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(54) Title: A PROCESS FOR THE TREATMENT OF ORGANOPHOSPHATE POISONING

(57) Abstract

The invention relates to a process for treating organophosphate poisoning in a mammal comprising the administration of an $A_1$ receptor adenosine agonist.

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Title: A process for the treatment of organophosphate poisoning

The invention relates to a process for the treatment of organophosphate poisoning.

The currently available treatment of organophosphate (OP)-poisoning (i.e. (ir)reversible inhibition of acetylcholinesterase) is mainly based on a combined administration of a cholinesterase reactivator (oxime), a muscarinic receptor antagonist (atropine) and an anticonvulsant (diazepam). Experiments with primates in the past, however, have demonstrated that such a treatment, even when carried out immediately after OP-exposure, do not rapidly restore electroencephalographic (EEG) activity and fail to prevent neuronal brain damage and postintoxication incapacitation (Dawson et al 1995, Van Helden et al 1996, Busker et al 1996, Lalleman et al 1998, Shih and McDonough 1997). Moreover, clinical experience has indicated that oximes, although designed to reactivate the inhibited acetylcholinesterase (AChE), are not always sufficiently effective as reactivators even when administered at very high dosages (Van Helden et al 1996). Furthermore, none of the oximes can be regarded as a 'broad spectrum' (generic) antidote, i.e., effective against all currently existing nerve agents.

Therefore, it seems justified to conclude that the therapeutic efficacy of available oximes in nerve gas poisoning is less than optimal, especially in case of rapidly ageing (a process leading to oxime-resistance) nerve agents such as soman, and seems to have reached its limits (Dawson et al 1995; Van Helden et al 1996). Therefore, new and preferably generic approaches are necessary to improve protection.

Accumulation of acetylcholine (ACh) in the synaptic cleft is generally considered as the main cause of the symptoms that ultimately lead to death in case of OP-poisoning. Thus, it is proposed that drug-induced decrease of

ACh-release in brain and muscle can prevent and counteract convulsions that are a result of OP-poisoning and improve survival rate.

In accordance with the present invention, it has been found that adenosine $A_1$ receptor agonists are well-suited for effectuating a decrease of ACh-release in the brain and in muscles. Hence, the invention provides a process for treating organophosphate poisoning in a mammal comprising the administration of an $A_1$ receptor adenosine agonist.

When compared with oxime treatment, the present approach provides a generic protection, i.e. protection against all nerve agents (including soman, sarin, tabun, VX and many other AChE-inhibitors, such as insecticides and pesticides), independent of ageing of the inhibited AChE. A process according to the invention is directed towards accumulation of ACh which causes the symptoms and death in OP-poisoning. Furthermore, lower dosages than in the oxime treatment are required in order to achieve an effective treatment.

Endogenous adenosine elicits a large variety of physiological effects through interaction with cell-surface adenosine receptors, which are heterogeneous ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ receptors) and widely spread throughout the body (Collis and Hourani 1993). This large variety of physiological effects elicited by adenosine provides a potential for therapeutic application of adenosine analogues.

Adenosine itself has been registered under the name of Adenocard as an anti-arrythmic drug and for controlled hypotension during aneurysm surgery. A1 adenosine agonists have been proposed as sedatives, in supraventricular tachycardia, in type II diabetes, stroke and seizures, whereas A2 adenosine agonists have been proposed as inhibitors of aggregation in thrombosis, in diagnosis of diseases in coronary arteries, in ischemia and reperfusion. Adenosine agonists for the A3 receptor have been proposed for use in certain behavorial disturbances. Other conditions for

which activation of the adenosine receptors may be useful are inflammation and some pathophysiological situations, such as anxiety and panic attacks, sleep disturbances, schizophrenia, depression, epilepsy and convulsions.

Inherent to the widespread distribution of adenosine receptor subtypes is the difficulty in obtaining desirable drug actions without concomitant side effects. For example, the profound hemodynamic disturbances observed with adenosine $A_1$ and $A_{2A}$ agonists have limited their use.

