



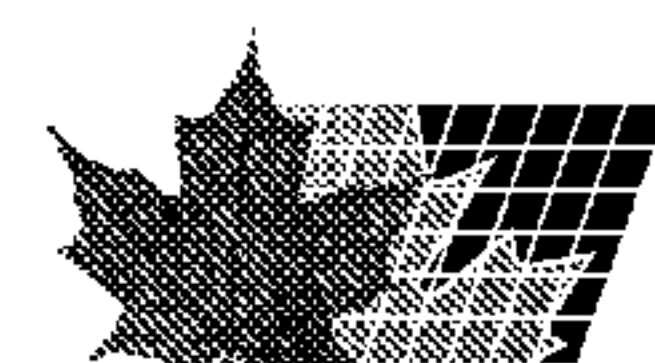
(86) Date de dépôt PCT/PCT Filing Date: 1998/07/09
(87) Date publication PCT/PCT Publication Date: 1999/01/21
(45) Date de délivrance/Issue Date: 2011/03/29
(85) Entrée phase nationale/National Entry: 1999/02/16
(86) N° demande PCT/PCT Application No.: BE 1998/000108
(87) N° publication PCT/PCT Publication No.: 1999/002675
(30) Priorité/Priority: 1997/07/09 (EP97870101.9)

(51) Cl.Int./Int.Cl. *C12N 15/12* (2006.01),
A01K 67/027 (2006.01), *A61K 31/70* (2006.01),
A61K 48/00 (2006.01), *C07K 14/705* (2006.01),
C07K 16/28 (2006.01), *C12Q 1/68* (2006.01),
G01N 33/566 (2006.01), *A61K 38/00* (2006.01)
(72) Inventeurs/Inventors:
COMMUNI, DIDIER, BE;
BOEYNAEMS, JEAN-MARIE, BE
(73) Propriétaire/Owner:
EUROSCREEN S.A., BE
(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : RECEPTEUR COUPLE A LA PROTEINE G PRESENTANT UNE AFFINITE SELECTIVE POUR L'ATP ET
MOLECULE D'ACIDE NUCLEIQUE CODANT CE RECEPTEUR
(54) Title: G-COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATP

(57) **Abrégé/Abstract:**

The present invention concerns a G-coupled receptor which has an amino acid sequence having more than 50 % homology with the amino acid sequence shown in Figure 1.



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :C12N 15/12, 5/10, C07K 14/705, 16/28,
C12Q 1/68, G01N 33/50, 33/566, A61K
31/70, A01K 67/027

A1

(11) International Publication Number:

WO 99/02675

(43) International Publication Date:

21 January 1999 (21.01.99)

(21) International Application Number: PCT/BE98/00108

(22) International Filing Date: 9 July 1998 (09.07.98)

(30) Priority Data:

97870101.9

9 July 1997 (09.07.97)

EP

(71) Applicant (for all designated States except US): EURO-
SCREEN S.A. [BE/BE]; Avenue des Becassines 7, B-1160
Bruxelles (BE).

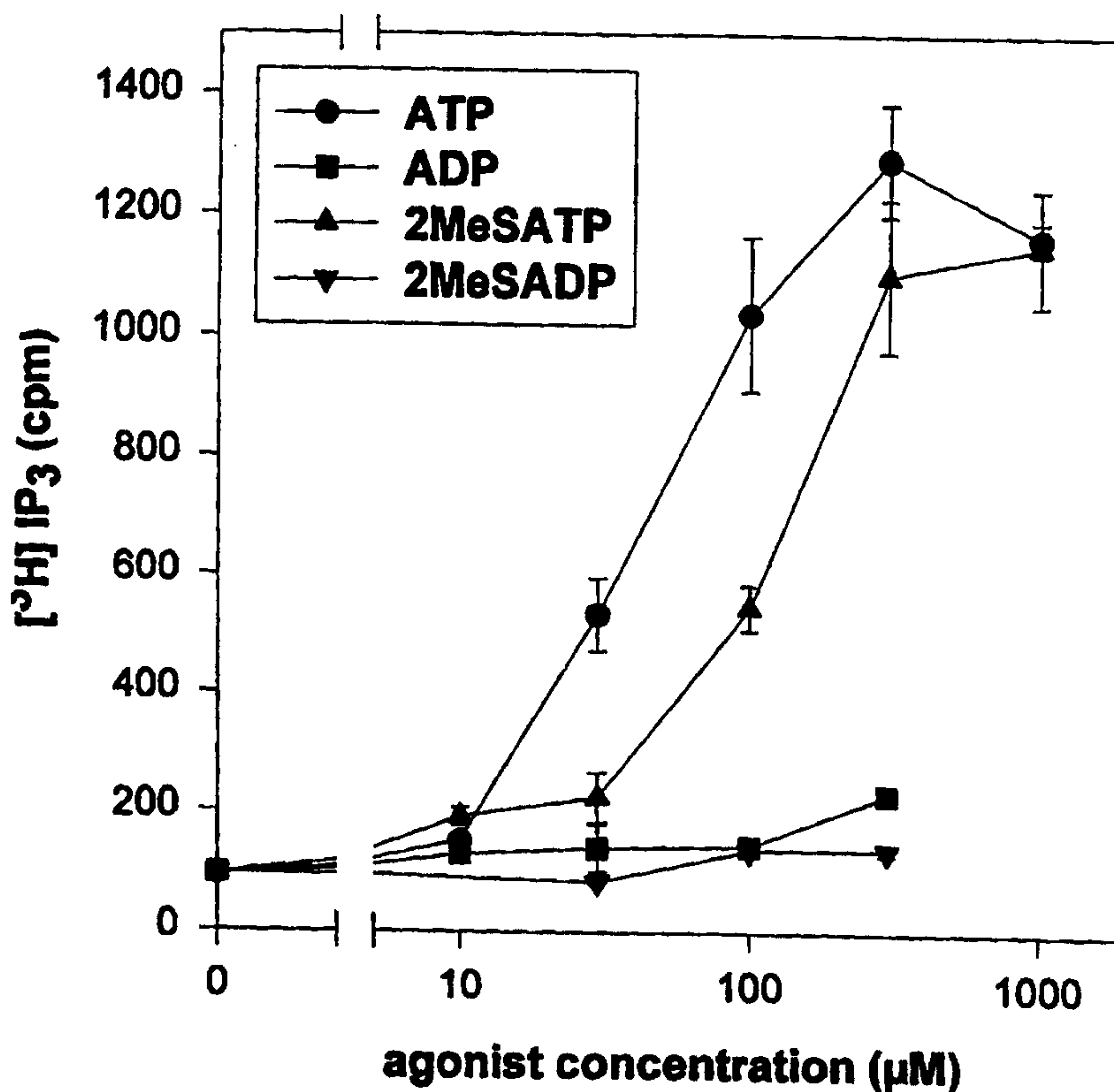
(72) Inventors; and

(75) Inventors/Applicants (for US only): COMMUNI, Didier
[BE/BE]; Groendallaan 19, B-1800 Vilvoorde (BE).
BOEYNAEMS, Jean-Marie [BE/BE]; Avenue Peter Benoît
5, B-1780 Wemmel (BE).(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen,
Place Reine Fabiola 6/1, B-1083 Brussels (BE).(81) Designated States: CA, JP, US, European patent (AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE).**Published***With international search report.**Before the expiration of the time limit for amending the
claims and to be republished in the event of the receipt of
amendments.*

(54) Title: G-COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATP

(57) Abstract

The present invention concerns a G-coupled receptor which has an amino acid sequence having more than 50 % homology with the amino acid sequence shown in Figure 1.



5

10

G-COUPLED RECEPTOR SHOWING SELECTIVE AFFINITY FOR ATPObject of the present invention

The present invention concerns a new G protein-coupled receptor having selective affinity for ATP and the nucleic acid molecule encoding said receptor, vectors comprising said nucleic acid molecule, cells transformed by said vector, antibodies directed against said receptor, nucleic acid probes directed against said nucleic acid molecule, pharmaceutical compositions comprising said products and non human transgenic animals expressing the receptor according to the invention or the nucleic acid molecule according to said receptor.

Background of the invention

An impressive number of P2 receptors subtypes has been cloned since 1993. A new molecular nomenclature has then been created in which G protein-coupled P2 receptors have been named P2Y while P2 receptors having an intrinsic ion channel activity have been named P2X. The P2Y family encompasses selective purinoceptors (the P2Y₁ receptor activated by ATP and ADP), nucleotide receptors responsive to both adenine and uracil nucleotides (P2Y₂ receptor: activated equipotentially by ATP and UTP) and pyrimidinoceptors (the P2Y₃ and P2Y₆ receptors activated by UDP; the P2Y₄ receptor: activated by UTP). The

P2Y₅ and P2Y₇ receptors display limited homologies with the other members of the P2Y family. They have been included in this family especially on the basis of radioligand binding studies showing affinities for adenine nucleotides (1-18).

5

Summary of the invention

The present invention concerns a new receptor having the amino acids sequence of figure 1 or any receptor which presents more than 50%, preferably more than 70%,
10 more preferably more than 85%, more specifically more than 95% homology with the amino acids sequence of figure 1.

The present invention concerns also the receptor having at least the amino acids sequence of figure 1 or a portion thereof, preferably an amino acids sequence
15 wherein the large extracellular part (the NH₂ portion of 450 amino acids sequence the end of which (♦) is represented on Fig. 1) has been truncated or active parts of said portion such as the sixth and seventh transmembrane domains comprising the amino acids : His⁶⁸⁶, Arg⁶⁸⁹ and
20 Arg⁷²⁸.

The present invention is also related to said NH₂ portion of 450 amino acids sequence, including peptides reproducing or mimicking a portion of this sequence or of organic molecules sharing the effects of these peptides.

25 Indeed, the inventors have discovered that either the whole receptor having the amino acids sequence of figure 1 or its portion (preferably the amino acids sequence wherein the large extracellular part of 450 amino acids has been truncated and starting from (♦) in Fig. 1)
30 seems to have the same industrial application (said portion will be identified hereafter as the P2Y₁₁ receptor or sequence).

The first industrial application of this receptor or its portions is the screening of agonists and

antagonists of said receptor which may have advantageous pharmaceutical or diagnostical properties. The second industrial application of the receptor according to the invention or of its portions or of active parts of its portions is the identification of patients who may present genetic disorders induced by an inactive receptor or by an inactive portion of said receptor.

