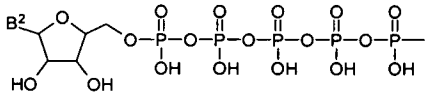
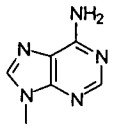
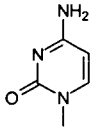
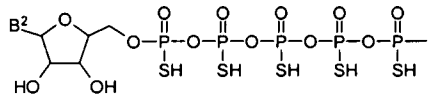
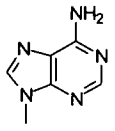
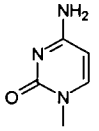
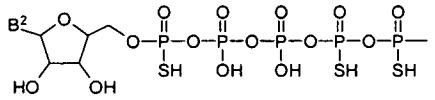
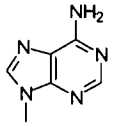
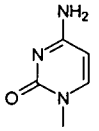
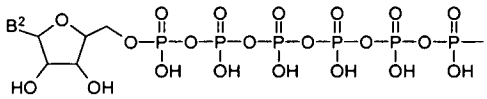
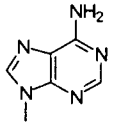
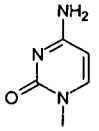
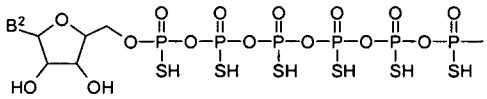
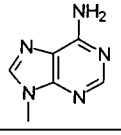
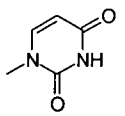
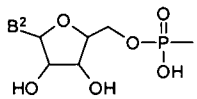
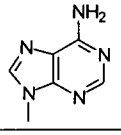
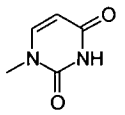
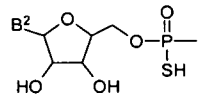
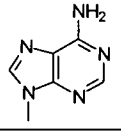
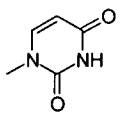
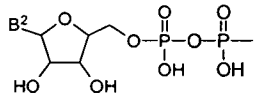
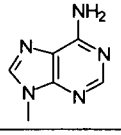
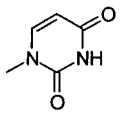
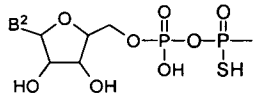
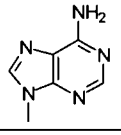
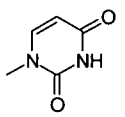
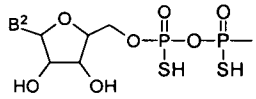
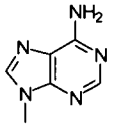
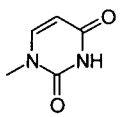
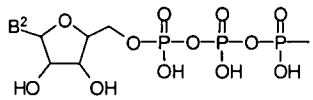
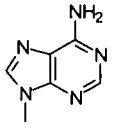
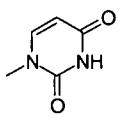
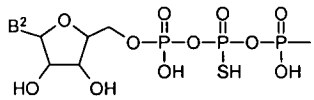
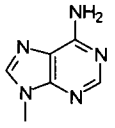
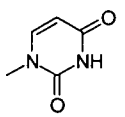
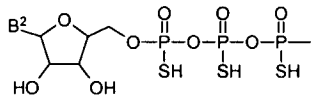
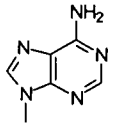
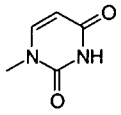
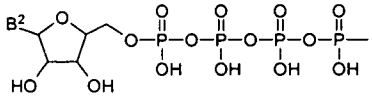
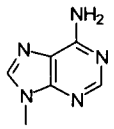
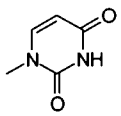
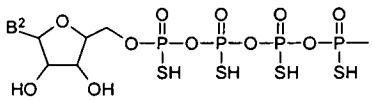
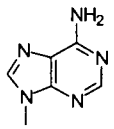
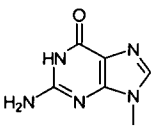
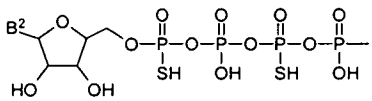
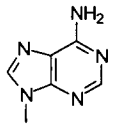
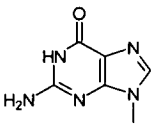
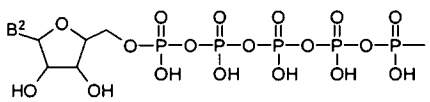
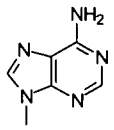
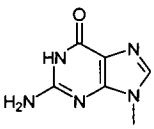
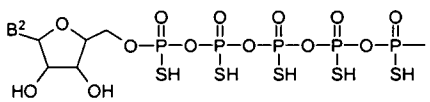
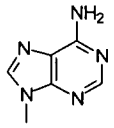
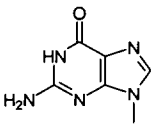
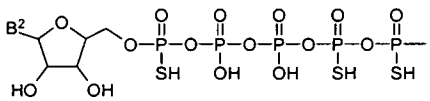
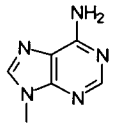
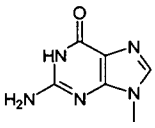
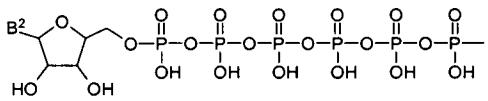
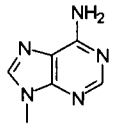
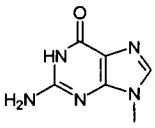
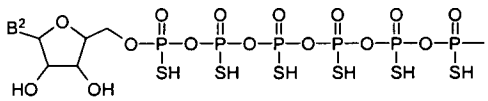
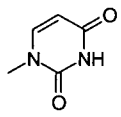
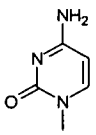
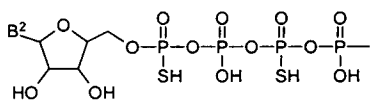
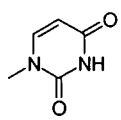
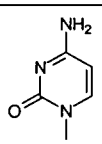
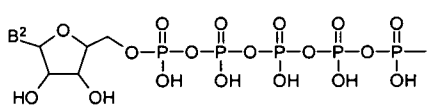
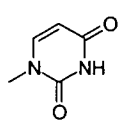
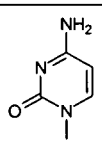
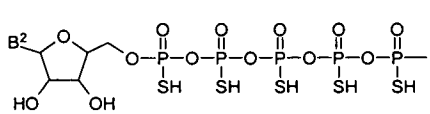
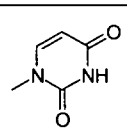
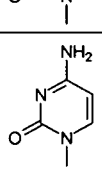
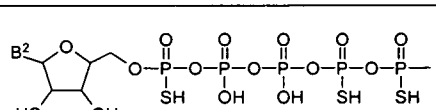
Cpd	B^1	R^1	R^2	X wherein $B^2 = B^1$
244		OH	OH	
245		OH	OH	
246		OH	OCH_3	
247		OH	OCH_3	
248			OH	
249			OH	
250		OH	OH	
251		OH	OH	
252		OH	OCH_3	
253		OH	OCH_3	
254			OH	
255			OH	
256			OH	

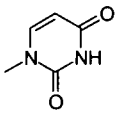
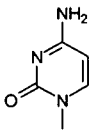
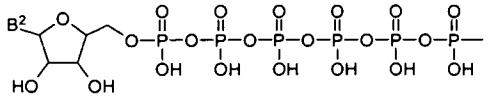
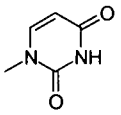
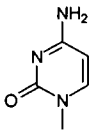
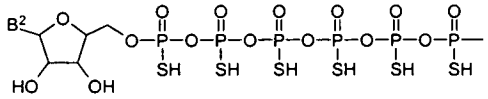
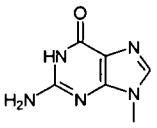
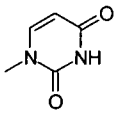
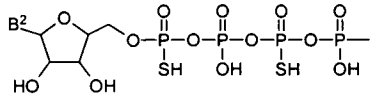
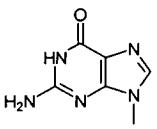
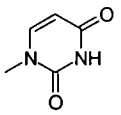
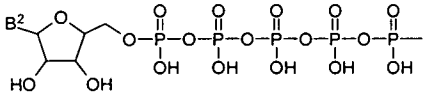
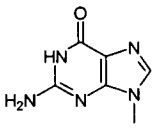
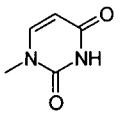
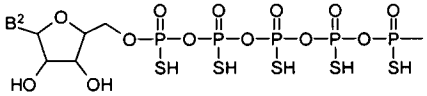
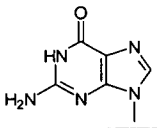
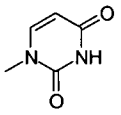
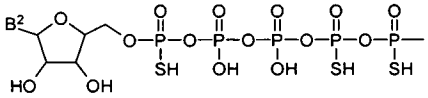
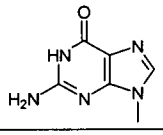
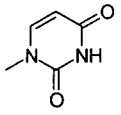
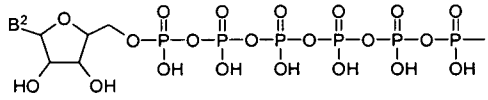
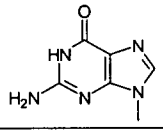
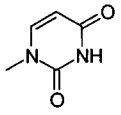
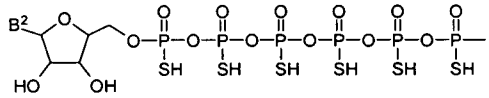
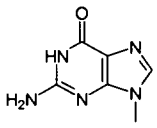
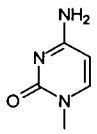
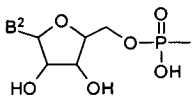
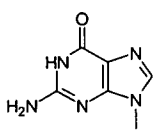
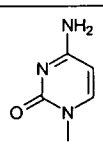
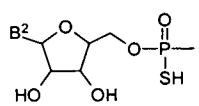
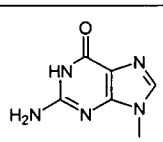
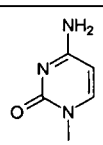
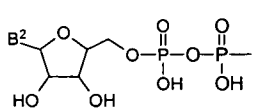
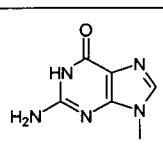
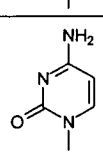
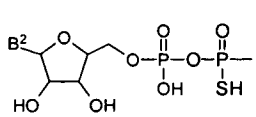
Table C