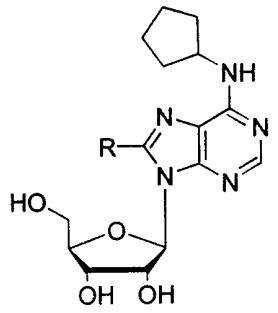
A new target is the inhibition of ACh-release to counteract organophosphate poisoning. This release inhibition is mediated by the $A_1$ adenosine receptor. Thus, in accordance with the invention, an $A_1$ receptor adenosine agonist is administered. In the context of the invention, an $A_1$ receptor adenosine agonist is an adenosine agonist which is selective for the $A_1$ receptor, i.e. interacts predominantly with the $A_1$ receptor, particularly at lower dosages.

In addition, it has been found that application of adenosine agonists with reduced intrinsic activity, i.e. partial $A_1$ adenosine agonists, is highly beneficial. In the context of the invention, a partial agonist is a compound that has a submaximal physiological effect at complete receptor occupancy in a certain system. It has been found that the activity of these drugs not only depends on receptor subtypes but on tissue differences (tissue selectivity) as well (Kenakin 1993). This results in less pronounced cardiovascular actions and a potential increase in selectivity of action, e.g., the inhibition of ACh-release in the brain.

Accordingly, in a preferred embodiment the present invention is directed to the treatment of organophosphate poisoning in mammals comprising the administration of a partial $A_1$ adenosine agonist. In accordance with this preferred embodiment, severe adverse effects of the treatment with respect to blood pressure and heart rate can be significantly reduced.

Particularly preferred partial $A_1$ adenosine agonists for use in a process according to the invention are 8-alkylamino-substituted analogues of $N^6$-cyclopentyladenosine, 8-substituted adenosine, 8-substituted theophylline-7-ribose analogues, and deoxyribose analogues of $N^6$-cyclopentyladenosine (CPA), $N^6$-cyclohexyladenosine (CHA), $N^6$-R-phenylisopropyladenosine (R-PIA) and $N^6$-S-phenylisopropyladenosine (S-PIA). These adenosine agonists have a highly beneficial therapeutic window. In other words, they combine a high activity and therapeutic efficacy with a low toxicity.

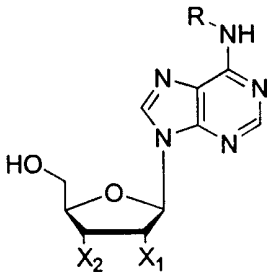
Suitable examples of the class of 8-alkylamino-substituted analogues of $N^6$-cyclopentyladenosine have the formula (I)




(I),

wherein R is $-NHCH_3$, $-NHCH_2CH_3$, $-NH(CH_2)_2CH_3$, $-NH(CH_2)_3CH_3$, or -NH-cyclopentyl. These compounds may be prepared in any known manner (e.g. Roelen et al 1996).

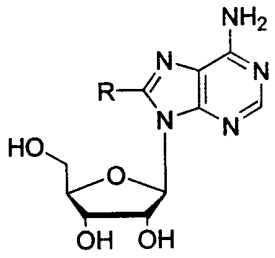
Suitable examples of the class of deoxyribose analogues of $N^6$-cyclopentyladenosine (CPA), $N^6$-cyclohexyladenosine (CHA), $N^6$-R-phenylisopropyladenosine (R-PIA) and $N^6$-S-phenylisopropyladenosine (S-PIA) have the formula (II)



(II),

wherein R is cyclopentyl, cyclohexyl, R-phenylisopropyl, or S-phenylisopropyl, and wherein $X_1$ and $X_2$ are different from each other and chosen from hydrogen and hydroxyl. These compounds have been described by Van der Wenden et al 1995a.