According to a preferred embodiment of the present invention, said receptor is a human receptor.

ATP seems to be the preferential natural agonist of this receptor : UTP, UDP, AP₄A, AP₆A, AMP and adenosine seem to be unable to stimulate the phosphoinositide pathway or were much less potent than ATP.

Therefore, the invention is also related to a new G-coupled receptor, its portions or active parts of its portions having a selective affinity for ATP. "A selective affinity for ATP" means that ATP is able to induce the formation of a functional response (preferably the accumulation of Inositol triphosphate IP₃ and a rise of intracellular Ca²⁺) in a short time of incubation with said agonist (preferably in less than 5 min, more preferably less than 1 min) while the other known agonists of P2Y (UTP, UDP, AP₄A, AP₆A, AMP and adenosine were unable to stimulate said receptor or were much less potent than ATP and induce a detectable functional response by said receptor.

The present invention is also related to a nucleic acid molecule, such as a DNA molecule or an RNA molecule, encoding the receptor, its portions or active parts of its portions according to the invention.

Preferably, said DNA molecule is a cDNA molecule or a genomic DNA molecule.

Preferably, said nucleic acid molecule has more than 50%, preferably more than 70%, more preferably

more than 85%, more specifically more than 95% homology with the DNA sequence shown in figure 1.

Preferably, the invention is related to a nucleic acid molecule which has more than 50%, preferably
5 more than 70%, more preferably more than 85%, more specifically more than 95% homology with this DNA sequence (shown in the figure 1), wherein the DNA sequence encoding the 450 amino acids of the NH₂ portion were truncated.

The present invention is also related to the
10 vector comprising the nucleic acid molecule according to the invention. Preferably, said vector is adapted for expression in a cell and comprises the regulatory elements necessary for expressing the amino acid molecule in said cell operatively linked to the nucleic acid sequence
15 according to the invention as to permit expression thereof.

Preferably, said cell is selected from the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells. The vector according to the invention is a plasmid or a virus, preferably a
20 baculovirus, an adenovirus or a semliki forest virus.

The present invention concerns also the cell transformed by the vector according to the invention, said cell is preferably non-neuronal in origin and is selected from the group consisting of a COS-7 cell, a CHO cell, an
25 LM(tk-) cell, an NIH-3T3 cell or a 1321N1 astrocytoma cell.

The present invention is also related to a nucleic acid probe comprising the nucleic acid molecule according to the invention, of at least 15 nucleotides capable of specifically hybridising with a unique sequence
30 included in the sequence of the nucleic acid molecule encoding the receptor according to the invention. Said nucleic acid probe may be a DNA or an RNA molecule.

The invention concerns also an antisense oligonucleotide having a sequence capable of specifically
35 hybridising to an mRNA molecule encoding the receptor according to the invention so as to prevent translation of

WO 99/02675

PCT/BE98/00108

5

said mRNA molecule or an antisense oligonucleotide having a sequence capable of specifically hybridising to the cDNA molecule encoding the receptor according to the invention.

Said antisense oligonucleotide may comprise
5 chemical analogs of nucleotide or substances which inactivate mRNA, or be included in an RNA molecule endowed with ribozyme activity.

Another aspect of the present invention concerns a ligand (preferably an antibody) other than known
10 molecules, especially the ATP, capable of binding to the receptor according to the invention and an anti-ligand (preferably also an antibody) capable of competitively inhibiting the binding of said ligand to the receptor according to the invention.

15 Preferably, said antibody is a monoclonal antibody directed to an epitope of the receptor according to the invention and present on the surface of a cell expressing said receptor.

The invention concerns also the
20 pharmaceutical composition comprising an effective amount of oligonucleotide according to the invention, effective to decrease the activity of said receptor by passing through a cell membrane and binding specifically with mRNA encoding the receptor according to the invention in the cell so as
25 to prevent its translation. The pharmaceutical composition comprises also a pharmaceutically acceptable carrier capable of passing through said cell membrane.

Preferably, in said pharmaceutical composition, the oligonucleotide is coupled to a substance,
30 such as a ribozyme, which inactivates mRNA.

Preferably, the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure. The structure of the pharmaceutically
35 acceptable carrier in said pharmaceutical composition is

capable of binding to a receptor which is specific for a selected cell type.

Preferably, said pharmaceutical composition comprises an amount of the antibody according to the invention effective to block the binding of a ligand to the receptor according to the invention and a pharmaceutically acceptable carrier.

The present invention concerns also a transgenic non human mammal overexpressing (or expressing ectopically) the nucleic acid molecule encoding the receptor according to the invention.

The present invention also concerns a transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to the invention.

According to a preferred embodiment of the invention, the transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid according to the invention is so placed as to be transcribed into antisense mRNA which is complementary to the mRNA encoding the receptor according to the invention and which hybridises to mRNA encoding said receptor, thereby reducing its translation. Preferably, the transgenic non human mammal according to the invention comprises a nucleic acid molecule encoding the receptor according to the invention and comprises additionally an inducible promoter or a tissue specific regulatory element.

Preferably, the transgenic non human mammal is a mouse.

The invention relates also to a method for determining whether a ligand as an agonist or an antagonist of the receptor according to the invention can be specifically bound to said receptor; said method comprising the steps of contacting a cell or a cell extract from cells transfected with a vector according to the invention and expressing the nucleic acid molecule encoding said

receptor, possibly isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction or with the cell under conditions permitting binding of said ligand to the receptor and detecting, possibly by means of a bioassay such as a modification in the second messenger concentration or a modification in the cellular metabolism (preferably determined by the acidification rate of the culture medium), an increase in the receptor activity), thereby determining whether the ligand binds to the receptor, possibly as an agonist or as an antagonist of said receptor.

Preferably, the second messenger assay comprises measurement of intracellular cAMP, intracellular inositol phosphate (IP3), intracellular diacylglycerol (DAG) concentration or intracellular calcium mobilisation.

Preferably, the cell used in said method is a mammalian cell non neuronal in origin, such as a COS-7 cell, a CHO cell, a LM(tk-) cell an NIH-3T3 cell or 1321N1 cell. In said method, the ligand is not previously known.

The invention is also related to the ligand isolated and detected by any of the preceding methods.

The present invention concerns also the pharmaceutical composition which comprises an effective amount of an agonist or an antagonist of the receptor according to the invention, effective to reduce the activity of said receptor and a pharmaceutically acceptable carrier.

The P2Y11 transcripts (obtained from the nucleotidic sequence starting from (♦) in Fig. 1) are detectable in HL-60 human leukaemia cells. Expression of P2Y11 receptor mRNA is increased by agents (riboic acid, DMSO) known to induce the granulocytic differentiations of HL-60 cells. However, the P2Y11 transcripts could not be detected in mature neutrophils. Therefore, a first industrial application of the product according to the invention is the diagnostic of leukaemia, preferably by

Northern blot analysis using the nucleotidic sequence encoding the P2Y11 receptor according to the invention.

The present invention is also related to a diagnostic device or kit comprising the elements for the
5 diagnostic of specific leukaemia, preferably HL-60 human leukaemia, comprising the receptor according to the invention, the nucleic acid sequence encoding said receptor, a nucleic acid probe comprising the nucleic acid molecule according to the invention of at least 15
10 nucleotides capable of specifically hybridising with a unique sequence included in the sequence of the nucleic acid molecule encoding the receptor according to the invention, such as an antisense oligonucleotide or a ligand such as an antibody, preferably a monoclonal antibody,
15 capable of binding or competitively inhibiting the binding of a ligand to the receptor according to the invention. Said diagnostic device or kit could be used for the specific diagnostic or for the monitoring of the evolution of tumoral cells, especially leukaemia HL-60 cells.

20 Therefore, the previously described methods may be used for the screening of drugs (having advantageously anti-tumoral properties) which specifically bind to the receptor according to the invention.

Another industrial application of the present
25 invention is related to the use of said drugs, preferably ligands or anti-ligands according to the invention, for the prevention and/or the treatment of specific diseases such as neutropenie or agranulocytose infections or cancer.

The invention is also related to the drugs
30 isolated and detected by any of these methods.

The present invention concerns also a pharmaceutical composition comprising said drugs and a pharmaceutically acceptable carrier.

The invention is also related to a method of
35 detecting expression of a receptor according to the invention by detecting the presence of mRNA coding for a

receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to the invention under hybridising conditions and detecting the presence of mRNA hybridised to the probe, thereby detecting the expression of the receptor by the cell.

The hybridisation conditions above-described are preferably standard stringent conditions as described by Sambrook et al. (§9.47-9.51 in Molecular Cloning : Laboratory Manual, Cold Spring Harbour, Laboratory Press, New York (1989)).

The present invention concerns also a method for diagnosing a predisposition to a disorder associated with the activity of the receptor according to the invention. Said method comprises:

- a) obtaining nucleic acid molecules of subjects suffering from said disorder;
- b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;
- 20 c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;
- d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridising to said nucleic acid molecule and labelled with a detectable marker;
- 25 e) detecting labelled bands which have hybridised to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;
- f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and
- 30 g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose
- 35

thereby predisposition to the disorder if the patterns are the same.

A last aspect of the present invention concerns a method of preparing the receptor according to the invention, which comprises:

- a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;
- b) inserting the vector of step a in a suitable host cell;
- c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;
- d) recovering the receptor so obtained; and
- e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

Brief description of the drawings

Figure 1 represents the nucleotide and deduced amino acid sequence of the new human P2Y receptor. The putative phosphorylation sites by protein kinase C or by calmodulin-dependent protein kinases are indicated respectively by a black circle (●) or a black diamond (◆). The potential N-glycosylation site is indicated by a black square (■).

Figure 2 represents dendrogram of the structural relatedness of the P2Y₁₁ receptor with the other P2Y subtypes. The plot was constructed using the multiple sequence alignment program Pileup of the GCG package. The P2Y₅-like published sequence (18) is identical to the P2Y₉ sequence submitted to the GenBank/EMBL Data Bank.