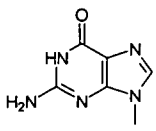
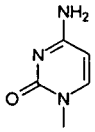
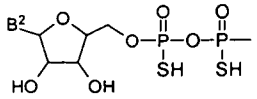
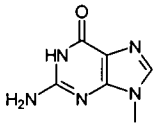
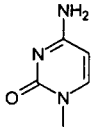
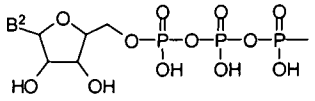
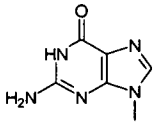
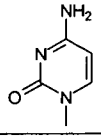
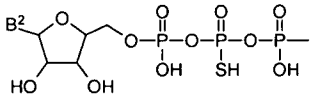
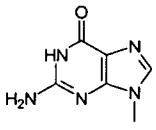
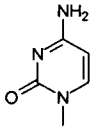
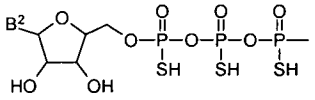
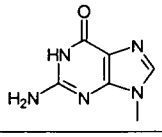
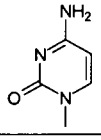
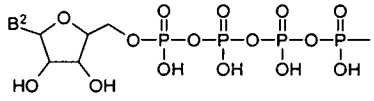
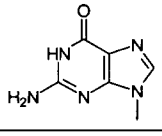
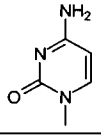
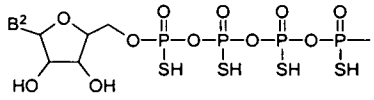
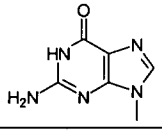
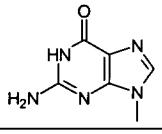
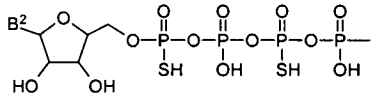
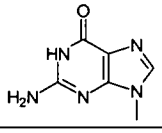
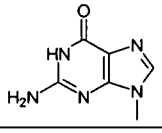
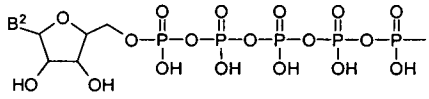
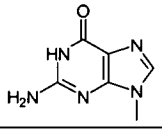
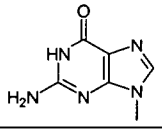
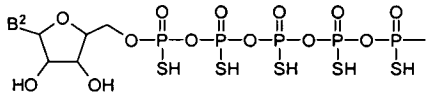
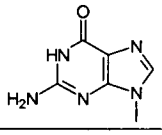
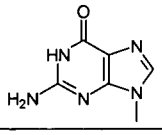
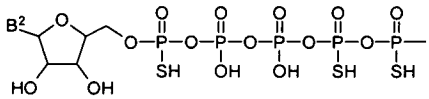
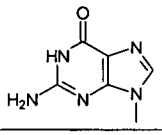
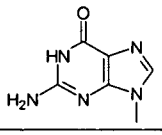
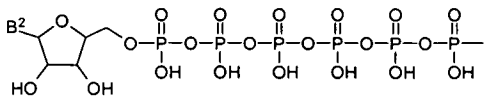
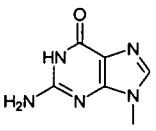
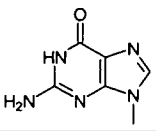
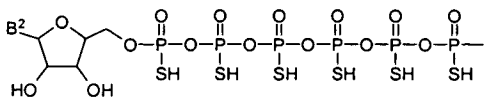
Cpd	B^1	B^2	R^1	R^2	X

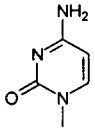
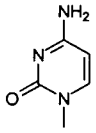
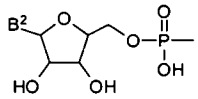
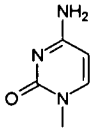
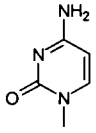
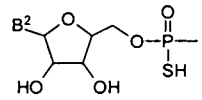
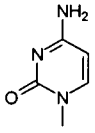
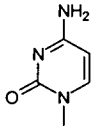
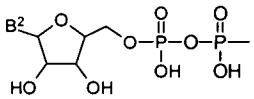
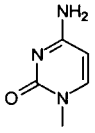
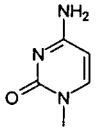
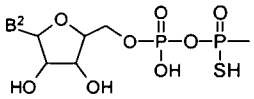
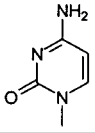
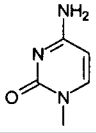
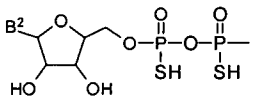
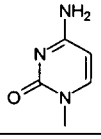
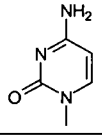
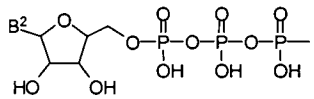
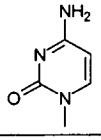
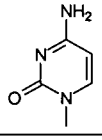
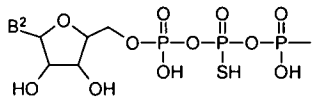
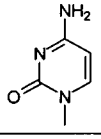
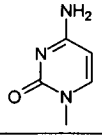
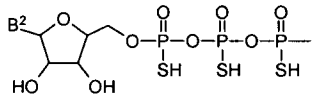
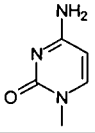
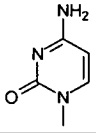
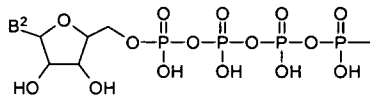
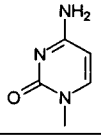
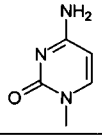
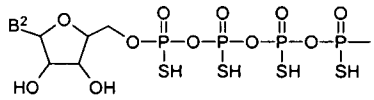
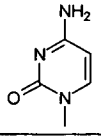
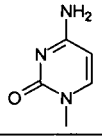
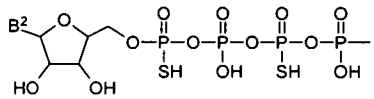
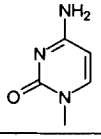
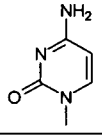
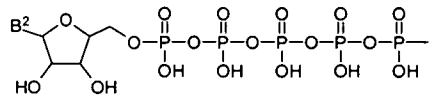
Cpd	B^1	B^2	R^1	R^2	X
257			OH	OH	
258			OH	OH	
259			OH	OH	
260			OH	OH	
261			OH	OH	
262			OH	OH	
263			OH	OH	
264			OH	OH	
265			OH	OH	
266			OH	OH	
267			OH	OH	
268			OH	OH	

Cpd	B^1	B^2	R^1	R^2	X
269			OH	OH	
270			OH	OH	
271			OH	OH	
272			OH	OH	
273			OH	OH	
274			OH	OH	
275			OH	OH	
276			OH	OH	
277			OH	OH	
278			OH	OH	
279			OH	OH	
280			OH	OH	

Cpd	B^1	B^2	R^1	R^2	X
281			OH	OH	
282			OH	OH	
283			OH	OH	
284			OH	OH	
285			OH	OH	
286			OH	OH	
287			OH	OH	
288			OH	OH	
289			OH	OH	
290			OH	OH	
291			OH	OH	
292			OH	OH	

Cpd	B^1	B^2	R^1	R^2	X
293			OH	OH	
294			OH	OH	
295			OH	OH	
296			OH	OH	
297			OH	OH	
298			OH	OH	
299			OH	OH	
300			OH	OH	
301			OH	OH	
302			OH	OH	
303			OH	OH	
304			OH	OH	

Cpd	B^1	B^2	R^1	R^2	X
305			OH	OH	
306			OH	OH	
307			OH	OH	
308			OH	OH	
309			OH	OH	
310			OH	OH	
311			OH	OH	
312			OH	OH	
313			OH	OH	
314			OH	OH	
315			OH	OH	
316			OH	OH	