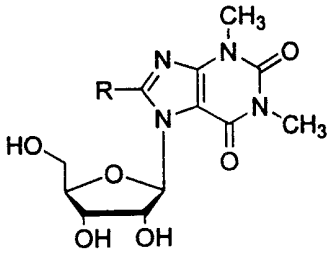
Suitable 8-substituted adenosines and 8-substituted theophylline-7-ribose have for instance been described by Van der Wenden et al 1995b. Preferred 8-substituted adenosines have the formula (III)



(III),

wherein R is methyl, ethyl, vinyl, thiophenyl, hydroxyl, oxymethyl, amino, aminoalkyl with from 1 to 5 carbon atoms, aminoalkylamine with from 1 to 5 carbon atoms, aminocyclopentyl, cyclohexyl, or halogen.

Preferred 8-substituted theophylline-7-riboses have the formula (IV)



(IV),

wherein R is hydrogen, amino, aminoalkyl with from 1 to 7 carbon atoms, or aminophenyl.

It will be clear that it is also possible to use suitable combinations of the above $A_1$ adenosine agonists for

treating an OP-poisoning. A treatment comprising such a combined administration of $A_1$ adenosine agonist is also encompassed by the invention.

As has been mentioned above, one of the great advantages of a process according to the invention is that it is a generic process. This means that it can be used to treat organophosphate poisoning resulting from substantially all nerve agents, such as soman, sarin, tabun, VX and so forth, as well as other AChE-inhibitors, such as a large number of insecticides and pesticides. An organophosphate poisoning has been found to be based on the inhibition of the enzyme acetylcholinesterase (AchE). Inactive AChE cannot hydrolyze acetylcholine (Ach) which will accumulate in the cholinergic synaps and as a result will paralyze the synaptic transmission. Apparent, outward symptoms are salivation, convulsions and respiratory paralysis.

The treatment of the invention can be applied to any mammal suffering from the effects of an OP-poisoning. However, it will be mostly applied to primates, in particular to humans.

As the effects of an OP-poisoning can be lethal within a very short period of time after the intoxication, i.e. the exposure to the poisonous compound(s), it is preferred that the treatment according to the invention is performed as soon as possible after said exposure. Desirably, the administration of an $A_1$ receptor adenosine agonist in accordance with the invention is carried out within one minute after acute intoxication or on guidance of symptoms. First mild symptoms are fatigue, headache, dizziness, numbness of extremities, nausea and vomiting, sweating, extreme salivation, diarrhoea, abdominal pain, frequent urination, and miosis. Moderate symptoms are generalized weakness, speech impediment, fasciculations, trembling, miosis, hampered motoric fasciculations, fever, tightness in the chest, involuntary urination and defecation.

The dosage in which the $A_1$ adenosine agonist can suitably be administered may vary within the range of 0.1-20 mg/kg, but is highly dependent on efficacy and adverse effects. Preferably, the dosage is chosen within the range of 1-2 mg/kg.

Effective manners in which the $A_1$ adenosine agonist may be administered include intramuscular, intravenous, and intranasal administration. The most preferred manners of administration are intramuscular and intravenous administration since after these application routes the $A_1$ adenosine agonist reaches the site of the $A_1$ receptor, where it is intended to effect a decrease of ACh-release, very fast.

For the above manners of administration, the $A_1$ adenosine agonist can most suitably be formulated in the form of a saline solution. However, in case the $A_1$ adenosine agonist appears insufficiently soluble in water, it may be useful to formulate them in DMSO or ethanol, diluted with a solution of sodium chloride in water (saline) to a final 10 to 30 vol.% DMSO solution, or a 5-10 vol.% ethanol solution.

OP-poisoning will be mostly encountered by people under harsh conditions, e.g. soldiers at war, anti-terrorist squads, and so forth. Moreover, it is of great importance that the treatment in accordance with the invention is performed as soon as possible after exposure to the poison. For these reasons, it is highly preferred to use a so-called 'auto-injector' device for the administration of the drug. This device has for instance been developed by Astra Tech AB, Mölndal, Sweden and by Meridian Medical Technologies, Columbia, Maryland, USA. In order to administer an $A_1$ receptor adenosine agonist using an auto-injector, the auto-injector is put on a muscle (e.g. a thigh muscle), and after pressing a button, a hollow needle penetrates the skin into the muscle and a unit-dose of the desired $A_1$ receptor adenosine agonist is injected into the muscle. Thus, the invention also encompasses an auto-injector holding a

formulation comprising an $A_1$ receptor adenosine agonist as disclosed hereinabove.