Figure 3 represents Northern blot analysis of P2Y₁₁ messenger expression. Each lane of the MTN blot contains 2 µg of polyA⁺ RNA from several human tissues. Each lane of the HL-60 blot contains 10 µg of total RNA from 5 differentiated or undifferentiated HL-60 cells. Hybridization with the probe was performed as described under Materials and Methods. The pictures of the MTNII blot and the HL-60 blot were obtained respectively, from an autoradiography and from a PhosphorImager SI (Molecular 10 Dynamics). The 2kb-length P2Y₁₁ transcripts are indicated by a black arrow.

Figure 4 represents concentration-action curves of several nucleotides on IP₃ and cAMP accumulation in cells transfected with the P2Y₁₁ receptor. 1321N1 and CHO-K1 15 transfected cells were assayed for the accumulation of, respectively, IP₃ (A) or cAMP (B) in response to various concentrations of the following nucleotides: ATP, 2MeSATP, ADP and 2MeSADP. Incubation times were 30 s for IP₃ measurements and 15 min for cAMP assays. The data represent 20 the means ± S.D. of triplicate experimental points and are representative of two independent experiments.

Description of a preferred embodiment of the present invention

25 Experimental Procedures

Materials

Trypsin was from Flow Laboratories (Bioggio, Switzerland). Culture media, G418, fetal calf serum (FCS), restriction enzymes and Taq polymerase were purchased from 30 GIBCO BRL (Grand Island, NY). The radioactive products myo-D-[2-³H]inositol (17.7 Ci/mmol) and [α ³²P]ATP (800 Ci/mmol) were from Amersham (Ghent, Belgium). Dowex TM AG1X8 (formate form) was from Bio-Rad Laboratories (Richmond, Calif.). ATP, ADP, AMP, adenosine, UTP, UDP, AP₄A, AP₆A, all-trans

retinoic acid (RA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-methylthio-ATP (2MeSATP), 2-methylthio-ADP (2MeSADP) and 8 (p-sulfophenyl) theophylline were from Research Biochemicals International (Natick, MA). Forskolin was purchased from Calbiochem (Bierges, Belgium). Indomethacin and dimethyl sulfoxide (DMSO) were from Merck (Netherlands). Rolipram was obtained from the Laboratoires Jacques Logeais (Trappes, France). The HL-60 human cell line was obtained from the American Type Culture Collection (Rockville, USA). The human genomic DNA library was from Stratagene (La Jolla, CA). pEFIN3 is an expression vector obtained from Euroscreen (Brussels, Belgium). Multiple Human Tissues Northern blot (MTN) were from Clontech (Palo Alto, CA).

Cloning and sequencing

A human placenta cDNA library was screened at moderate stringency with an [$\alpha^{32}\text{P}$] dATP labelled P2Y₄ receptor probe corresponding to a partial sequence covering the third to the seventh transmembrane segments. Three overlapping clones encoding a new G protein-coupled receptor were isolated, but did not contain the 3' end of the coding region. A human genomic DNA library was then screened with this partial sequence to obtain the complete sequence of this new receptor. The hybridization conditions for screening the two libraries were 6 X SSC (1 X SSC: 0.15 M NaCl, 0.015 M sodium citrate) and 40% formamide at 42 °C for 14 hours and the final washing conditions were 0.5 X SSC, 0.1% SDS at 60 °C. Four genomic clones were purified and shown to contain the 3' end of the open reading frame missing in the cDNA clones. The sequence was obtained on both strands after subcloning of overlapping restriction fragments in M13mp18 and M13mp19 using the Sanger dideoxy nucleotide chain termination method.

Northern blot analysis

Two blots of human organs (MTN I and MTN II: 2 µg polyA⁺ RNA/lane) and a blot containing total RNA from differentiated and undifferentiated HL-60 cells (10 µg of total RNA/lane) were hybridized with a probe corresponding to the new receptor in order to characterize its tissue distribution. The HL-60 cells were maintained in RPMI 1640 supplemented with 10% FCS, 5 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin at 37 °C with 5% CO₂. The HL-60 cells were incubated during six days with or without 1 µM retinoic acid or 1.25% DMSO or during eight hours with 25 nM TPA. The RNA from the differentiated or undifferentiated HL-60 cells was prepared with the RNeasy kit (Quiagen). The blots were prehybridized 8 hours at 42 °C in a 50% formamide, 2% SDS solution and hybridized for 18 hours in the same solution supplemented with the [α^{32} P] labelled probe. The final washing conditions were 0.1 X SSC and 0.1% SDS at 55 °C. The blots were exposed during twelve days and visualized as an autoradiography or using the PhosphorImager SI (Molecular Dynamics).

Cell culture and transfection

The complete sequence of the new receptor according to the invention was subcloned between the Hind III and Nhe I sites of the bicistronic pEFIN3 expression vector. 1321N1 and CHO-K1 cells were transfected with the recombinant pEFIN3 plasmid or with the plasmid alone using the calcium phosphate precipitation method as described (19). The transfected cells were selected with 400 µg/ml G418 in complete medium (10% FCS, 100 units/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin B in Dulbecco's modified Eagle's medium (DMEM)) two days after transfection and maintained in the same medium (10).

Inositol phosphates (IP) measurement

1321N1 cells were labelled for 24 hours with 10 mCi/ml [³H] inositol in inositol free DMEM containing 5% FCS, antibiotics, amphotericin, sodium pyruvate and 400 µg/ml G418. Cells were washed twice with Krebs-Ringer Hepes (KRH) buffer of the following composition (124 mM NaCl, 5 mM KCl, 1.25 mM MgSO₄, 1.45 mM CaCl₂, 1.25 mM KH₂PO₄, 25 mM Hepes (pH:7.4) and 8 mM glucose) and incubated in the same medium for 30 min. The cells were then challenged by various nucleotides for 30 s. The incubation was stopped by the addition of an ice cold 3% perchloric acid solution. IP were extracted and separated on Dowex columns as previously described (20).

15 Cyclic AMP measurements

Stably transfected CHO-K1 or 1321N1 cell lines were spread on Petri dishes (150.000 cells per dish) and cultured in Ham's F12 or DMEM medium containing 10% FCS, antibiotics, amphotericin, sodium pyruvate and 400 µg/ml G418. Cells were preincubated for 30 min in KRH buffer with rolipram (25 µM) and incubated for different times in the presence of the agonists (15 min in most experiments). The incubation was stopped by the addition of 1 ml HCl 0.1 M. The incubation medium was dried up, resuspended in water and diluted as required. Cyclic AMP was quantified by radioimmunoassay after acetylation as previously described (21).

Results30 Cloning and Sequencing

A human cDNA placenta library was screened at moderate stringency with a human P2Y₄ probe. Nine clones which hybridized weakly with the P2Y₄ probe were obtained, purified and analyzed. Six of them corresponded to the

sequence of the P2Y₆ receptor (10) while three overlapping clones corresponded to a partial sequence encoding a new G protein-coupled receptor, displaying about 30% identity with the other P2Y receptors. The partial open reading
5 frame started with an ATG-codon in a Kozak consensus but the 3' end was missing in all three cDNA clones. The Inventors screened a human genomic DNA library using this partial sequence as a probe. Four overlapping genomic clones were obtained. Mapping of the coding sequence and
10 partial sequencing allowed to determine that the gene encoding the new receptor contains an intron interrupting the coding sequence at the 5' end of the gene. This intron separates the three first codons from the rest of the coding sequence. Beside these first codons, the four
15 genomic clones contained the complete open reading frame including the 3' end missing in the cDNA clones. The full open reading frame appeared as 1113 base pairs (bp) long and encoded a protein of 371 amino acids containing one potential site for N-linked glycosylation and two potential
20 sites for phosphorylation by protein kinase C or calmodulin-dependent protein kinases (Fig. 1). The new receptor, provisionally named P2Y₁₁, displays significant homologies with the other P2Y receptors (Fig. 2). In particular, 33% and 28 % amino acid identity were observed
25 respectively with the human P2Y₁ and P2Y₂ receptors.

Tissue distribution of the new receptor

The tissue distribution of the new receptor transcripts was investigated by Northern blotting (Fig. 3)
30 by using a probe corresponding to a partial sequence encoding transmembrane segments 3 to 7. The strongest signal was observed for human spleen and corresponded to a 2 kilobase (kb)-length messenger RNA (MTN II). A weaker signal was observed in small intestine (MTN II). All the
35 lanes in MTN I (heart, brain, placenta, lung, liver,

skeletal muscle, kidney, pancreas) were negative. The Inventors also detected specific 2 kb-length transcripts in HL-60 cells. The signal was very weak in the undifferentiated HL-60 cells but increased when the cells
5 had been treated with retinoic acid or DMSO. No increase was observed when the HL-60 cells were stimulated with TPA. A weak non-specific hybridization with 18S rRNA was observed. These data were confirmed with a non-overlapping probe corresponding to the first 300 bp of the coding
10 region, presenting limited homologies with the other P2Y subtypes.

Functional Expression of the new receptor in 1321N1 astrocytoma cells

The complete sequence of the new receptor was
15 introduced in the pEFIN3 expression vector in order to transfect the 1321N1 astrocytoma cell line, used previously to characterize several P2Y subtypes (6, 10, 12). The pool of G418-resistant clones was tested for its functional response to several nucleotides. ATP (100 μ M) induced a
20 strong inositol trisphosphate (IP₃) accumulation in cells transfected with the recombinant plasmid, whereas ADP, AMP, adenosine, UTP, UDP, AP₄A and AP₆A were inactive at the same concentration. All nucleotides were totally inactive on the cells transfected with the vector alone. We then
25 tested ATP, 2MeSATP, ADP and 2MeSADP in a large range of concentrations. As shown in Fig. 4A, ATP was the most potent agonist (EC_{50} ATP = 38 ± 7 μ M; EC_{50} 2MeSATP = 118 ± 15 μ M; means $\pm$ range of two independent experiments). The effect of ADP and 2MeSADP were minimal. Pertussis toxin
30 (50 ng/ml; 24 h pretreatment) had no effect on the ATP response, whereas a lower concentration of pertussis toxin was previously shown to abolish the response to UTP in P2Y₄ transfected 1321N1 astrocytoma cells (22). A response to ATP (10 μ M) was also obtained following $[Ca^{2+}]_i$

measurements performed on the 1321N1 transfected cells while ADP was inactive at this concentration.