Cpd	B^1	B^2	R^1	R^2	X
317			OH	OH	
318			OH	OH	
319			OH	OH	
320			OH	OH	
321			OH	OH	
322			OH	OH	
323			OH	OH	
324			OH	OH	
325			OH	OH	
326			OH	OH	
327			OH	OH	
328			OH	OH	

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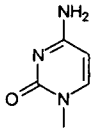
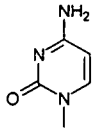
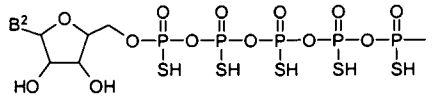
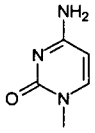
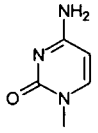
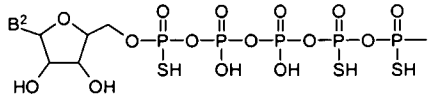
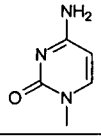
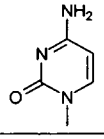
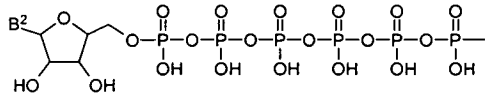
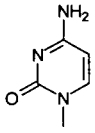
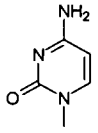
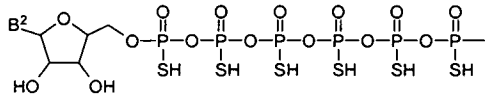
329			OH	OH	
330			OH	OH	
331			OH	OH	
332			OH	OH	

The invention will be more readily understood by reference to the following examples and figures, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A to 1D represent graphs plotting the production of TSP-1 by human monocyte-derived DCs in response to nucleotides. (A) DCs were stimulated for various periods of time with either ATPγS (100 µM), PGE_2 (500 nM) or LPS (100 ng/ml). (B) DCs were challenged with various concentrations of ATPγS or PGE_2 during 24 h. (C) DCs were challenged with various concentrations of ATPγS or ATP during 24 h. (D) DCs were stimulated during 24 h with ATPγS, ARC-67085MX, BzATP and ADPβS at 100 µM and with ATP, ADP, UTP, UDP and UDP-glucose at 300 µM. Supernatants of treated DCs were harvested for ELISA measurements of human TSP-1. Results are expressed as ng/10^6 cells/ml and represent the mean ± range of duplicate points in one representative out of three (A) or two (B,C,D) independent experiments.

Figures 2A and 2B represent the results of Western Blotting experiments for proteomics analysis of TSP-1 production by DCs in response to ATPγS. (A) DCs were either untreated (Ctrl) or treated with ATPγS (100 µM) or PGE_2 (500 nM) for different periods of time in complete medium. Supernatants were collected at the end of incubation to perform Western blotting using an anti-TSP-1 rabbit polyclonal antibody (1:200). (B) DCs were treated with ATPγS (100 µM) for 4, 8 or 24 h in serum-free medium. At the end of incubation, supernatants were collected, proteins were extracted and loaded on a 8% SDS-polyacrylamide gel. After colloidal Coomassie blue staining, proteins of interest (black arrows A and B) were excised and in-gel digested by trypsin.

Figures 3A to 3G represent graphs plotting the effect of supernatants from ATP-treated DCs on naive T $CD4^+$ cells. Naive T $CD4^+$ cells were partially activated (A) - with coated anti-CD3 - or fully

activated (C) - with coated anti-CD3 and soluble anti-CD28 - in the presence of supernatant from non-treated DCs (CTRL) or ATP-treated DCs (ATP) during 48 h. Cell suspensions were then additionally incubated for 16 h in presence of [^{3}H]thymidine (1 µCi/well). The effect of human recombinant TSP-1 (7 µg/ml) was also tested on thymidine incorporation of partially activated (B) or fully activated (D) naive T CD4$^+$ cells in fresh medium. (E) Supernatants from non-treated DCs (left) or ATP-treated DCs (right) were previously incubated 2 h at room temperature in the presence (black bars) or the absence (white bars) of neutralizing anti-TSP-1 mAb at 25 µg/ml (clone C6.7; that selectively interferes with TSP-1/CD47 interaction). Fully activated T CD4$^+$ cells were incubated in the presence of these DCs supernatants for thymidine incorporation experiments. (F,G) Fully activated T CD4$^+$ cells were incubated in the presence of supernatants from non-treated or ATP-treated DCs. After 48 h, IFN-γ (F) or IL-10 (G) levels were measured by ELISA. Data represent the mean ± SD of triplicate experimental points obtained in one representative experiment out of five (A, B, C, D) or two (E, F, G).

Figures 4A and 4B represent respectively the result of Western Blotting experiments and a graph showing the synergy between ATP/ATPγS and IFN-γ on the functional expression of IDO. (A) DCs were either untreated or treated with ATP (300 µM) or ATPγS (100 µM) alone or in the presence of IFN-γ (10 or 100 U/ml) for 24 h in complete medium. The same amount of total protein were analyzed by Western blotting with an anti-human IDO rabbit polyclonal antibody. (B) After a similar treatment with ATP (300 µM) or ATPγS (100 µM), DCs were washed and incubated for five additional hours in red-phenol-free RPMI supplemented with 300 µM L-tryptophan. Kynurenine levels were determined in each supernatants by HPLC. Data were obtained in an experiment performed in duplicate and representative of three independent experiments.

EXAMPLES

In these examples, ATP and derivatives thereof are shown to induce immunotolerance through their action on DCs. Microarray analysis of ATP-stimulated human DCs revealed *inter alia* a drastic up-regulation of two genes encoding mediators involved in immunotolerance: thrombospondin-1 (TSP-1) and indoleamine 2,3-dioxygenase (IDO). The release of TSP-1 by DCs in response to ATP was confirmed at the protein level by ELISA, immunodetection and mass spectrometry analysis and has a tolerance inducing effect through TSP-1/CD47 interaction. The present pharmacological data shows the involvement of $P2Y_{11}$ receptor in this ATP-mediated TSP-1 secretion. It is demonstrated also that ATP significantly potentiates the up-regulation of IDO - a negative regulator of T lymphocyte proliferation - and kynurenine production initiated by IFN-γ in human DCs.

Thus, extracellular ATP released from damaged cells and previously considered as danger signal is also a potent regulator of mediators playing key roles in immune tolerance. Consequently nucleotides derivatives are useful tools for DC-based immunotherapies and are particularly useful either alone or combined with immunogenic agents for inducing immunotolerance.

Methods

Reagents - ATP, adenosine 5'-*O*-(3-thiotriphosphate) (ATPγS), adenosine 5'-*O*-(2-thiodiphosphate) (ADPβS), 2'- 3'-*O*-(4-benzoylbenzoyl)-ATP (BzATP), PGE_2, LPS, forskolin, A23187, apyrase, adenosine deaminase and pertussis toxin were obtained from Sigma (St. Louis, MO). 2-propylthio-β,γ-dichloromethylene-D-ATP (AR-C67085MX) was a generous gift from Drs. J.D. Turner and D. Shah (AstraZeneca, Wilmington, DE). The anti-CD3 mAb OKT3 (Orthoclone OKT3) was provided by Janssen-Cilag (Berchem, Belgium), and the anti-CD28 mAb (clone CD28.2) was supplied by BD PharMingen (San Diego, CA). [^{3}H]thymidine (25 Ci/mmol) was from Moravek Biochemicals (Brea, CA). The C6.7 mAb that interferes with the ligation of human TSP-1 to CD47 was from LabVision (Fremont, CA).

Preparation of Monocyte-Derived Dendritic Cells – Immature human DCs were derived from adherent peripheral blood monocytes obtained from buffy coats of healthy volunteers donors as described previously (Romani N et al., J Exp Med. 1994;180:83-93). After 5 or 6 days of culture in the presence of 800 U/ml of GM-CSF and 500 U/ml of IL-4, cells were replated at 1×10^6 cells/ml in 24-multiwells in complete medium with GM-CSF and IL-4. Nucleotides or other tested agents were then added for different periods of time.

Flow cytometric analysis - The purity of each cell preparation was evaluated using FITC-conjugated anti-human CD3, CD4, CD8, CD45RA (for lymphocytes) and CD1a, CD3, CD14, CD80, CD83, CD86 and HLA-DR (for DCs) supplied by PharMingen (San Diego, CA) on 2.10^5 cells in 100 µl of PBS with 0.1% sodium azide of 30 min in the dark at 4°C. Cells were washed with 1 ml of PBS and analyzed on a FACSort® (Becton Dickinson, Franklin Lakes, NJ). Data were analyzed using Cellquest software (Becton Dickinson, Franklin Lakes, NJ); the number of events was at least 10,000.

Microarray analysis – Immature DCs were stimulated by ATPγS (100 µM) for 6 h in the complete RPMI medium. The stimulation was stopped by addition of TRIZOL® reagent (Life Technologies, Groningen, Nederland). RNA was extracted using Trizol reagent (Life Technologies) and purified on a RNeasy kit column (Qiagen, Hilden, Germany). Amplified antisense RNA (5 µg) was labeled by reverse transcription using SuperScript II (Invitrogen, Carlsbad, CA) and hybridized to an array containing on average 21,000 human cDNA fragments (VIB microarray Facility, Leuven, Belgium) (Puskas LG, et al. Biotechniques. 2002;32:1330-1334, 1336, 1338, 1340). The experiments were made including colorflip with two preparations of DCs from two independent donors.