The invention will now be elucidated by the following, non-restricting examples.

**Example I**

An experiment was carried out using 5'-N-ethylcarboxamido-adenosine (NECA), an agonist of $A_1$ and $A_2$ receptors, to treat OP-poisoning, using the nerve agent soman as an irreversible model AChE-inhibitor.

Based on in vitro experiments in which 300 nM of NECA appeared to decrease the release of ACh from a rat diaphragm endplate zone by 70%, NECA was tested in an in vivo experiment. The in vitro concentration was extrapolated to an in vivo dose. Assuming that NECA distributes homogeneously in the body and that 300 nM is the effective concentration in the brain (300 nM = 300 nMol/lit/kg $\approx$ 0.1 mg/kg i.m.), 0.1 mg/kg of NECA was administered intramuscularly (i.m.). In a practical protocol, this calculated dose was administered intramuscularly at 1 min following a subcutaneous poisoning with 1.5 or 2 $LD_{50}$ soman in unanaesthesized rats. Symptoms and survival were registered. The results of this pilot are presented in Table 1 and show that 0.1 mg/kg (i.m.) NECA prevents and postpones the appearance of convulsive activity, and tends to improve the survival rate.

**Example II**

In this experiment cyclopentyl adenosine (CPA), a highly specific $A_1$ adenosine receptor agonist, was tested using a similar protocol as described in Example I. The therapeutic efficacy of two doses (1 and 2 mg/kg, i.m.) of the latter compound was tested against 1.9 $LD_{50}$ soman in a similar way as described in Example I for NECA. The results are presented in Table 2 showing that administration of CPA prevented convulsions and led to survival of each animal in a healthy condition judging from clinical observation.

**Example III**

In separate experiments, using a limited number of unanaesthesized rats, it was investigated to what degree CPA (2 mg/kg i.m.) would be able to prevent the accumulation of ACh in the striate body (corpus striatum) of the brain upon soman poisoning (2 $LD_{50}$ s.c.) by performing brain microdialysis according to the method described by Moor et al (1994). Briefly, rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and a dialysis probe was implanted in the striate body. The actual dialysis experiments were conducted around 20 h after surgery.

Probes were perfused with artificial cerebrospinal fluid containing in mM: NaCl 147, KCl 3.0, $CaCl_2$ 1.2, and $MgCl_2$ 1.2. The AChE-inhibitor neostigmine bromide ($10^{-8}$ M) was added to this perfusion fluid to obtain detectable quantities of base-line ACh. The artificial cerebrospinal fluid was delivered by a syringe pomp (Carnegy Medicine, Sweden) at a rate of 2 µl/min. Ten min samples were collected in a 50-µl loop of an injection valve that was automatically activated by an electronic timer.

After stabilisation of the ACh levels, soman (1-2 $LD_{50}$) was injected subcutaneously followed by intramuscular injection of CPA (2 mg/kg) 1 minute later. These preliminary results demonstrated a low level of extracellular brain ACh (0-50 fold increase in ACh) following CPA treatment of soman poisoning, which was in contrast to a large increase in the amount of extracellular ACh in soman-poisoned animals not treated with CPA (180-400 fold increase in ACh). This low level of ACh-release in the brain following soman poisoning and CPA treatment was associated with postponement or lack of symptoms, and survival of the animals. Soman poisoned animals (controls) showed convulsions and died within 20 minutes.

**Example IV**

A number of partial $A_1$ receptor agonists was tested in a similar way as described for NECA and CPA in Examples I

and II. Advantageous therapeutic efficacy against soman and sarin in rats and guinea pigs was demonstrated while the adverse effects on blood pressure and heart rate were less than in case of NECA and CPA.