Functional expression of the new receptor in CHO-K1 cells

5 The 1321N1 cells transfected with the new receptor displayed a strong cAMP increase in response to ATP. A much lower but significant endogeneous response due to the degradation of adenine nucleotides into adenosine was also obtained in the 1321N1 cells transfected with the
10 vector alone. The CHO-K1 cells express an endogeneous P2Y₂ receptor coupled to the phosphoinositide pathway (23) but do not possess adenosine receptors coupled to adenylyl cyclase. We therefore used CHO-K1 cells in order to characterize the coupling of the new receptor to the cAMP
15 pathway. A pool of G418 resistant CHO-K1 clones was first tested for its response to several nucleotides at a concentration of 100 μ M. ATP was able to induce a strong increase in the cAMP content, whereas it was inactive on cells transfected with the vector alone. ADP, AMP,
20 adenosine, UTP and UDP were completely inactive. Concentration-action curves were established for ATP, 2MeSATP, ADP and 2MeSADP (Fig. 4B). The rank order of potency was the same as in the inositol phosphate study on 1321N1 cells. The curves were obtained after 15 min of
25 stimulation by the agonists; however a significant cAMP response to ATP was already obtained after 2 min of stimulation. The response to ATP (30 μ M) was inhibited neither by indomethacin (10 μ g/ml, present from 30 minutes before the stimulation and readded in the stimulation
30 medium) nor by 8 (p-sulfophenyl) theophylline (100 μ M).

 The receptor according to the invention presents some structural peculiarities which differentiate it from some other P2Y subtypes. Concerning its gene structure, the coding sequence is interrupted by an intron.
35 Comparison between the cDNA and the genomic DNA sequences has clearly demonstrated the absence of intron in the

coding region of the human P2Y₁ receptor (24, 25), the rat P2Y₂ receptor (26) and the rat P2Y₆ receptor (11). In terms of protein structure, the second and third extracellular loops are significantly longer than those of the other P2Y receptors. The homology with the other subtypes is relatively weak (about 30%). The closest G-coupled receptor is the human P2Y₁ receptor (33%) which is also a receptor responsive to adenine nucleotides (3, 4). Mutagenesis experiments with the P2Y₂ receptor have identified three positively charged amino acids in the sixth and seventh transmembrane domains (His²⁶², Arg²⁶⁵ and Arg²⁹²), which play a crucial role in nucleotide binding (presumably by neutralizing the negative charge of the phosphate groups) (27). These three residues are conserved in this new receptor.

So far, eight P2Y receptor subtypes are described in the literature (P2Y₁-P2Y₈). In addition, two sequences related to the P2Y₅ receptor and named P2Y₉ and P2Y₁₀, have been recently submitted to the GenBank/EMBL Data Bank. The P2Y₉ sequence is identical to that recently published under the name "P2Y₅-like" (18). Therefore the new receptor described in this paper might be called P2Y₁₁. However, it is already clear that the nomenclature needs a revision. It was recently demonstrated that the P2Y₇ receptor is actually a receptor for leukotriene B₄ (16) and there is no functional evidence that the P2Y₅ and related receptors (P2Y₅-like or P2Y₉, P2Y₁₀) are nucleotide receptors (17, 18).

Among the sixteen human organs tested by Northern blotting, P2Y₁₁ transcripts of 2 kb size were only detectable in spleen, and with lower intensity in small intestine. This distribution is reminiscent of that of the human P2Y₆ 1.7 kb-messenger. The observation of the

expression of the P2Y₁₁ receptor in the HL-60 cell line shows that this expression was strongly increased following treatment by DMSO or retinoic acid, two agents known to induce the differentiation of these cells into granulocytes (28). On the contrary, TPA, which is known to induce the monocytic differentiation of the HL-60 cells (29), did not stimulate the expression of the P2Y₁₁ receptor. The confirmation of these data with a second probe of the P2Y₁₁ cDNA, that shares little similarity with other P2Y sequences, excludes possible cross-hybridization with another P2Y receptor transcript. In view of the Northern blots results, it is tempting to speculate that the P2Y₁₁ receptor is involved in the recently described accumulation of cAMP in ATP-stimulated HL-60 cells (30).

Among the P2Y receptors, the P2Y₁₁ subtype has the unique property to activate both the phosphoinositide and the cAMP pathways. Other cloned P2Y receptors are coupled to phospholipase C exclusively. The rank order of potency of agonists was the same for the two pathways. ATP was clearly much more potent than ADP. This difference may be even underestimated as a result of low level ATP contamination in ADP preparation or conversion of ADP into ATP during assays (4, 11). On the other hand, 2MeSATP had the same maximal effect than ATP but presented a lower potency, while 2MeSADP, a potent activator of the P2Y₁ and P2_T subtypes (4), was almost inactive. The EC₅₀ values were comparable to those obtained in the study concerning the effects of extracellular nucleotides on the cAMP accumulation in the HL-60 cells (30).

Stimulatory effects of adenine nucleotides on the cAMP pathway have been described in different cell types (31, 32). In most cases, the stimulatory effect of nucleotides was inhibited by xanthines. These data suffer from the fact that it is difficult to exclude that the effect of adenine nucleotides is mediated by their

degradation into adenosine due to the ubiquitous presence of ectonucleotidases expressed at the cell surface. The cAMP study has been performed with CHO-K1 cells to avoid the endogeneous cAMP response to adenosine in the
5 astrocytoma cell line. Neither in untransfected CHO-K1 cells nor in P2Y₁₁-transfected CHO-K1 cells did adenosine increase cAMP accumulation. Furthermore the cAMP response to ATP was insensitive to xanthine inhibition. It was also insensitive to indomethacin, indicating that is not
10 mediated by the release of prostaglandins. It is unlikely that the cAMP response would be an indirect consequence of the calcium response since the use of ATP, which activates the phosphoinositide pathway by the activation of P2Y₂ endogeneous receptors, or the use of calcium ionophores in
15 the CHO-K1 cells failed to stimulate cAMP accumulation (33). Therefore these data constitute the first strong evidence that a P₂ receptor can be coupled to the stimulation of adenylyl cyclase.

REFERENCES

1. Abbrachio, M.P. and Burnstock, G. (1994) *Pharmacol. Ther.* **64**, 445-475.
2. Fredholm, B.B. et al. (1997) *Trends Pharmacol. Sci.* **18**, 79-82.
3. Webb, T.E. et al. (1993) *FEBS Lett.* **324**, 219-225.
4. Léon, C. et al. (1997) *FEBS Lett.* **403**, 26-30.
5. Lustig, K.D. et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5113-5117.
- 10 6. Parr, C.E. et al. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3275-3279.
7. Bogdanov, Y. et al. (1997) *J. Biol. Chem.* **272**, 12583-12590.
8. Webb, T.E. et al. (1996) *Mol. Pharmacol.* **50**, 258-265.
- 15 9. Chang, K. et al. (1995) *J. Biol. Chem.* **270**, 26152-26158.
10. Communi, D. et al; (1996) *Biochem. Biophys. Res. Commun.* **222**, 303-308.
11. Nicholas, R.A. et al. (1996) *Mol. Pharmacol.* **50**, 224-229.
- 20 12. Communi, D. et al. (1995) *J. Biol. Chem.* **270**, 30849-30852.
13. Nguyen, T. et al. (1995) *J. Biol. Chem.* **270**, 30845-30848.
- 25 14. Webb, T.E. et al. (1996) *Biochem. Biophys. Res. Commun.* **219**, 105-110.
15. Akbar, G.K.M. et al. (1996) *J. Biol. Chem.* **271**, 18363-18367.
16. Yokomizo, T. et al; (1997) *Nature* **387**, 620-624.
- 30 17. Schachter, J.B. and Harden, T.K. (1997) *Br. J. Pharmacol.* **121**, 338-344.
18. Janssens, R. et al. (1997) *Biochem. Biophys. Res. Commun.* **226**, 106-112.

19. Velu, T.J. et al. (1989) *J. Cell. Biochem.* 39, 153-166.
20. Communi, D. et al. (1995) *Circ. Res.* 76, 191-198.
21. Brooker, G. et al. (1979) *Adv. Cyclic Nucleotide Res.*
5 10, 1-33.
22. Communi, D. et al. (1996) *Eur. J. Pharmacol.* 317, 383-389.
23. Iredale, P.A. and Hill, S.J. (1993) *Br. J. Pharmacol.* 110, 1305-1310.
- 10 24. Schachter, J.B. et al. (1996) *Br. J. Pharmacol.* 118, 167-173.
25. Janssens, R. et al. (1996) *Biochem. Biophys. Res. Commun.* 221, 588-593.
26. Godecke, S. et al. (1996) *Am. J. Physiol.* 270, C570-
15 C577.
27. Erb, L. et al. (1995) *J. Biol. Chem.* 270, 4185-4188.
28. Breitman, T.R. et al. (1980) *Proc. Natl. Acad. Sci. USA* 77, 2936-2940.
29. Blaineau, C. et al. (1983) *Embo J.* 11, 2007-2012.
- 20 30. Jiang, L. et al. (1997) *Biochem. Biophys. Res. Commun.* 232, 626-630.
31. Matsuoka, I. et al. (1995) *Mol. Pharmacol.* 47, 855-862.
32. Song, S.L. and Chueh, S.H. (1996) *Brain Res.* 734, 243-
25 251.
33. Thekkumkara, T.J. et al. (1995) *Mol. Cell Biochem.* 152, 77-86.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: EUROSCREEN S.A.
 (B) STREET: Avenue des Becassines 7
 (C) CITY: BRUSSELS
 (E) COUNTRY: BELGIUM
 (F) POSTAL CODE (ZIP): B-1160

(A) NAME: COMMUNI Didier
 (B) STREET: Groendallaan 19
 (C) CITY: VILVOORDE
 (E) COUNTRY: BELGIUM
 (F) POSTAL CODE (ZIP): B-1800

(A) NAME: BOEYNAEMS Jean-Marie
 (B) STREET: Avenue Peter Benoit 5
 (C) CITY: WEMMEL
 (E) COUNTRY: BELGIUM
 (F) POSTAL CODE (ZIP): B-1780

(ii) TITLE OF INVENTION: G-COUPLED RECEPTOR SHOWING SLECTIVE AFFINITY
 FOR ATP AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: WO PCT/BE 98/00108

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2427 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 40..2424