ELISA - Human thrombospondin (TSP-1) was measured in DC supernatants by ELISA using commercially available kit from Chemicon International (Temecula, USA). IL-10 and IFN-□ were measured in supernatants by ELISA using commercially available kits from BioSource (Nivelles, Belgium).

Western blotting experiments - Immature DCs were seeded (10^6 cells/ml) and treated for different periods of time with or without different agents (PGE_2, ATP, ATP□S and/or IFN-□). For

TSP-1 detection, supernatants were collected at the end of incubation, and centrifuged to remove non-adherent cells. 40 µl of each supernatant was loaded on a 7.5% SDS-polyacrylamide gel. For IDO detection, cells were washed once with PBS and lysed on ice in 150 µl of Laemmli buffer. The protein concentration was determined using the method of Minamide and Bamburg (Minamide LS et al., Anal Biochem. 1990;190:66-70.). The same amount of protein (25 µg) for each condition was loaded and separated on a 12 % SDS-polyacrylamide gel. Proteins were then transferred overnight at 60 V and 4 °C onto a nitrocellulose membrane using 20 mM Tris, 154 mM glycine, 20% (v/v) methanol as a transfer buffer. Immunodetection was achieved with anti-TSP-1 (Abcam, UK,1:200) or anti-IDO antibody (1:500, a generous gift from Pr. B. van den Eynde), antibodies using the Western Lightning™ Chemiluminescence Reagent Plus detection system (PerkinElmer, Belgium) with a biotinylated secondary rabbit antibody (Amersham Bioscience).

Mass Spectrometry Analysis- Immature DCs were washed four times with serum-free medium, seeded (10^6 cells/ml) in RPMI without serum and treated for different periods of time with or without ATPγS (100 µM). Supernatants (4 ml/condition) were collected and centrifuged to remove floating cells. Proteins were extracted from supernatants with StrataClean™ resin (Stratagene, La Jolla, CA) and loaded on a 8% SDS-polyacrylamide gel. After colloidal Coomassie blue staining, proteins of interest were excised and in-gel digested using trypsin (Promega, Madison, WI) (Shevchenko A, et al., Anal Chem. 1996;68:850-858). Mass spectrometry analysis was performed on a Q-TOF Ultima Global mass spectrometer equipped with a MALDI source (Micromass, Manchester, UK). Microsequencing was performed by Argon-induced fragmentation after selection of the parent ion.

T cell proliferation assays – DCs were prepared as described above and were stimulated by ATP 300 µM in complete medium. After 24 h, supernatants of untreated and ATP-treated DCs were collected. In parallel, naive T $CD4^+$ lymphocytes were prepared using T $CD4^+$ isolation kit II and CD45RO microbeads (Miltenyi Biotec, Bergish Gladbach, Germany) as previously described (la Sala A, et al., J Immunol. 2001;166:1611-1617). The $CD4^+$ $CD45RA^+$ T cells (2.10^5/well) were resuspended in 100 µl of fresh medium and mixed with 100 µl of DC supernatant. Then, they were activated in flat-bottom 96-well plates precoated with the anti-CD3 mAb (10 µg/ml) alone or in combination with soluble anti-CD28 mAb (1 µg/ml). After 48 h of culture, proliferation was assessed by [^{3}H]-thymidine (1 µCi/well) (ICN Biochemicals, Belgium) uptake during the next 16 h. Each experimental condition was tested in triplicate.

Kynurenine measurements - DCs were stimulated with IFN-γ (10 and 100 U/ml) alone or in combination with ATP or ATPγS during 24 h. Then cells were washed and resuspended in red-phenol free complete medium supplemented with 300 µM L-tryptophan. After 5 h supernatants were collected and kynurenine concentration was quantified by HPLC (Takikawa O, et al., J Biol Chem. 1986;261:3648-3653). Culture supernatants (400 µl) were extracted with 80 µl of trichloroacetic acid 10 %, the precipitate was removed by centrifugation, and the supernatant was diluted in the initial mobile phase, composed of deionized water, 5% (v/v) methanol, 1% (v/v) acetic acid and 5mM of hexane sulfonic acid. Samples were injected onto a µBondapak C18 reverse

phase (3.9 x 300 mm, Waters, Milford, MA) and eluted with a linear gradient of methanol (5-40% over 35 min) at 1 ml/min. Absorbance was measured at 370 nm and compared against a standard curve of L-kynurenine.

Statistical analysis – Student's t-test were performed using Prism software (GraphPad, CA).

Results

Identification of major target genes of adenosine triphosphates in human DCs

Microarray experiments were performed to select relevant target genes in the gene expression profile of adenosine triphosphates in human monocyte-derived DCs. ATPγS, which is more resistant to degradation by ectonucleotidases, was used instead of ATP to avoid additional gene regulations due to its degradation products, more particularly ADP and adenosine. Immature monocyte-derived DCs (10^6/ml) have been incubated during 6 h with or without ATPγS 100 µM. RNAs were then extracted, amplified and labeled to hybridize an array containing 21.005 human sequences. The experiment has been reproduced for two independent donors. From these experiments, it appeared that about 200 sequences were at least two times up-regulated or down-regulated in response to ATPγS. Table 1 shows regulated sequences reflecting DC maturation: up-regulation of CXCR4, CCR7, CD83, CD86 mRNAs, and down-regulation of CCR1, CCR5 and DC-STAMP mRNAs (Table 1).

Table 1. ATPγS-regulated markers of DC maturation.

Origin	GeneBank	*Unigene*	Name	Description	*Ratio*
RZPD	AA479357	Hs.421986	CXCR4	chemokine (C-X-C motif) receptor 4	39.2
RZPD	AI682706	Hs.1652	CCR7	chemokine (C-C motif) receptor 7	3.9
RZPD	AA443748	Hs.79197	CD83	CD83 antigen	3.5
RZPD	H16746	Hs.27954	CD86	CD86 antigen	1.8
RZPD	AI268407	Hs.211458	DC-STAMP	DC-specific transmembrane protein	0.18
RZPD	AI417775	Hs.511796	CCR5	chemokine (C-C motif) receptor 5	0.13
GS	D10925	Hs.301921	CCR1	chemokine (C-C motif) receptor 1	0.13

Table 2 shows regulated genes involved in interactions between DCs and T lymphocytes such as interleukins (IL-10 and IL-23) or chemokines (CXCL10). The following experiments focused on two major target genes up-regulated by ATPγS which are involved in immune tolerance (Urban BC et al., Proc Natl Acad Sci U S A. 2001;98:8750-8755; Avice MN, et al., J Immunol. 2000;165:4624-4631; Avice MN, et al., J Immunol. 2001;167:2459-2468; Doyen V et al., J Exp Med. 2003;198:1277-1283; Mellor AL, Munn DH. Nat Rev Immunol. 2004;4:762-774; Grohmann U Trends Immunol. 2003;24:242-248): thrombospondin-1 (TSP-1) which was clearly the most up-regulated gene and indoleamine 2,3-dioxygenase (IDO) (Table 2).

Table 2. ATPγS-regulated genes involved in DC-T lymphocyte interactions.

Origin	GeneBank	*Unigene*	Name	Description	*Ratio*
RZPD	AA043285	Hs.164226	TSP-1	thrombospondin-1	199.6
GS	AU138239	Hs.840	INDO	indoleamine-pyrrole –2,3 dioxygenase	7.8
RZPD	AA418747	Hs.98309	IL-23A	Interleukin 23, p19	3.1
GS		Hs.193717	IL-10	Interleukin 10	2.3
RZPD	AA150307	Hs.413924	CXCL10	chemokine (C-X-C motif) ligand 10	0.5

ATP is a potent activator of TSP-1 release in human DCs

TSP-1 production from stimulated DCs was quantified by ELISA. DCs were stimulated for various periods of time with ATPγS, PGE_2 and LPS. These last two agents were chosen because LPS is a major DC maturation signal and PGE_2 displays similar effects as ATP in DCs, through cAMP activation. As shown in Fig.1A and 1B, ATPγS is more efficient than PGE_2 to stimulate TSP-1 release, whereas LPS had no effect. Moreover, TSP-1 production was observed already after 3 h for ATPγS and rose to its maximum after 12 h of stimulation. At 24 h of stimulation, PGE_2 effect represents 34.8 ± 6.0% of the ATPγS effect which represents a peak of 10 µg/ml of TSP-1 (mean ± S.D. of three independent experiments). In addition, concentration-action curves of ATPγS and the physiological ligand ATP have been compared (Fig.1C). ATPγS was more potent than ATP with EC_{50} values of 6.1 ± 1.3 µM for ATPγS and 17.4 ± 3.2 µM for ATP (Fig.1C) (mean ± range of two independent experiments). Comparable TSP-1 production was observed in response to other ATP derivatives like ARC-67085MX (2-propyl-β,γ-dichloromethylene-D-ATP) and BzATP (2'- and 3'-O-(4-benzoyl-benzoyl)ATP), known to be $P2Y_{11}$ agonists. The cAMP-elevating agent forskolin (10 µM) was also able to initiate TSP-1 release (Fig. 1D) whereas a preincubation of DCs with pertussis toxin (100 ng/ml; 16 hours before stimulation with ATP) did not affect ATP-mediated TSP-1 production (data not shown). These pharmacological data support the involvement of $P2Y_{11}$ receptor in ATP-mediated TSP-1 production. Whether the strong effect of ATPγS and ATP compared to PGE_2 or forskolin on TSP-1 production was due to activation of both cAMP and phosphoinositide pathways was investigated next. Calcium-elevating agents such as UTP or calcium ionophore A23187 were not able to induce any TSP-1 production (Fig 1D and data not shown) or to potentiate PGE_2-mediated TSP-1 production (data not shown).