The protocol in which the efficacy of the partial $A_1$ receptor agonists against AChE-inhibitors was tested, was not standard; both repetitive - and prophylactic administration (intramuscularly or intravenously) were investigated. Neither the level of intoxication was standard; it was in the range of 0.5-3 $LD_{50}$ of AChE-inhibitors tested.

**Table 1.** Protection of NECA (0.1 mg/kg intramuscularly) injected 1 min. after soman-poisoning in rats (n indicates the number of animals tested).

| **Treatment** | **Survival time** | **Symptoms** |
|---|---|---|
| Soman (n=5) (2 $LD_{50}$ s.c.) | < 15 min. | starting at 5-7 min.: salivation, convulsions, respiratory distress |
| Soman (2 $LD_{50}$ s.c.) + NECA (n=5) | > 24 h;<br>> 24 h;<br>33 min.;<br>90 min.;<br>75 min. | normal respiration;<br>alert, dry mouth, drank water;<br>convulsions at t=21 min.;<br>convulsions at t=50 min.;<br>convulsions at t=40 min. |
| Soman (1.5 $LD_{50}$ s.c.) (n=3) | > 24 h | starting at 2-10 min.: convulsive activity for hours on end |
| Soman (1.5 $LD_{50}$ s.c.) +NECA (n=3) | > 24 h | no convulsions at all |
| NECA (n=2) | > 24 h | no physical signs |

**Table 2.** Protection of CPA (intramuscular) injected 1 min. after soman-poisoning (1.9 $LD_{50}$ s.c.) in rats (n indicates the number of animals tested).

| **Treatment** | **Survival time** | **Symptoms** |
|---|---|---|
| Soman (n=6) | 17-28 min. | after 5-7 min.: chewing, salivation, convulsions, respiratory distress |
| Soman + CPA (1 mg/kg) (n=6) | > 24 h | 3 rats: no symptoms;<br>1 rat: chewing after 32 min., then salivation, convulsions and decreased respiration;<br>1 rat: decreased respiration frequently, and salivation next morning;<br>1 rat: chewing after 10 min. |
| Soman + CPA (2 mg/kg) (n=6) | > 24 h | 5 rats: no symptoms, normal respiration, alert, dry mouth, drank water;<br>1 rat: some traces of blood around mouth next morning. |
| CPA (2 mg/kg) (n=2) | > 24 h | no physical signs |

**References**

*Busker RW, Zijlstra JJ, Philippens, Groen B, Melchers BPC (1996) Comparison of the efficacy of single or repeated HI-6 treatment following soman poisoning in guinea pigs and marmoset monkeys. Toxicol 112: 183-194

*Collis MG and Hourani SMO (1993) Adenosine receptor subtypes. TiPS 14: 360-366

*Correio-de-Sa P, Sebastiao AM, Ribeiro JA (1991) Inhibitory and excitatory effects of adenosine receptor agonists on evoked transmitter release from phrenic nerve ending of the rat. Br J Pharmacol 103 92): 1614-1620

*Dawson RM (1994) Review of oximes available for treatment of nerve agent poisoning. J Appl Toxicol 14:317-31

*Kenakin TP (1993) Pharmacological analysis of drug-receptor interaction, pp441-468, Raven Press, New York

*Lallement G, Clarencon D, Masqueliez C, Baubichon D, Galonnier M, Burckhart M-F, Peoc'h M, Mestries J-C (1998) Nerve agent poisoning in primates: antilethal, anti-epileptic and neuroprotective effects of GK-11. Arch Toxicol 72:84-92

* Moor E, De Boer P, Beldhuis HJA, Westerink BHC (1994) A novel approach for studying septo-hippocampal cholinergic neurons in freely moving rats: a microdialysis study with dual-probe design. Brain Res 648: 32-38

*Roelen H, Veldman N, Spek AL, Von Frijtag Drabbe Kunzel JK, Mathot RAA, IJzerman AP (1996) N6, C8-disubstituted adenosine derivatives as partial agonists for adenosine A1 receptors. J Med Chem 39: 1463-1471