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAATTCGGCA CGAGGAGGCC TCGTGGAGGA CACAGCAGC ATG GGA CAG TCA GGG	54
Met Gly Gln Ser Gly	
1 5	
AGG TCC CGG CAC CAG AAG CGC GCC CGC GCC CAG GCG CAG CTC CGC AAC	102
Arg Ser Arg His Gln Lys Arg Ala Arg Ala Gln Ala Gln Leu Arg Asn	

24

10								15				20				
CTC	GAG	GCC	TAT	GCC	GCG	AAC	CCG	CAC	TCG	TTC	GTG	TTC	ACG	CGA	GGC	150
Leu	Glu	Ala	Tyr	Ala	Ala	Asn	Pro	His	Ser	Phe	Val	Phe	Thr	Arg	Gly	
			25					30					35			
TGC	ACG	GGT	CGC	AAC	ATC	CGG	CAG	CTC	AGC	CTG	GAC	GTG	CGG	CGG	GTC	198
Cys	Thr	Gly	Arg	Asn	Ile	Arg	Gln	Leu	Ser	Leu	Asp	Val	Arg	Arg	Val	
		40					45					50				
ATG	GAG	CCG	CTC	ACT	GCC	AGC	CGT	CTG	CAG	GTT	CGT	AAG	AAG	AAC	TCG	246
Met	Glu	Pro	Leu	Thr	Ala	Ser	Arg	Leu	Gln	Val	Arg	Lys	Lys	Asn	Ser	
	55					60					65					
CTG	AAG	GAC	TGC	GTG	GCA	GTG	GCT	GGG	CCC	CTC	GGG	GTC	ACA	CAC	TTT	294
Leu	Lys	Asp	Cys	Val	Ala	Val	Ala	Gly	Pro	Leu	Gly	Val	Thr	His	Phe	
70					75				80						85	
CTG	ATC	CTG	AGC	AAA	ACA	GAG	ACC	AAT	GTC	TAC	TTT	AAG	CTG	ATG	CGC	342
Leu	Ile	Leu	Ser	Lys	Thr	Glu	Thr	Asn	Val	Tyr	Phe	Lys	Leu	Met	Arg	
				90				95						100		
CTC	CCA	GGA	GGC	CCC	ACC	TTG	ACC	TTC	CAG	GTG	AAG	AAG	TAC	TCG	CTG	390
Leu	Pro	Gly	Gly	Pro	Thr	Leu	Thr	Phe	Gln	Val	Lys	Lys	Tyr	Ser	Leu	
			105					110					115			
GTG	CGT	GAT	GTG	GTC	TCC	TCA	CTG	CGC	CGG	CAC	CGC	ATG	CAC	GAG	CAG	438
Val	Arg	Asp	Val	Val	Ser	Ser	Leu	Arg	Arg	His	Arg	Met	His	Glu	Gln	
		120					125					130				
CAG	TTT	GCC	CAC	CCA	CCC	CTC	CTG	GTA	CTC	AAC	AGC	TTT	GGC	CCC	CAT	486
Gln	Phe	Ala	His	Pro	Pro	Leu	Leu	Val	Leu	Asn	Ser	Phe	Gly	Pro	His	
	135					140					145					
GGT	ATG	CAT	GTG	AAG	CTC	ATG	GCC	ACC	ATG	TTC	CAG	AAC	CTG	TTC	CCC	534
Gly	Met	His	Val	Lys	Leu	Met	Ala	Thr	Met	Phe	Gln	Asn	Leu	Phe	Pro	
150					155				160						165	
TCC	ATC	AAC	GTG	CAC	AAG	GTG	AAC	CTG	AAC	ACC	ATC	AAG	CGC	TGC	CTC	582
Ser	Ile	Asn	Val	His	Lys	Val	Asn	Leu	Asn	Thr	Ile	Lys	Arg	Cys	Leu	
				170				175						180		
CTC	ATC	GAC	TAC	AAC	CCC	GAC	TCC	CAG	GAG	CTG	GAC	TTC	CGC	CAC	TAT	630
Leu	Ile	Asp	Tyr	Asn	Pro	Asp	Ser	Gln	Glu	Leu	Asp	Phe	Arg	His	Tyr	
			185					190					195			
AGC	ATC	AAA	GTT	GTT	CCT	GTG	GGC	GCG	AGT	CGC	GGG	ATG	AAG	AAG	CTG	678
Ser	Ile	Lys	Val	Val	Pro	Val	Gly	Ala	Ser	Arg	Gly	Met	Lys	Lys	Leu	
		200					205					210				
CTC	CAG	GAG	AAG	TTC	CCC	AAC	ATG	AGC	CGC	CTG	CAG	GAC	ATC	AGC	GAG	726
Leu	Gln	Glu	Lys	Phe	Pro	Asn	Met	Ser	Arg	Leu	Gln	Asp	Ile	Ser	Glu	
	215					220					225					
CTG	CTG	GCC	ACG	GGC	GCG	GGG	CTG	TCG	GAG	AGC	GAG	GCA	GAG	CCT	GAC	774
Leu	Leu	Ala	Thr	Gly	Ala	Gly	Leu	Ser	Glu	Ser	Glu	Ala	Glu	Pro	Asp	
230					235				240						245	
GGC	GAC	CAC	AAC	ATC	ACA	GAG	CTG	CCT	CAG	GCT	GTC	GCT	GGC	CGT	GGC	822
Gly	Asp	His	Asn	Ile	Thr	Glu	Leu	Pro	Gln	Ala	Val	Ala	Gly	Arg	Gly	
				250				255						260		

AAC	ATG	CGG	GCC	CAG	CAG	AGT	GCA	GTG	CGG	CTC	ACC	GAG	ATC	GGC	CCG	870
Asn	Met	Arg	Ala	Gln	Gln	Ser	Ala	Val	Arg	Leu	Thr	Glu	Ile	Gly	Pro	
			265					270					275			
CGG	ATG	ACA	CTG	CAG	CTC	ATC	AAG	GTC	CAG	GAG	GGC	GTC	GGG	GAG	GGC	918
Arg	Met	Thr	Leu	Gln	Leu	Ile	Lys	Val	Gln	Glu	Gly	Val	Gly	Glu	Gly	
		280					285					290				
AAA	GTG	ATG	TTC	CAC	AGT	TTT	GTG	AGC	AAG	ACG	GAG	GAG	GAG	CTG	CAG	966
Lys	Val	Met	Phe	His	Ser	Phe	Val	Ser	Lys	Thr	Glu	Glu	Glu	Leu	Gln	
	295					300					305					
GCC	ATC	CTG	GAA	GCC	AAG	GAG	AAG	AAG	CTG	CGG	CTG	AAG	GCG	CAG	AGG	1014
Ala	Ile	Leu	Glu	Ala	Lys	Glu	Lys	Lys	Leu	Arg	Leu	Lys	Ala	Gln	Arg	
310					315				320						325	
CAG	GCC	CAG	CAG	GCC	CAG	AAT	GTG	CAG	CGC	AAG	CAG	GAG	CAG	CGG	GAG	1062
Gln	Ala	Gln	Gln	Ala	Gln	Asn	Val	Gln	Arg	Lys	Gln	Glu	Gln	Arg	Glu	
				330				335						340		
GCC	CAC	AGA	AAG	AAG	AGC	CTG	GAG	GGC	ATG	AAG	AAG	GCA	CGG	GTC	GGG	1110
Ala	His	Arg	Lys	Lys	Ser	Leu	Glu	Gly	Met	Lys	Lys	Ala	Arg	Val	Gly	
			345					350					355			
GGT	AGT	GAT	GAA	GAG	GCC	TCT	GGG	ATC	CCT	TCA	AGG	ACG	GCG	AGC	CTG	1158
Gly	Ser	Asp	Glu	Glu	Ala	Ser	Gly	Ile	Pro	Ser	Arg	Thr	Ala	Ser	Leu	
		360					365					370				
GAG	TTG	GGT	GAG	GAC	GAT	GAT	GAA	CAG	GAA	GAT	GAT	GAC	ATC	GAG	TAT	1206
Glu	Leu	Gly	Glu	Asp	Asp	Asp	Glu	Gln	Glu	Asp	Asp	Asp	Ile	Glu	Tyr	
	375					380					385					
TTC	TGC	CAG	GCG	GTG	GGC	GAG	GCG	CCC	AGT	GAG	GAC	CTG	TTC	CCC	GAG	1254
Phe	Cys	Gln	Ala	Val	Gly	Glu	Ala	Pro	Ser	Glu	Asp	Leu	Phe	Pro	Glu	
390					395				400						405	
GCC	AAG	CAG	AAA	CGG	CTT	GCC	AAG	TCT	CCA	GGG	CGG	AAG	CGG	AAG	CGG	1302
Ala	Lys	Gln	Lys	Arg	Leu	Ala	Lys	Ser	Pro	Gly	Arg	Lys	Arg	Lys	Arg	
				410				415					420			
TGG	GAA	ATG	GAT	CGA	GGT	GCC	AAG	TCC	TGC	CCT	GCC	AAC	TTC	TTG	GCA	1350
Trp	Glu	Met	Asp	Arg	Gly	Ala	Lys	Ser	Cys	Pro	Ala	Asn	Phe	Leu	Ala	
			425					430					435			
GCT	GCC	GAC	GAC	AAA	CTC	AGT	GGG	TTC	CAG	GGG	GAC	TTC	CTG	TGG	CCC	1398
Ala	Ala	Asp	Asp	Lys	Leu	Ser	Gly	Phe	Gln	Gly	Asp	Phe	Leu	Trp	Pro	
		440					445					450				
ATA	CTG	GTG	GTT	GAG	TTC	CTG	GTG	GCC	GTG	GCC	AGC	AAT	GGC	CTG	GCC	1446
Ile	Leu	Val	Val	Glu	Phe	Leu	Val	Ala	Val	Ala	Ser	Asn	Gly	Leu	Ala	
	455					460					465					
CTG	TAC	CGC	TTC	AGC	ATC	CGG	AAG	CAG	CGC	CCA	TGG	CAC	CCC	GCC	GTG	1494
Leu	Tyr	Arg	Phe	Ser	Ile	Arg	Lys	Gln	Arg	Pro	Trp	His	Pro	Ala	Val	
470					475				480						485	
GTC	TTC	TCT	GTC	CAG	CTG	GCA	GTC	AGC	GAC	CTG	CTC	TGC	GCT	CTG	ACG	1542
Val	Phe	Ser	Val	Gln	Leu	Ala	Val	Ser	Asp	Leu	Leu	Cys	Ala	Leu	Thr	
				490				495					500			
CTG	CCC	CCG	CTG	GCC	GCC	TAC	CTC	TAT	CCC	CCC	AAG	CAC	TGG	CGC	TAT	1590
Leu	Pro	Pro	Leu	Ala	Ala	Tyr	Leu	Tyr	Pro	Pro	Lys	His	Trp	Arg	Tyr	