Proteomics analysis of nucleotide-mediated TSP-1 production by DCs

Time courses of TSP-1 secretion in response to ATPγS and PGE_2 was compared by Western blotting experiments (Fig. 2A). Three bands were detected using a polyclonal anti-TSP-1 antibody. A detectable signal (upper band A; 180 kDa) was observed after 6 h and a significant signal after 22 h of stimulation with ATPγS whereas a weak signal was obtained only after 22 h of stimulation with PGE_2.

To confirm that the bands revealed in the immunodetection experiments (Fig.2A) correspond to human TSP-1, a 8% SDS-polyacrylamide gel was performed using supernatants from unstimulated DCs and DCs stimulated for various periods of time with ATPγS. As shown in Fig. 2B, colloidal Coomassie blue staining revealed one highly modulated band (A) presenting an apparent MW

around 180,000 (band A) and another modulated band with an apparent MW of 150,000 (band B). A third lower band was slightly regulated and is likely to correspond to the lower band detected with anti-TSP-1 antibody. Intensities of bands A and B were sufficient to expect protein identification by mass spectrometry. They were excised and in-gel digested with trypsin. Peptide mass fingerprinting and microsequencing after fragmentation (sequenced peptide: N^{1114}ALWHTGNTPGQVR^{1127}) permitted to unambiguously identify bands A and B as intact human TSP-1, most probably with distinct post-translational modifications.

ATP inhibits T CD4$^+$ lymphocyte proliferation through TSP-1 release by DCs

To investigate the effect of soluble factors like TSP-1 secreted by DCs in response to ATP, the capacity of supernatants of these DCs to modulate proliferation of activated naive T CD4$^+$ cells has been evaluated (Fig. 3). T cell activation was obtained using coated anti-CD3 mAb (10 μg/ml) alone (Fig.3A and B) - to mimic TCR activation - or in combination with soluble anti-CD28 mAb (1 μg/ml) (Fig. 3C,D,E,F,G) – to mimic full activation. In both cases, supernatants from ATP-treated DCs reduce the proliferation of activated T CD4$^+$ (Fig.3A and 3C): 86.7 ± 29.7 % and 72.3 ± 25.9 % of inhibition in the presence of anti-CD3 mAb alone or the combination of anti-CD3 and anti-CD28 mAbs, respectively (mean ± S.D. of five independent experiments; $P < 0.01$ according Student's t-test). The effect of human recombinant TSP-1 (7 μg/ml) - diluted in fresh medium - on T cell proliferation was tested using the same preparation of DCs (Fig. 3B and 3D).

CD47 receptor mediates inhibitory effects of TSP-1 on T lymphocyte early activation and proliferation (Li Z et al., J Immunol. 2001;166:2427-2436.). Whether TSP-1 was one of the soluble factors responsible of the observed immunosuppressive effect mediated by ATP was evaluated: DCs supernatants were pre-incubated with an anti-human TSP-1 neutralizing antibody named C6.7 (Fig. 3E) which blocks binding of TSP-1 to CD47 receptors (Doyen V, et al., J Exp Med. 2003;198:1277-1283.). There was a consistent reduction of the ATP-mediated inhibition of T lymphocyte proliferation in the presence of anti-TSP-1 antibody (C6.7): from 71.5 ± 3.5% of inhibition to 23.3 ± 16.5% of inhibition (mean ± range from two independent experiments; $P < 0.05$ according Student's t-test). IFN-γ and IL-10 levels have been quantified by ELISA in these experiments. When T CD4$^+$ lymphocytes were incubated with supernatants from ATP-treated DCs, Th1 and Th2 cytokine production was abolished (Fig.3F and 3G). Control experiments were also performed using supernatants of ATP-treated or untreated DCs preincubated during 30 min at room temperature with apyrase (50 U/ml) and/or adenosine deaminase (5 U/ml) to avoid any effect of residual ATP or degradation products such as ADP and adenosine. The use of these enzymes separately or in combination had no effect on T CD4$^+$ proliferation and did not affect inhibition of proliferation observed with ATP-treated DC supernatants (data not shown).

ATP regulates IDO expression and activity in human DCs

IDO - an enzyme involved in the catabolism of tryptophan - was shown to be a target gene of ATPγS in our microarray experiments. RT-PCR experiments confirmed that ATPγS and ATP - but

not PGE_2 - were able to up-regulate IDO mRNA in monocyte-derived DCs (data not shown). To confirm that DCs treated with ATPγS (100 μM) express IDO protein, Western blotting experiments were performed with a rabbit polyclonal antibody against human IDO. Figure 4A shows that no detectable signal was observed with ATPγS or ATP alone whereas a signal was already detectable with 10 U/ml IFN-γ. Strikingly ATPγS 100 μM and ATP 300 μM synergize with IFN-γ, at both 10 U/ml and 100 U/ml, to upregulate IDO expression, ATPγS being more efficient.

As previously shown by other groups, expression of IDO is not always correlated with full enzymatic activity. To check its activity an HPLC-based assay of kynurenine quantification - the main product of tryptophan degradation by IDO was performed (Mellor AL, Munn DH., Nat Rev Immunol. 2004;4:762-774). Figure 4B shows that ATP or ATPγS alone do not induce a significant production of kynurenine whereas IFN-γ does. Interestingly ATP and ATPγS potentiate the effect of IFN-γ and leads to an increase of kynurenine production of 168.8 ± 5.4% and 201.4± 3.0% respectively compared to IFN-γ 100 U/ml alone (mean ± S.D. of three independent experiments; $P < 0.01$ according Student's t-test).

Discussion

The present experiments demonstrate the link between the semi-mature state of DC and immune tolerance by focusing analysis of gene profiling experiments on genes associated with tolerance. More particularly, TSP-1 and IDO are two major target genes strongly up-regulated by ATPγS and involved in immunotolerance.

First the present data demonstrate that stimulation of monocyte-derived DCs with extracellular ATP induces a strong and early expression and secretion of TSP-1 using microarray, ELISA and immunodetection. Additional mass spectrometry analysis has revealed that different forms of intact TSP-1 were up-regulated by ATPγS. The pharmacological profile supports the involvement of $P2Y_{11}$ receptor in ATP-mediated TSP-1 production. Indeed ATPγS was more potent than ATP and comparable effects of other ATP derivatives like ARC-67085MX and BzATP, known agonists of $P2Y_{11}$ receptor were observed. Moreover the importance of cAMP increase in TSP-1 production was highlighted by the effect of forskolin and PGE_2 – which elevates cAMP level through activation EP_2 receptors. Calcium-elevating agents were not able to induce any TSP-1 production or to potentiate PGE_2-mediated TSP-1 production. The stronger effect of ATPγS and ATP on TSP-1 production could be explained by the activation of additional specific signal transduction proteins.

A proliferation assay was designed using supernatants from DCs treated or not with ATP. These experiments show that the high TSP-1 secretion by DCs in response to ATP leads to inhibition of T $CD4^+$ cell proliferation through TSP-1/CD47 interaction as demonstrated by the use an antibody blocking this interaction. The observed IFN-γ and IL-10 down-regulations suggest that ATP/DC conditioned medium affects proliferation of both Th1 and Th2 lymphocytes.

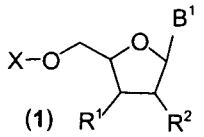
ATP is therefore able to induce immunotolerance indirectly through the activation of $P2Y_{11}$ receptors expressed on immature DCs and subsequent TSP-1 secretion.

Among the ATP-regulated sequences, another major gene involved in immunotolerance has been identified which encodes IDO protein - an intracellular enzyme involved in the catabolism of tryptophan (Mellor AL, Munn DH. Nat Rev Immunol. 2004;4:762-774.; Grohmann U et al., Trends Immunol. 2003;24:242-248). In humans, IDO has been shown to play a crucial role in tumoral immune resistance by depleting tryptophan locally and blocking the proliferation of alloreactive T lymphocytes (Uyttenhove C, et al., Nat Med. 2003;9:1269-1274.). IDO up-regulation at the mRNA level in response to ATP alone was observed. However, the presence of IFN-γ was necessary to observe the regulation of IDO protein and kynurenine production by ATP and ATPγS. In the present experiments - the high production of kynurenine (up to 150 μM) in response to ATP in combination with IFN-γ is sufficient to induce human T cell immunosuppression through tryptophan metabolites.

In conclusion, the present examples demonstrate that ATP and derivatives thereof potentiates IDO regulation and induces high TSP-1 release from human DCs that consequently affects immunotolerance. Accordingly, it is possible to manipulate pharmacologically the immune tolerance at the level of DCs. The compounds of formula (1) for use in the present invention are therefore particularly suitable vaccination adjuvant for inducing immunotolerance.