*Sebastiao AM, Stone TW, Ribeiro JA (1990) The inhibitory adenosine receptor at the neuromuscular junction and hippocampus of the rat: antagonism by 1,3,8-substituted xanthines. Br J Pharmacol 101, 453-459

*Shih TM, McDonough JH (1997) Neurochemical mechanisms in soman-induced seizures. J Appl Toxicol 17: 255-264

*Van der Wenden EM, Von Frijtag Drabbe Kunzel JK, Mathot RAA, Danhof M, IJzerman AP, Soudijn W (1995a) Ribose-modified adenosine analogs as potential partial agonists for the adenosine receptor. J Med Chem 38: 4000-4006

*Van der Wenden EM, Hartog-Witte HR, Roelen HCPF, Von Frijtag Drabbe Kunzel JK, Pirovano IM, Mathot RAA, Danhof M, Van Aerschot A, Lidaks MJ, IJzerman AP, Soudijn W (1995b) 8-Substituted adenosine and theophylline-7-riboside analogs as potential partial agonists for the adenosine A1 receptor. Eur J Pharmacol Mol Pharmacol Sect 290: 189-199

*Van Helden HPM, Busker RW, Melchers BPC, Bruijnzeel PLB (1996) Pharmacological effects of oximes: how relevant are they? Arch Toxicol 70:779-786

*Van Schaick EA, Mathot AA, Gubbens-Stibbe JM, Langemeijer MWE, Roelen HCPF, IJzerman AP, Danhof M (1997) 8-Alkylamino-substituted analogs of N6-cyclopentyladenosine are partial agonists for the cardiovascular adenosine A1 receptors in vivo. J Pharmacol Exp Ther 283: 800-808

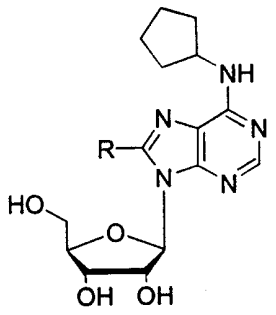
## Claims

1. A process for treating organophosphate poisoning in a mammal comprising the administration of an $A_1$ receptor adenosine agonist.

2. A process according to claim 1, wherein a partial $A_1$ receptor adenosine agonist is administered.

3. A process according to claim 2, wherein the partial $A_1$ receptor adenosine agonist is chosen from the group of 8-alkylamino-substituted analogues of $N^6$-cyclopentyladenosine, 8-substituted adenosine, 8-substituted theophylline-7-ribose analogues, and deoxyribose analogues of $N^6$-cyclopentyladenosine (CPA), $N^6$-cyclohexyladenosine (CHA), $N^6$-R-phenylisopropyladenosine (R-PIA) and $N^6$-S-phenylisopropyladenosine.

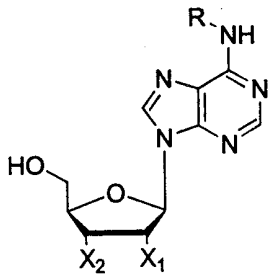
4. A process according to claim 3, wherein the partial $A_1$ adenosine agonist is a 8-alkylamino-substituted analogue of $N^6$-cyclopentyladenosine having the formula (I)




(I),

wherein R is $-NHCH_3$, $-NHCH_2CH_3$, $-NH(CH_2)_2CH_3$, $-NH(CH_2)_3CH_3$, or -NH-cyclopentyl.

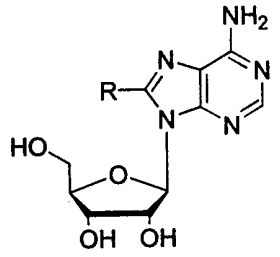
5. A process according to claim 3, wherein the partial $A_1$ adenosine agonist is a deoxyribose analogue of $N^6$-cyclopentyladenosine (CPA), $N^6$-cyclohexyladenosine (CHA), $N^6$-R-phenylisopropyladenosine (R-PIA) or $N^6$-S-phenylisopropyladenosine having the formula (II)



(II),

wherein R is cyclopentyl, cyclohexyl, R-phenylisopropyl, or S-phenylisopropyl, and wherein $X_1$ and $X_2$ are different from each other and chosen from hydrogen and hydroxyl.