505							510					515					
GGG Gly	GAG Glu	GCC Ala 520	GCG Ala	TGC Cys	CGC Arg	CTG Leu	GAG Glu 525	CGC Arg	TTC Phe	CTC Leu	TTC Phe	ACC Thr 530	TGC Cys	AAC Asn	CTG Leu	1638	
CTG Leu 535	GGC Gly 535	AGC Ser	GTC Val	ATC Ile	TTC Phe 540	ATC Ile 540	ACC Thr	TGC Cys	ATC Ile	AGC Ser	CTC Leu 545	AAC Asn	CGC Arg	TAC Tyr	CTG Leu	1686	
GGC Gly 550	ATC Ile	GTG Val	CAC His	CCC Pro	TTC Phe 555	TTC Phe	GCC Ala	CGA Arg	AGC Ser	CAC His 560	CTG Leu	CGA Arg	CCC Pro	AAG Lys	CAC His 565	1734	
GCC Ala	TGG Trp	GCC Ala	GTG Val 570	AGC Ser 570	GCT Ala	GCC Ala	GGC Gly	TGG Trp	GTC Val 575	CTG Leu	GCC Ala	GCC Ala	CTG Leu 580	CTG Leu 580	GCC Ala	1782	
ATG Met	CCC Pro	ACA Thr	CTC Leu 585	AGC Ser	TTC Phe	TCC Ser	CAC His	CTG Leu 590	AAG Lys	AGG Arg	CCG Pro	CCG Pro	CAG Gln 595	CAG Gln	GGG Gly	1830	
GCG Ala	GGC Gly 600	AAC Asn 600	TGC Cys	AGC Ser	GTG Val	GCC Ala	AGG Arg 605	CCC Pro	GAG Glu	GCC Ala	TGC Cys 610	ATC Ile 610	AAG Lys	TGT Cys	CTG Leu	1878	
GGG Gly 615	ACA Thr 615	GCA Ala	GAC Asp	CAC His	GGG Gly 620	CTG Leu 620	GCG Ala	GCC Ala	TAC Tyr	AGA Arg	GCG Ala 625	TAT Tyr	AGC Ser	CTG Leu	GTG Val	1926	
CTG Leu 630	GCG Ala	GGG Gly	TTG Leu	GGC Gly	TGC Cys 635	GGC Gly	CTG Leu	CCG Pro	CTG Leu	CTG Leu 640	CTC Leu	ACG Thr	CTG Leu	GCA Ala	GCC Ala 645	1974	
TAC Tyr	GGC Gly	GCC Ala	CTC Leu	GGG Gly 650	CGG Arg	GCC Ala	GTG Val	CTA Leu	CGC Arg 655	AGC Ser	CCA Pro	GGC Gly	ATG Met 660	ACT Thr 660	GTG Val	2022	
GCC Ala	GAG Glu	AAG Lys 665	CTG Leu 665	CGT Arg	GTG Val	GCA Ala	GCG Ala	TTG Leu 670	GTG Val	GCC Ala	AGT Ser	GGT Gly 675	GTG Val 675	GCC Ala	CTC Leu	2070	
TAC Tyr	GCC Ala 680	AGC Ser 680	TCC Ser	TAT Tyr	GTG Val	CCC Pro	TAC Tyr 685	CAC His	ATC Ile	ATG Met	CGG Arg 690	GTG Val 690	CTC Leu	AAC Asn	GTG Val	2118	
GAT Asp 695	GCT Ala 695	CGG Arg	CGG Arg	CGC Arg	TGG Trp	AGC Ser 700	ACC Thr	CGC Arg	TGC Cys	CCG Pro	AGC Ser 705	TTT Phe	GCA Ala	GAC Asp	ATA Ile	2166	
GCC Ala 710	CAG Gln	GCC Ala	ACA Thr	GCA Ala	GCC Ala 715	CTG Leu 715	GAG Glu	CTG Leu	GGG Gly	CCC Pro 720	TAC Tyr	GTG Val	GGC Gly	TAC Tyr	CAG Gln 725	2214	
GTG Val	ATG Met	CGG Arg	GGC Gly 730	CTC Leu 730	ATG Met	CCC Pro	CTG Leu	GCC Ala	TTC Phe 735	TGT Cys	GTC Val	CAC His	CCT Pro 740	CTA Leu 740	CTC Leu	2262	
TAC Tyr	ATG Met	GCC Ala 745	GCA Ala 745	GTG Val	CCC Pro	AGC Ser	CTG Leu 750	GGC Gly 750	TGC Cys	TGC Cys	TGC Cys	CGA Arg 755	CAC His 755	TGC Cys	CCC Pro	2310	

GGC TAC AGG GAC AGC TGG AAC CCA GAG GAC GCC AAG AGC ACT GGC CAA	2358
Gly Tyr Arg Asp Ser Trp Asn Pro Glu Asp Ala Lys Ser Thr Gly Gln	
760 765 770	
GCC CTG CCC CTC AAT GCC ACA GCC GCC CCT AAA CCG TCA GAG CCC CAG	2406
Ala Leu Pro Leu Asn Ala Thr Ala Ala Pro Lys Pro Ser Glu Pro Gln	
775 780 785	
TCC CGT GAG CTG AGC CAA TGA	2427
Ser Arg Glu Leu Ser Gln	
790 795	

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 795 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Gln Ser Gly Arg Ser Arg His Gln Lys Arg Ala Arg Ala Gln	
1 5 10 15	
Ala Gln Leu Arg Asn Leu Glu Ala Tyr Ala Ala Asn Pro His Ser Phe	
20 25 30	
Val Phe Thr Arg Gly Cys Thr Gly Arg Asn Ile Arg Gln Leu Ser Leu	
35 40 45	
Asp Val Arg Arg Val Met Glu Pro Leu Thr Ala Ser Arg Leu Gln Val	
50 55 60	
Arg Lys Lys Asn Ser Leu Lys Asp Cys Val Ala Val Ala Gly Pro Leu	
65 70 75 80	
Gly Val Thr His Phe Leu Ile Leu Ser Lys Thr Glu Thr Asn Val Tyr	
85 90 95	
Phe Lys Leu Met Arg Leu Pro Gly Gly Pro Thr Leu Thr Phe Gln Val	
100 105 110	
Lys Lys Tyr Ser Leu Val Arg Asp Val Val Ser Ser Leu Arg Arg His	
115 120 125	
Arg Met His Glu Gln Gln Phe Ala His Pro Pro Leu Leu Val Leu Asn	
130 135 140	
Ser Phe Gly Pro His Gly Met His Val Lys Leu Met Ala Thr Met Phe	
145 150 155 160	
Gln Asn Leu Phe Pro Ser Ile Asn Val His Lys Val Asn Leu Asn Thr	
165 170 175	
Ile Lys Arg Cys Leu Leu Ile Asp Tyr Asn Pro Asp Ser Gln Glu Leu	
180 185 190	
Asp Phe Arg His Tyr Ser Ile Lys Val Val Pro Val Gly Ala Ser Arg	
195 200 205	

Gly	Met	Lys	Lys	Leu	Leu	Gln	Glu	Lys	Phe	Pro	Asn	Met	Ser	Arg	Leu
210						215					220				
Gln	Asp	Ile	Ser	Glu	Leu	Leu	Ala	Thr	Gly	Ala	Gly	Leu	Ser	Glu	Ser
225					230					235					240
Glu	Ala	Glu	Pro	Asp	Gly	Asp	His	Asn	Ile	Thr	Glu	Leu	Pro	Gln	Ala
				245					250					255	
Val	Ala	Gly	Arg	Gly	Asn	Met	Arg	Ala	Gln	Gln	Ser	Ala	Val	Arg	Leu
			260					265					270		
Thr	Glu	Ile	Gly	Pro	Arg	Met	Thr	Leu	Gln	Leu	Ile	Lys	Val	Gln	Glu
		275					280					285			
Gly	Val	Gly	Glu	Gly	Lys	Val	Met	Phe	His	Ser	Phe	Val	Ser	Lys	Thr
	290					295					300				
Glu	Glu	Glu	Leu	Gln	Ala	Ile	Leu	Glu	Ala	Lys	Glu	Lys	Lys	Leu	Arg
305					310					315					320
Leu	Lys	Ala	Gln	Arg	Gln	Ala	Gln	Gln	Ala	Gln	Asn	Val	Gln	Arg	Lys
				325					330					335	
Gln	Glu	Gln	Arg	Glu	Ala	His	Arg	Lys	Lys	Ser	Leu	Glu	Gly	Met	Lys
			340					345					350		
Lys	Ala	Arg	Val	Gly	Gly	Ser	Asp	Glu	Glu	Ala	Ser	Gly	Ile	Pro	Ser
		355					360					365			
Arg	Thr	Ala	Ser	Leu	Glu	Leu	Gly	Glu	Asp	Asp	Asp	Glu	Gln	Glu	Asp
	370					375					380				
Asp	Asp	Ile	Glu	Tyr	Phe	Cys	Gln	Ala	Val	Gly	Glu	Ala	Pro	Ser	Glu
385					390					395					400
Asp	Leu	Phe	Pro	Glu	Ala	Lys	Gln	Lys	Arg	Leu	Ala	Lys	Ser	Pro	Gly
				405					410					415	
Arg	Lys	Arg	Lys	Arg	Trp	Glu	Met	Asp	Arg	Gly	Ala	Lys	Ser	Cys	Pro
			420					425					430		
Ala	Asn	Phe	Leu	Ala	Ala	Ala	Asp	Asp	Lys	Leu	Ser	Gly	Phe	Gln	Gly
		435					440					445			
Asp	Phe	Leu	Trp	Pro	Ile	Leu	Val	Val	Glu	Phe	Leu	Val	Ala	Val	Ala
	450					455					460				
Ser	Asn	Gly	Leu	Ala	Leu	Tyr	Arg	Phe	Ser	Ile	Arg	Lys	Gln	Arg	Pro
465					470					475					480
Trp	His	Pro	Ala	Val	Val	Phe	Ser	Val	Gln	Leu	Ala	Val	Ser	Asp	Leu
				485					490					495	
Leu	Cys	Ala	Leu	Thr	Leu	Pro	Pro	Leu	Ala	Ala	Tyr	Leu	Tyr	Pro	Pro
			500					505					510		
Lys	His	Trp	Arg	Tyr	Gly	Glu	Ala	Ala	Cys	Arg	Leu	Glu	Arg	Phe	Leu
		515					520					525			
Phe	Thr	Cys	Asn	Leu	Leu	Gly	Ser	Val	Ile	Phe	Ile	Thr	Cys	Ile	Ser
	530					535					540				