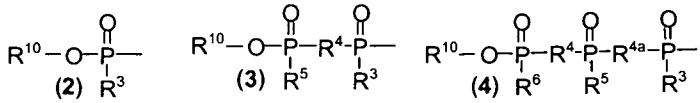
CLAIMS

1. Use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for inducing immunotolerance to an antigen whereby the individual is immunized with an antigen in combination with the agonist of formula (1)



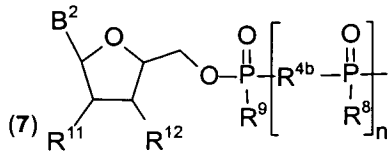
(1)

wherein B^1 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, R^1 and R^2 represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



(2) (3) (4)

wherein R^{10} represents hydrogen or is of formula (7),



(7)

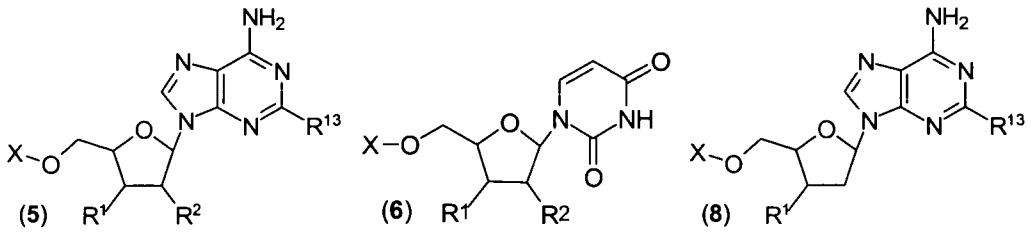
wherein B^2 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, wherein n is 0, 1, 2, 3 or 4, and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6, R^8, R^9 represent each independently OH or SH, and wherein R^{11} and R^{12} represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 is as defined above.

2. Use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) as defined above for the preparation of a medicament for inducing immunotolerance.

3. Use of at least one purinergic and pyrimidinergic receptor agonist of formula (1) as defined above for the preparation of a medicament for inducing immunotolerance to an antigen in an individual whereby said agonist of formula (1) is administered to an individual which has previously been exposed to an antigen.

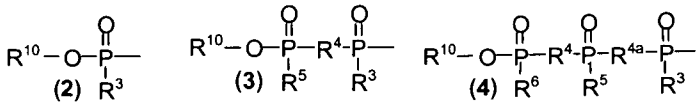
4. Use according to claim 1, 2 or 3, for the preparation of a tolerogenic vaccine for inducing immunotolerance to an antigen.

5. Use according to any of claims 1 to 4, wherein said purinergic or pyrimidinergic receptor agonist is of formula (5), (6) or (8) or a pharmaceutically acceptable salt thereof,



wherein R^1, R^2 and X have the same meaning as defined above and R^{13} is selected from hydrogen, thioalkyl, thioaryl or thioalkylaryl.

6. Use according to claim 5, wherein R^{13} is selected from hydrogen, thiomethyl, thiobutyl, thiopentyl, thiophenyl or thiobenzyl, and R^1 and R^2 are each independently selected from OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



wherein R^{10} represents hydrogen and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6 represent each independently OH or SH.

7. Use according to any of claims 1 to 6, wherein said purinergic or pyrimidinergic receptor agonist of formula (1) is selected from the group comprising ATP, adenosine 5'-O-(3-thiotriphosphate) (ATPγS), 2'- and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP), 2-propylthio-β,γ-dichloromethylene-D-ATP, 2-methylthio-ATP (2-MeSATP), ADP, 2-methylthio-ADP (2-MeSADP), adenosine 5'-O-(2-thiodiphosphate) (ADPβS), P^1,P^3-di(adenosine-5')triphosphate (Ap_3A), P^1,P^4-di(adenosine-5')tetraphosphate (Ap_4A), P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A), P^1,P^6-di(adenosine-5')hexaphosphate (Ap_6A), a deoxyderivative thereof or a pharmaceutically acceptable salt thereof.

8. Use according to any of claims 1 to 7, wherein said purinergic or pyrimidinergic receptor agonist of formula (1) is selected from the group as depicted in tables A, B or C.

9. Use according to any of claims 1 to 8, wherein said antigen is selected from the group comprising antigens responsible of asthma including bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, allergic rhinitis, conjunctivitis, keratitis, keratoconjunctivitis, uveitis, psoriasis, eczema, atopic dermatitis, contact dermatitis, cutaneous T cell lymphoma (CTCL), Sezary syndrome, pemphigus vulgaris, bullous pemphigoid, pemphigus foliaceus, dermatomyositis, erythema nodosum, scleroderma, Bechet's disease, sarcoidosis, Sjögren's syndrome, rheumatoid arthritis, juvenile arthritis, Reiter's syndrome, lupus erythematosus, polymyositis, myocarditis, primary biliary cirrhosis, Crohn's disease, ulcerative colitis, multiple sclerosis and other demyelinating diseases, idiopathic thrombocytopenic purpura, Graves' disease and Hashimoto's disease, Addison's disease, insulin-dependent diabetes mellitus (type 1), transplant rejection or graft-versus-host disease

10. Use according to claim 9, wherein said antigen is selected from the group comprising antigens responsible of autoimmune disease including psoriasis, rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus (type 1), of allergic disease including bronchial asthma and atopic dermatitis, or transplant rejection and graft-versus-host disease.

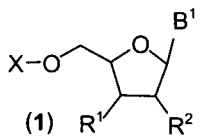
11. Pharmaceutical composition comprising at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined above, a pharmaceutically acceptable carrier and at least one antigen.

12. Tolerogenic vaccine comprising at least one purinergic and pyrimidinergic receptor agonists of formula (1) as defined above as an adjuvant for inducing immune tolerance to an antigen.

13. Tolerogenic vaccine of claim 12, which further comprises an antigen.

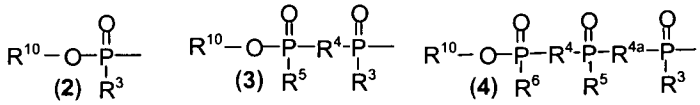
14. Method to stimulate the production of TSP-1 in dendritic cells comprising submitting said cell to at least one purinergic and pyrimidinergic receptor agonists of formula (1).

15. Method of inducing immunotolerance to an antigen comprising administering to an individual in need thereof, a therapeutically effective amount of a purinergic or pyrimidinergic receptor agonist of formula (1)



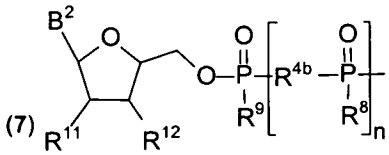
(1)

wherein B^1 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, R^1 and R^2 represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



(2) (3) (4)

wherein R^{10} represents hydrogen or is of formula (7),



(7)

wherein B^2 is selected from the group comprising adenine, uracil, thymine, cytosine, guanine, xanthine and hypoxanthine, optionally substituted by thioalkyl, thioaryl, or thioalkylaryl, wherein n is 0, 1, 2, 3 or 4, and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6, R^8, R^9 represent each independently OH or SH, and wherein R^{11} and R^{12} represent each independently OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 is as defined above.

16. Method according to claim 15, comprising stimulating the production of TSP-1 by dendritic cells using at least one purinergic and pyrimidinergic receptor agonists of formula (1).

17. Method according to claim 15, wherein said administration is by oral, and/or parenteral, and/or intranasal, and/or dermal administration.

18. Method according to any of claims 15 to 17, comprising co-administering an antigen.

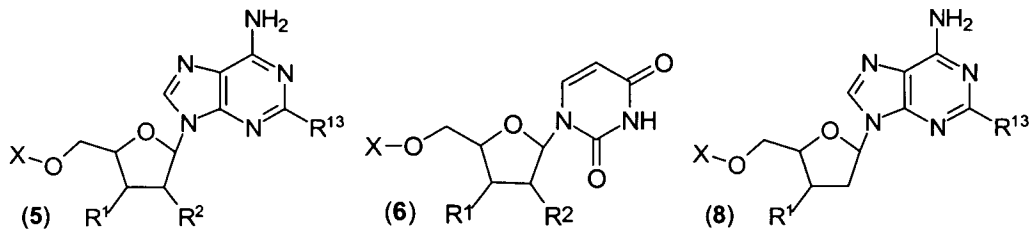
19. Method according to claim 18, wherein said co-administration is sequential.

20. Method according to claim 18, wherein said co-administration is simultaneous.

21. Method according to any of claims 14 to 20 wherein said antigen is responsible of asthma including bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma, allergic rhinitis, conjunctivitis, keratitis, keratoconjunctivitis, uveitis, psoriasis, eczema, atopic dermatitis, contact dermatitis, cutaneous T cell lymphoma (CTCL), Sezary syndrome, pemphigus vulgaris, bullous pemphigoid, pemphigus foliaceus, dermatomyositis, erythema nodosum, scleroderma, Bechet's disease, sarcoidosis, Sjögren's syndrome, rheumatoid arthritis, juvenile arthritis, Reiter's syndrome, lupus erythematosus, polymyositis, myocarditis, primary biliary cirrhosis, Crohn's disease, ulcerative colitis, multiple sclerosis and other demyelinating diseases, idiopathic thrombocytopenic purpura, Graves' disease and Hashimoto's disease, Addison's disease, insulin-dependent diabetes mellitus (type 1), transplant rejection or graft-versus-host disease

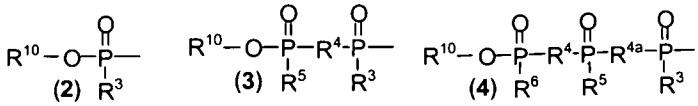
22. Method according to claim 21, wherein said antigen is selected from the group comprising antigens responsible of autoimmune disease including psoriasis, rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus (type 1), of allergic disease including bronchial asthma and atopic dermatitis, or transplant rejection and graft-versus-host disease.