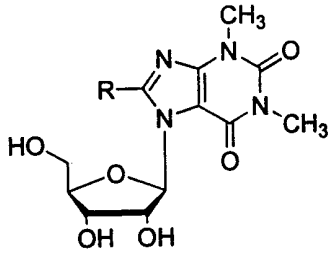
6. A process according to claim 3, wherein the partial $A_1$ adenosine agonist is an 8-substituted adenosine having the formula (III)



(III),

wherein R is methyl, ethyl, vinyl, thiophenyl, hydroxyl, methyoxy, amino, aminoalkyl with from 1 to 5 carbon atoms, aminoalkylamine with from 1 to 5 carbon atoms, aminocyclopentyl, cyclohexyl, or halogen.

7. A process according to claim 3, wherein the partial $A_1$ adenosine agonist is an 8-substituted theophylline-7-ribose having the formula (IV)



(IV),

wherein R is hydrogen, amino, aminoalkyl with from 1 to 7 carbon atoms, or aminophenyl.

8. A process according to any of the preceding claims, wherein a human is treated for organophosphate poisoning.

9. A process according to any of the preceding claims, wherein the $A_1$ receptor adenosine agonist is administered in a dosage of 0.1-20 mg/kg.

10. A process according to any of the preceding claims, wherein the $A_1$ receptor adenosine agonist is administered intramuscularly or intravenously.

11. A process according to claim 10, wherein the $A_1$ receptor adenosine agonist is administered in the form of a saline solution.

12. A process according to claim 11, wherein the saline solution further comprises 10-30 vol.% dimethylsulfoxide.

13. A process according to claim 11 or 12, wherein the saline solution further comprises 5-10 vol.% ethanol.

14. A process according to any of the preceding claims, wherein the $A_1$ receptor adenosine agonist is administered by use of an injector, preferably an auto-injector.

15. An injector, preferably an auto-injector, comprising an $A_1$ receptor adenosine agonist.

16. Use of an $A_1$ receptor adenosine agonist for preparing a medicament for treating organophosphate poisoning in mammals.

# INTERNATIONAL SEARCH REPORT

I ational Application No
PCT/NL 98/00343

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/70

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | W.-M. LAU ET AL.: "Effects of some Organophosphorus Compounds on the Binding of a Radioligand (8-Cyclopentyl-1,3-'3H!dipropylxanthine) to Adenosine Receptors in Ovine Cardiac Membranes" J.APPL.TOXICOL., vol. 11, no. 6, 1991, pages 411-414, XP002093243 the whole document --- -/-- | 1-16 |

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

[X] Patent family members are listed in 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 |
|---|---|
| 12 July 1999 | 24.08.99 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Theuns, H |