Leu	Asn	Arg	Tyr	Leu	Gly	Ile	Val	His	Pro	Phe	Phe	Ala	Arg	Ser	His
545					550					555					560
Leu	Arg	Pro	Lys	His	Ala	Trp	Ala	Val	Ser	Ala	Ala	Gly	Trp	Val	Leu
				565					570					575	
Ala	Ala	Leu	Leu	Ala	Met	Pro	Thr	Leu	Ser	Phe	Ser	His	Leu	Lys	Arg
				580				585						590	
Pro	Pro	Gln	Gln	Gly	Ala	Gly	Asn	Cys	Ser	Val	Ala	Arg	Pro	Glu	Ala
		595					600					605			
Cys	Ile	Lys	Cys	Leu	Gly	Thr	Ala	Asp	His	Gly	Leu	Ala	Ala	Tyr	Arg
	610					615					620				
Ala	Tyr	Ser	Leu	Val	Leu	Ala	Gly	Leu	Gly	Cys	Gly	Leu	Pro	Leu	Leu
625					630					635					640
Leu	Thr	Leu	Ala	Ala	Tyr	Gly	Ala	Leu	Gly	Arg	Ala	Val	Leu	Arg	Ser
				645					650					655	
Pro	Gly	Met	Thr	Val	Ala	Glu	Lys	Leu	Arg	Val	Ala	Ala	Leu	Val	Ala
			660					665						670	
Ser	Gly	Val	Ala	Leu	Tyr	Ala	Ser	Ser	Tyr	Val	Pro	Tyr	His	Ile	Met
		675					680					685			
Arg	Val	Leu	Asn	Val	Asp	Ala	Arg	Arg	Arg	Trp	Ser	Thr	Arg	Cys	Pro
	690					695					700				
Ser	Phe	Ala	Asp	Ile	Ala	Gln	Ala	Thr	Ala	Ala	Leu	Glu	Leu	Gly	Pro
705					710					715					720
Tyr	Val	Gly	Tyr	Gln	Val	Met	Arg	Gly	Leu	Met	Pro	Leu	Ala	Phe	Cys
				725					730					735	
Val	His	Pro	Leu	Leu	Tyr	Met	Ala	Ala	Val	Pro	Ser	Leu	Gly	Cys	Cys
			740					745					750		
Cys	Arg	His	Cys	Pro	Gly	Tyr	Arg	Asp	Ser	Trp	Asn	Pro	Glu	Asp	Ala
		755					760					765			
Lys	Ser	Thr	Gly	Gln	Ala	Leu	Pro	Leu	Asn	Ala	Thr	Ala	Ala	Pro	Lys
	770					775					780				
Pro	Ser	Glu	Pro	Gln	Ser	Arg	Glu	Leu	Ser	Gln					
785					790					795					

CLAIMS

1. Receptor comprising more than 70% identity with the amino acid sequence starting from amino acid 424 until amino acid 795 of SEQ ID NO: 2 and having an affinity for ATP.
2. Receptor according to claim 1 comprising more than 85% identity with the amino acid sequence starting from amino acid 424 until amino acid 795 of SEQ ID NO: 2 and having an affinity for ATP.
3. Receptor according to claim 1 comprising more than 95% identity with the amino acid sequence starting from amino acid 424 until amino acid 795 of SEQ ID NO: 2 and having an affinity for ATP.
4. Receptor according to claim 1 comprising at least the amino acid sequence starting from amino acid 424 until amino acid 795 of SEQ ID NO: 2 or a portion thereof, wherein said portion has a selective affinity for ATP.
5. Receptor according to any one of the claims 1 to 4, belonging to the P2Y receptor family.
6. Receptor according to any one of the claims 1 to 5, being a G protein-coupled receptor.
7. Receptor according to any one of the claims 1 to 6, being a human receptor.
8. A nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising more than 70% identity with the amino acid sequence starting from amino acid 424 until 795 of SEQ ID NO: 2 which has an affinity for ATP.
9. Nucleic acid molecule according to claim 8, wherein the nucleic acid molecule is DNA or mRNA molecule.

10. Nucleic acid molecule according to claim 9, wherein the DNA is a cDNA molecule or a genomic DNA molecule.
11. Nucleic acid molecule according to claim 10 having more than 70% identity to the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1, and said nucleic acid molecule encoding a receptor having a selective affinity for ATP.
12. Nucleic acid molecule according to claim 11 having more than 85% identity to the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1, and said nucleic acid molecule encoding a receptor having a selective affinity for ATP.
13. Nucleic acid molecule according to claim 11 having more than 95% identity to the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1, and said nucleic acid molecule encoding a receptor having a selective affinity for ATP.
14. The nucleic acid molecule according to claim 13, which has at least the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1 or a portion thereof, wherein said portion encodes an amino acid sequence having a selective affinity for ATP.
15. Vector comprising the nucleic acid molecule according to any one of the claims 8 to 14.
16. Vector according to claim 15, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any one of the claims 8 to 14 as to permit expression thereof.
17. Vector of claim 16, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells.

18. Vector according to any one of the claims 15 to 17, wherein the vector is a plasmid or a virus.
19. The vector according to claim 18, wherein said virus is chosen from the group consisting of baculovirus, adenovirus and Semliki Forest virus.
20. Cell comprising the vector according to any one of the claims 15 to 19.
21. Cell of claim 20, wherein the cell is a mammalian cell.
22. The cell according to claim 21, wherein said cell is non neuronal in origin.
23. Cell of claim 22, wherein the cell is selected from the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells and 1321 N1 astrocytoma cells.
24. A nucleic acid probe for detecting the target sequence of SEQ ID NO: 1, said probe comprising a nucleotide sequence of at least 15 nucleotides that is fully complementary to the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1.
25. Nucleic acid probe of claim 24 wherein the nucleic acid is DNA or RNA.
26. Antisense oligonucleotide for preventing translation of the mRNA defined by SEQ ID NO: 1, comprising a nucleotide sequence that is fully complementary to an mRNA molecule as defined by the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1.
27. Antisense oligonucleotide for preventing translation of the mRNA defined by SEQ ID NO: 1, having a sequence that specifically hybridises to the DNA sequence starting at nucleotide position 1309 to nucleotide position 2424 of SEQ ID NO: 1, whereby the hybridisation conditions are as follows: buffer used: 6 X SSC (1 X SSC: 0.15 M NaCl, 0.015 M sodium citrate) and 40% formamide, hybridization performed at 42°C for 14 hours and the final washing conditions were 0.5 X SSC, 0.1% SDS at 60°C.

28. Antisense oligonucleotide according to claim 26 or 27, comprising chemical analogs of nucleotides.
29. Antibody that binds specifically to a receptor according to any one of the claims 1 to 7.
30. The antibody according to claim 29, which is a monoclonal antibody.
31. The antibody according to claim 29 or 30 that binds specifically to a receptor according to any one of the claims 1 to 7, present on the surface of a cell expressing said receptor.
32. Isolated non-human mammalian cell transfected with and expressing the nucleic acid molecule as defined in any one of the claims 8 to 14.
33. An in vitro method for determining the presence or absence of binding of a ligand to a receptor as defined in any one of claims 1 to 7, said method comprising the steps of:
 - a. contacting the ligand with a cell or cell extract from cells expressing said receptor;
 - b. performing a bioassay to measure bound ligand; and
 - c. wherein a positive signal is indicative of binding of the ligand to the receptor.
34. The method according to claim 33, wherein said ligand is an agonist or an antagonist of said receptor.
35. The method according to claim 33 or 34, wherein a membrane fraction from the cell extract is isolated.
36. The method according to any one of claims 33 to 35, wherein said presence is detected by means of a bioassay.
37. The method according to any one of claims 33 to 36, wherein said bioassay detects the modification of the production of a second messenger.

38. The method according to claim 37, wherein the second messenger assay comprises measurement of intracellular cAMP, intracellular Inositol phosphate, intracellular diacylglycerol concentration and intracellular calcium mobilisation.

39. Method according to any one of claims 33 to 38, wherein the cell is a mammalian cell.

40. The method according to claim 39, wherein said cell is non neuronal in origin.

41. The method according to claim 40, wherein said cell is selected from the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells and 1321 N1 cells.

42. An in vitro method of screening drugs to identify drugs which specifically bind to the receptor as defined in any one of claims 1 to 7 on the surface of a cell, said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;
- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. contacting the control cells and the transfected cells with a plurality of drugs;
- iv. performing a detection assay to determine binding to the transfected cells and the control cells; and
- v. determining which drugs bind specifically to the receptor on the transfected cells.

43. An in vitro method of screening drugs to identify drugs which specifically bind to the receptor as defined in any one of the claims 1 to 7 on the surface of the cell, said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;

- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. preparing a cell extract from the control and transfected cells;
- iv. isolating a membrane fraction from the cells extract;
- v. contacting the membrane fraction with a plurality of drugs; and
- vi. determining those drugs which bind to the membrane fraction, thereby identifying drugs which specifically bind to said receptor.

44. An in vitro method of screening drugs to identify drugs which act as agonists of the receptor as defined in any one of the claims 1 to 7, said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;
- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. contacting the transfected cells with a plurality of drugs;
- iv. performing a bioassay to determine if a drug induces a functional receptor response; and
- v. identifying drugs which act as receptor agonists.