23. Method according to any of claims 14 to 22, wherein said purinergic or pyrimidinergic receptor agonist is of formula (5), (6) or (8) or a pharmaceutically acceptable salt thereof,



wherein R^1, R^2 and X have the same meaning as defined above and R^{13} is selected from hydrogen, thioalkyl, thioaryl or thioalkylaryl.

24. Method according to claim 23, wherein R^{13} is selected from hydrogen, thiomethyl, thiobutyl, thiopentyl, thiophenyl or thiobenzyl, and R^1 and R^2 are each independently selected from OH, H, OR^7, $OCOR^7$, $OCOR^7COR^7$, wherein R^7 represents alkyl or aryl and X is of formula (2), (3) or (4),



wherein R^{10} represents hydrogen and wherein R^4, R^{4a} and R^{4b} represent each independently O, NH, alkylene, monohaloalkylene or dihaloalkylene and R^3, R^5, R^6 represent each independently OH or SH.

25. Method according to any of claims 14 to 24, wherein said purinergic or pyrimidinergic receptor agonist of formula (1) is selected from the group as depicted in tables A, B or C.

26. Method according to any of claims 14 to 25, wherein said purinergic receptor agonist of formula (1) is selected from the group comprising ATP, adenosine 5'-O-(3-thiotriphosphate) (ATPγS), 2-propylthio-βγ-dichloromethylene-D-ATP, 2'- and 3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP), 2-methylthio-ATP (2-MeSATP), ADP, 2-methylthio-ADP (2-MeSADP), adenosine 5'-O-(2-thiodiphosphate) (ADPβS), P^1,P^3-di(adenosine-5')triphosphate (Ap_3A), P^1,P^4-di(adenosine-5')tetraphosphate (Ap_4A), P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A), P^1,P^6-di(adenosine-5')hexaphosphate (Ap_6A), deoxyderivative thereof or a pharmaceutically acceptable salt thereof.

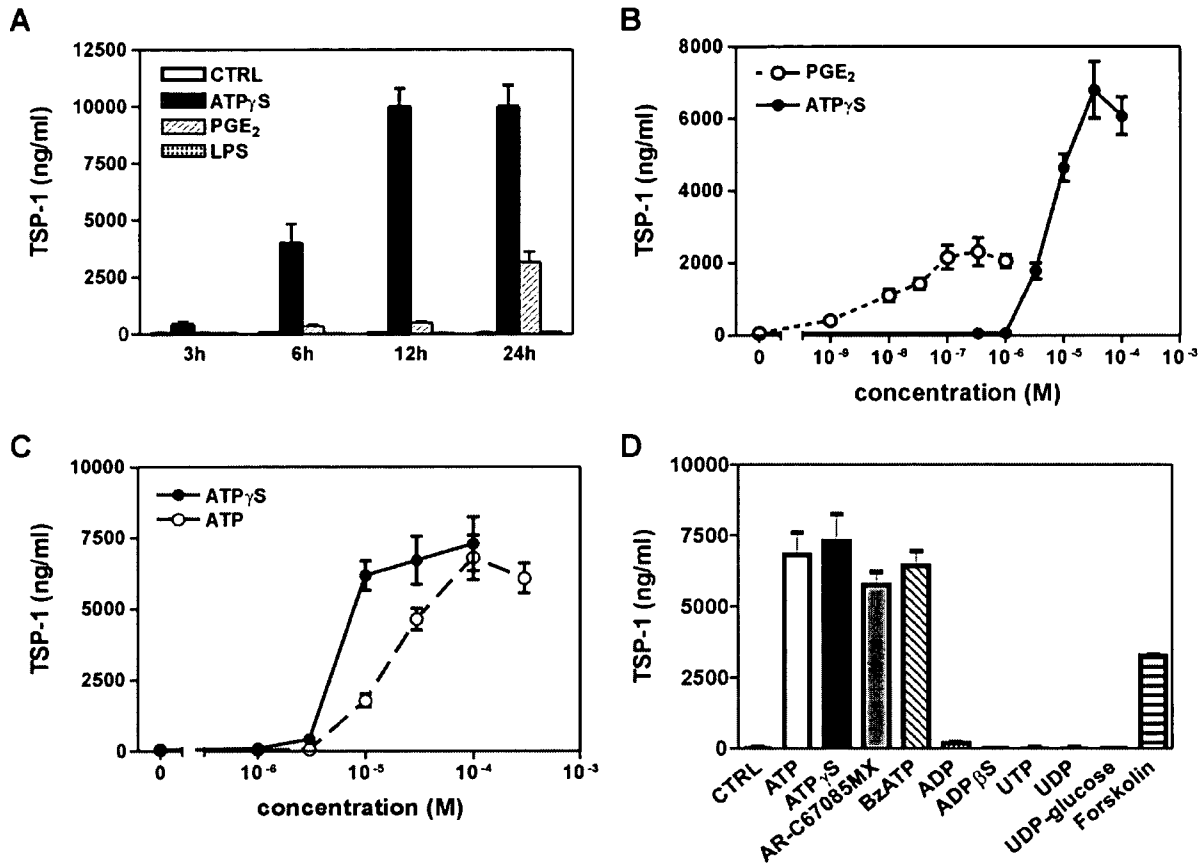
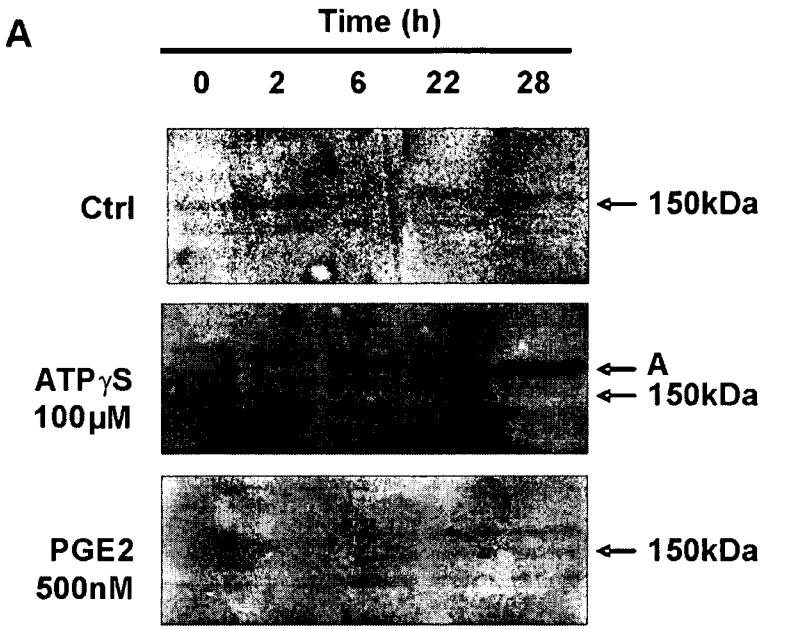

A
TSP-1 (ng/ml)
12500
10000
7500
5000
2500
0
CTRL
ATPγS
PGE2
LPS
3h
6h
12h
24h
B
TSP-1 (ng/ml)
8000
6000
4000
2000
0
PGE2
ATPγS
0
10-9
10-8
10-7
10-6
10-5
10-4
10-3
concentration (M)
C
TSP-1 (ng/ml)
10000
7500
5000
2500
0
ATPγS
ATP
0
10-6
10-5
10-4
10-3
concentration (M)
D
TSP-1 (ng/ml)
10000
7500
5000
2500
0
CTRL
ATP
ATPγS
AR-C67085MX
BzATP
ADP
ADPβS
UTP
UDP
UDP-glucose
Forskolin