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | W.-M. LAU ET AL.: "Binding of some organophosphorus compounds at adenosine receptors in guinea pig brain membranes" NEUROSCI.LETT., vol. 94, no. 1-2, 22 November 1988 (1988-11-22), pages 125-130, XP002093244 the whole document | 1-16 |
| A | F. FONNUM ET AL.: "Modulation of the Cholinergic Activity of Bronchial Muscle during Inhalation of Soman" FUNDAM. APPL. TOXICOL., vol. 4, no. 2 Part 2, 1984, pages 52-57, XP002093245 the whole document | 1-16 |
| A | L.E.GUSTAFSSON ET AL.: "Characterization of pre- and post-junctional adenosine receptors in guinea-pig ileum" ACTA PHYSIOL. SCAND., vol. 123, no. 2, February 1985 (1985-02), pages 195-203, XP002093246 abstract | 1-16 |
| A | M. DE ZWART ET AL.: "A FUNCTIONAL SCREENING OF ADENOSINE ANALOGUES AT THE ADENOSINE A2B RECEPTOR: A SEARCH FOR POTENT AGONISTS" NUCLEOSIDES NUCLEOTIDES, vol. 17, no. 6, 1998, pages 969-985, XP002108781 abstract | 1-16 |
| A | WO 95 02604 A (THE UNITED STATES OF AMERICA) 26 January 1995 (1995-01-26) page 2 page 36 | 1-16 |
| A | H.O.KIM ET AL.: "Selective Ligands for Rat A3 Adenosine Receptors: Structure-Activity Relationships of 1,3-Dialkylxanthine 7-Riboside Derivatives" J.MED.CHEM., vol. 37, no. 23, 1994, pages 4020-4030, XP002108782 abstract | 1-16 |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | A.P.IJZERMAN ET AL.: "Partial agonism of theophylline-7-riboside on adenosine receptors" NAUNYN-SCHMIEDEBERG'S ARCH PHARMACOL, vol. 350, no. 6, December 1994 (1994-12), pages 638-645, XP002108783 abstract | 1-16 |
| A | JP 53 056690 A (RIKAGAKU RES LABS KK) 23 May 1978 (1978-05-23) the whole document | 1-16 |
| A | A.S. CLANACHAN : "Antagonism of presynaptic adenosine receptors by theophylline 9-beta-D-riboside and 8-phenyltheophylline" CAN J PHYSIOL PHARMACOL, vol. 59, no. 6, June 1981 (1981-06), pages 603-606, XP002108784 abstract | 1-16 |
| A | V.OZOLA ET AL.: "SYNTHESIS OF 8-SUBSTITUTED THEOPHYLLINE BETA-D-RIBOFURANOSIDES" NUCLEOSIDES NUCLEOTIDES, vol. 12, no. 8, 1993, pages 827-839, XP002108785 abstract | 1-16 |
| A | P.J.M. VAN GALEN ET AL.: "XANTHINE-7-RIBOSIDES AS ADENOSINE A1 RECEPTOR ANTAGONISTS: FURTHER EVIDENCE FOR ADENOSINE'S ANTI MODE OF BINDING" NUCLEOSIDES NUCLEOTIDES, vol. 9, no. 2, 1990, pages 275-291, XP002108786 abstract | 1-16 |
| A | E M VAN DER WENDEN ET AL.: "8Substituted adenosine and theophylline-7-riboside analogues as potential partial agonists for the adenosine A1 receptor" EUR J PHARMACOL, MOL PHARMACOL SECT, vol. 290, no. 3, 1995, pages 189-199, XP002108787 abstract | 1-16 |
| A | M A N MOSSELHI: "NUCLEOSIDES, I; RIBOSYLATION OF 8-SUBSTITUTED THEOPHYLLINE DERIVATIVES" NUCLEOSIDES NUCLEOTIDES, vol. 12, no. 5, 1993, pages 431-439, XP002108788 abstract | 1-16 |

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# INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/NL 98/00343

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | R F BRUNS: "ADENOSINE ANTAGONISM BY PURINES, PTERIDINES AND BENZOPTERIDINES IN HUMAN FIBROBLASTS" BIOCHEM PHARMACOL, vol. 30, no. 4, 1981, pages 325-333, XP002108789 abstract | 1-16 |

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 98/00343

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:

Remark: Although claim(s) 1-14
is(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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

see additional sheet

as a result of the prior review under R.40.2(e) PCT, no additional fees are to be refunded

1. [X] 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**

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

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

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/NL 98/00343

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 4-6, and partially 1-3, 8-16

   Use of adenosine analogues of formulas (I) to (III) for preparing a medicament for treating organophosphate poisoning.

2. Claims: 7 and partially 1-3, 8-16

   Use of theophylline derivatives of formula (IV) for preparing a medicament for treating organophosphate poisoning.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/NL 98/00343

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9502604 A | 26-01-1995 | AU 7331094 A | 13-02-1995 |
| | | EP 0708781 A | 01-05-1996 |
| | | US 5773423 A | 30-06-1998 |
| | | US 5688774 A | 18-11-1997 |
| JP 53056690 A | 23-05-1978 | JP 1023006 C | 28-11-1980 |
| | | JP 55012917 B | 04-04-1980 |