FIG. 1


A
Time (h)
0
2
6
22
28
Ctrl
150kDa
ATPγS
100µM
A
150kDa
PGE2
500nM
150kDa

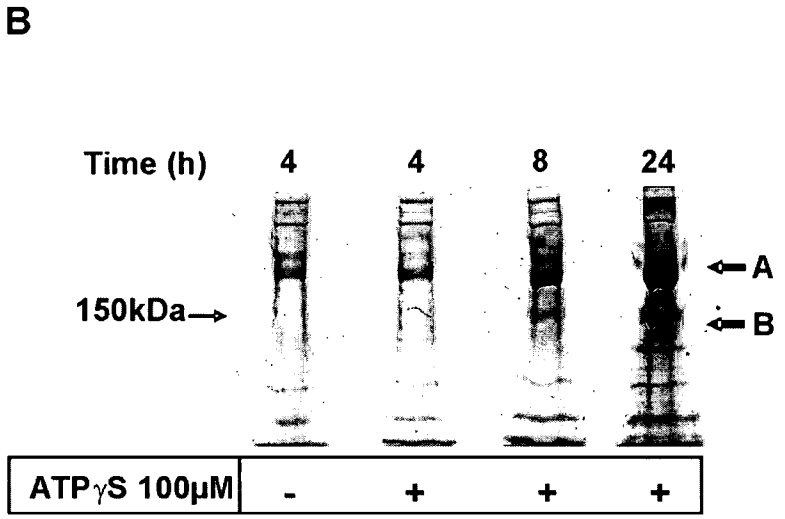

B
Time (h)
4
4
8
24
150kDa
A
B
ATPγS 100µM
-
+
+
+

FIG. 2

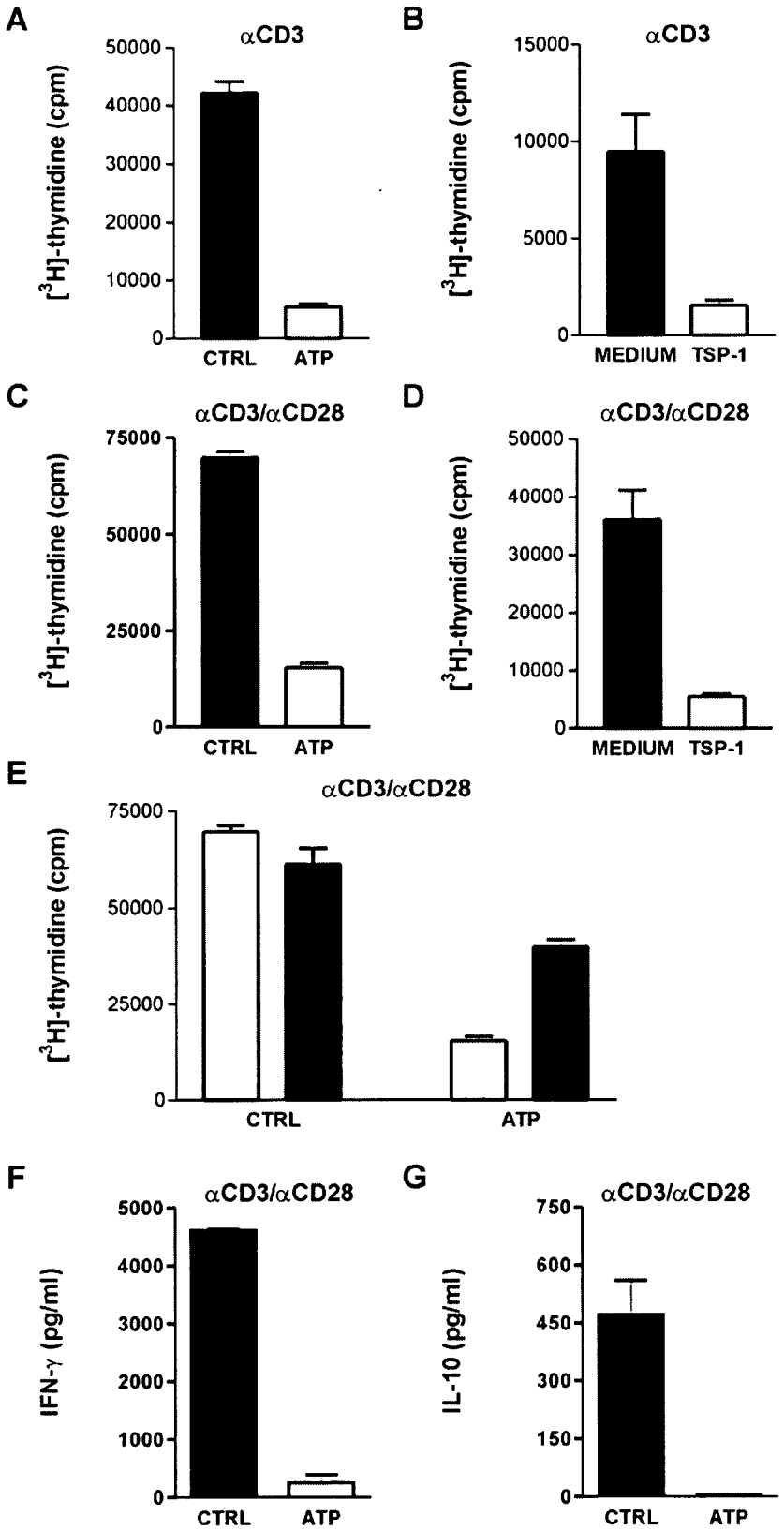
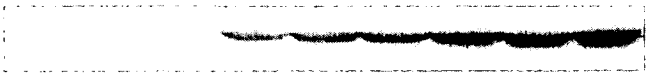

A
αCD3
[3H]-thymidine (cpm)
50000
40000
30000
20000
10000
0
CTRL
ATP
B
αCD3
[3H]-thymidine (cpm)
15000
10000
5000
0
MEDIUM
TSP-1
C
αCD3/αCD28
[3H]-thymidine (cpm)
75000
50000
25000
0
CTRL
ATP
D
αCD3/αCD28
[3H]-thymidine (cpm)
50000
40000
30000
20000
10000
0
MEDIUM
TSP-1
E
αCD3/αCD28
[3H]-thymidine (cpm)
75000
50000
25000
0
CTRL
ATP
F
αCD3/αCD28
IFN-γ (pg/ml)
5000
4000
3000
2000
1000
0
CTRL
ATP
G
αCD3/αCD28
IL-10 (pg/ml)
750
600
450
300
150
0
CTRL
ATP

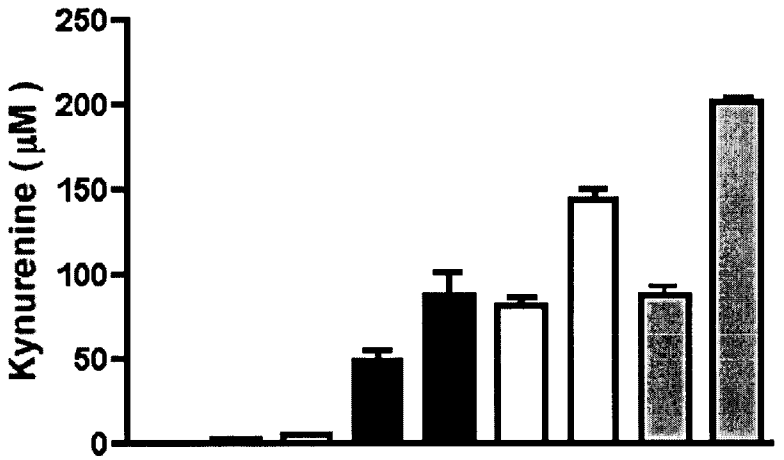
FIG. 3

A



ATP	-	+	-	-	-	+	+	-	-
ATPγS	-	-	+	-	-	-	-	+	+
IFN-γ (U/ml)	0	0	0	10	100	10	100	10	100

B



ATP	-	+	-	-	-	+	+	-	-
ATPγS	-	-	+	-	-	-	-	+	+
IFN-γ (U/ml)	0	0	0	10	100	10	100	10	100

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/007966

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/52 A61K31/513 A61K31/522 A61K39/00 A61P37/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/028712 A (UNIVERSITE LIBRE DE BRUXELLES; SCHANDENE, LILIANE; GOLDMAN, MICHEL; DU) 10 April 2003 (2003-04-10) cited in the application tables A-C	2,3, 5-10, 15-17, 21-26
Y	claims 1,6,9	1-26
Y	US 2003/212223 A1 (FAVROT JEAN-MICHEL ET AL) 13 November 2003 (2003-11-13) abstract claims 1-28 examples 1-6 paragraph [0026] paragraph [0028] paragraph [0037] - paragraph [0041]	1-26

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 2006	12/12/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Giacobbe, Simone

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/007966

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/053612 A1 (GRANSTEIN RICHARD D ET AL) 10 March 2005 (2005-03-10) cited in the application paragraphs [0050], [0055], [0070] paragraph [0074] examples 1-6,9	1-26
Y	LA SALA A ET AL: "Dendritic cells exposed to extracellular adenosine thriphosphate acquire the migratory properties of mature cells and show a reduced capacity to attract type 1T lymphocytes" BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, US, vol. 99, no. 5, 1 March 2002 (2002-03-01), pages 1715-1722, XP002988064 ISSN: 0006-4971 abstract page 1721, column 1, line 1 - line 27	1-26
P,Y	MARTEAU FRÉDÉRIC ET AL: "Thrombospondin-1 and indoleamine 2,3-dioxygenase are major targets of extracellular ATP in human dendritic cells." BLOOD. 1 DEC 2005, vol. 106, no. 12, 1 December 2005 (2005-12-01), pages 3860-3866, XP002362635 ISSN: 0006-4971 the whole document	1-26
T	TRAN MINH D ET AL: "Purinergic signaling induces thrombospondin-1 expression in astrocytes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 103, no. 24, June 2006 (2006-06), pages 9321-9326, XP002407675 ISSN: 0027-8424 abstract	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/007966

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claims 14-26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

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

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/007966

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03028712 A	10-04-2003	NONE	
US 2003212223 A1	13-11-2003	US 2003195309 A1	16-10-2003
US 2005053612 A1	10-03-2005	NONE	