45. An in vitro method of screening drugs to identify drugs which act as agonists of the receptor as defined in any one of the claims 1 to 7 said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;
- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. preparing a cell extract transfected cells;
- iv. isolating a membrane fraction from the cell extract;
- v. contacting the membrane fraction with a plurality of drugs;
- vi. performing a bioassay to determine those drugs which induce a detectable receptor response; and
- vii. identifying drugs which act as receptor agonists.

46. An in vitro method of screening drugs to identify drugs which act as antagonists of the receptor as defined in any one of the claims 1 to 7, said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;
- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. contacting a transfected cell with a plurality of drugs in the presence of ATP or 2MeSATP;
- iv. performing a bioassay to determine those drugs which inhibit the activation of the receptor; and
- v. identifying drugs which act as receptor antagonists.

47. An in vitro method of screening drugs to identify drugs which act as antagonists of the receptor as defined in any one of the claims 1 to 7, said method comprising:

- i. obtaining a population of control cells that do not normally express a receptor as defined in any one of claims 1 to 7;
- ii. transfecting a subpopulation of control cells with a vector that expresses the nucleic acid molecule encoding the receptor to obtain a population of transfected cells;
- iii. preparing a cell extract from transfected cells;
- iv. isolating a membrane fraction from the cell extract;
- v. contacting the membrane fraction with a plurality of drugs in presence of ATP or 2MeSATP;
- vi. performing a bioassay to determine those drugs which inhibit the activation of the receptor; ; and
- vii. identifying drugs which act as receptor antagonists.

48. Method of screening drugs according to any one of claims 44 to 47, wherein said bio-assay determines a modification in a second messenger concentration.

49. Method of detecting the expression of the receptor as defined in any one of claims 1 to 7, by detecting the presence of mRNA coding said receptor, which

comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to claim 24 and detecting the presence of an mRNA/probe duplex thereby detecting the expression of the receptor by the cell.

50. Method of detecting the presence of the receptor according to any one of the claims 1 to 7 on the surface of a cell, which comprises contacting the cell with the antibody of claim 29 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

51. An in vitro method of preparing the purified receptor according to any one of the claims 1 to 7, which comprises:

- a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;
- b) inserting the vector of step a in a suitable host cell;
- c) incubating the cell of step b under conditions allowing the expression of the receptor as defined in any of claim 1 to 7;
- d) recovering the receptor of step c); and
- e) purifying the receptor of step d), thereby preparing a purified receptor according to any one of the claims 1 to 7.

52. A pharmaceutical composition comprising an amount of the antisense oligonucleotide according to any of the claims 26 to 28, effective to decrease the activity of a receptor having a selective affinity for ATP according to any one of claims 1-7, by passing through a cell membrane and binding specifically with mRNA encoding said receptor in a cell so as to prevent his translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

53. The pharmaceutical composition according to claim 52 wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

54. The pharmaceutical composition according to claim 53 wherein the substance which inactivates mRNA is a ribozyme.

55. The pharmaceutical composition according to any of the preceding claims 52 to 53 wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

56. The pharmaceutical composition according to the claim 55 wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

P2Y₁₁ sequence

FIG.1a

1		M G Q S G R S	20
1	GAATTCGGCACGAGGAGGCCTCGTGGAGGACACAGCAGCATGGGACAGTCAGGGAGGTCC		60
21	R H Q K R A R A Q A Q L R N L E A Y A A		40
61	CGGCACCAGAAGCGCGCCCGCGCCCGAGGCGCAGCTCCGCAACCTCGAGGCCTATGCCGCG		120
41	N P H S F V F T R G C T G R N I R Q L S		60
121	AACCCGCACTCGTTCGTGTTACGCGAGGCTGCACGGGTGCGCAACATCCGGCAGCTCAGC		180
61	L D V R R V M E P L T A S R L Q V R K K		80
181	CTGGACGTGCGGCGGGTCATGGAGCCGCTCACTGCCAGCCGTCTGCAGGTTTCGTAAGAAG		240
81	N S L K D C V A V A G P L G V T H F L I		100
241	AACTCGCTGAAGGACTGCGTGGCAGTGGCTGGGCCCCCTCGGGGTACACACTTTCTGATC		300
101	L S K T E T N V Y F K L M R L P G G P T		120
301	CTGAGCAAAACAGAGACCAATGTCTACTTTAAGCTGATGCGCCTCCCAGGAGGCCCCACC		360
121	L T F Q V K K Y S L V R D V V S S L R R		140
361	TTGACCTTCCAGGTGAAGAAGTACTCGCTGGTGCCTGATGTGGTCTCCTCACTGCGCCGG		420
141	H R M H E Q Q F A H P P L L V L N S F G		160
421	CACCGCATGCACGAGCAGCAGTTTGCCCAACCCACCCCTCCTGGTACTCAACAGCTTTGGC		480
161	P H G M H V K L M A T M F Q N L F P S I		180
481	CCCCATGGTATGCATGTGAAGCTCATGGCCACCATGTTCCAGAACCTGTTCCCCTCCATC		540
181	N V H K V N L N T I K R C L L I D Y N P		200
541	AACGTGCACAAGGTGAACCTGAACACCATCAAGCGCTGCCTCCTCATCGACTACAACCCC		600
201	D S Q E L D F R H Y S I K V V P V G A S		220
601	GACTCCCAGGAGCTGGACTTCCGCCACTATAGCATCAAAGTTGTTCTGTGGGCGCGAGT		660
221	R G M K K L L Q E K F P N M S R L Q D I		240
661	CGCGGGATGAAGAAGCTGCTCCAGGAGAAGTTCCCCAACATGAGCCGCCTGCAGGACATC		720
241	S E L L A T G A G L S E S E A E P D G D		260
721	AGCGAGCTGCTGGCCACGGGCGCGGGGCTGTCTGGAGAGCGAGGCAGAGCCTGACGGCGAC		780
261	H N I T E L P Q A V A G R G N M R A Q Q		280
781	CACAACATCACAGAGCTGCCTCAGGCTGTCTGCTGGCCGTGGCAACATGCGGGCCCCAGCAG		840
281	S A V R L T E I G P R M T L Q L I K V Q		300
841	AGTGCAGTGCGGCTCAGCGAGATCGGCCCCGCGGATGACACTGCAGCTCATCAAGGTCCAG		900
301	E G V G E G K V M F H S F V S K T E E E		320
901	GAGGGCGTCCGGGAGGGGCAAGTGATGTTCCACAGTTTTTGTGAGCAAGACGGAGGAGGAG		960
321	L Q A I L E A K E K K L R L K A Q R Q A		340
961	CTGCAGGCCATCCTGGAAGCCCAAGGAGAAGAAGCTGCGGCTGAAGGCGCAGAGGCAGGCC		1020
341	Q Q A Q N V Q R K Q E Q R E A H R K K S		360
1021	CAGCAGGCCCCAGATGTGTGAGCGCAGCAGGAGCAGCGGGAGGCCCCACAGAAAGAAGAGC		1080

WO 99/02675

2/6

PCT/BE98/00108

FIG. 1b

361	L E G M K K A R V G G S D E E A S G I P	380
1081	CTGGAGGGCATGAAGAAGGCACGGGTGCGGGGTAGTGATGAAGAGGCCTCTGGGATCCCT	1140
381	S R T A S L E L G E D D D E Q E D D D I	400
1141	TCAAGGACGGCGAGCCTGGAGTTGGGTGAGGACGATGATGAACAGGAAGATGATGACATC	1200
401	E Y F C Q A V G E A P S E D L F P E A K	420
1201	GAGTATTTCTGCCAGGCGGTGGGCGAGGCGCCAGTGAGGACCTGTTCCCCGAGGCCAAG	1260
421	Q K R L A K S P G R K R K R W E M D R G	440
1261	CAGAAACGGCTTGCCAAGTCTCCAGGGCGGAAGCGGAAGCGGTGGGAAATGGATCGAGGT	1320
441	A K S C P A N F L A A A D D K L S G F Q	460
1321	GCCAAGTCCTGCCCTGCCAACTTCTTGGCAGCTGCCGACGACAACTCAGTGGGTTCAG	1380
461	G D F L W P I L V V E F L V A V A S N G	480
1381	GGGGACTTCCTGTGGCCATACTGGTGGTTGAGTTCCTGGTGGCCGTGGCCAGCAATGGC	1440
481	L A L Y R F S I R K Q R P W H P A V V F	500
1441	CTGGCCCTGTACCGCTTCAGCATCCGGAAGCAGCGCCCATGGCACCCCGCCGTGGTCTTC	1500
501	S V Q L A V S D L L C A L T L P P L A A	520
1501	TCTGTCCAGCTGGCAGTCAGCGACCTGCTCTGCGCTCTGACGCTGCCCCCGCTGGCCGCC	1560
521	Y L Y P P K H W R Y G E A A C R L E R F	540
1561	TACCTCTATCCCCCAAGCACTGGCGCTATGGGGAGGCGCGTGGCGCCTGGAGCGCTTC	1620
541	L F T C N L L G S V I F I T C I S L N R	560
1621	CTCTTCACCTGCAACCTGCTGGGCAGCGTCATCTTCATCACCTGCATCAGCCTCAACCGC	1680
561	Y L G I V H P F F A R S H L R P K H A W	580
1681	TACCTGGGCATCGTGCACCCCTTCTTCGCCCCGAAGCCACCTGCGACCCCAAGCACGCCTGG	1740
581	A V S A A G W V L A A L L A M P T L S F	600
1741	GCCGTGAGCGCTGCCGGCTGGGTCTGGCCGCCCTGCTGGCCATGCCACACTCAGCTTC	1800
601	S H L K R P P Q Q G A G N C S V A R P E	620
1801	TCCCACCTGAAGAGGCCGCGCAGCAGGGGGCGGGCAACTGCAGCGTGGCCAGGCCCGAG	1860
621	A C I K C L G T A D H G L A A Y R A Y S	640
1861	GCCTGCATCAAGTGTCTGGGGACAGCAGACCACGGGCTGGCGGCCTACAGAGCGTATAGC	1920
641	L V L A G L G C G L P L L L T L A A Y G	660
1921	CTGGTGCTGGCGGGGTTGGGCTGCGGCCTGCCGCTGCTGCTCACGCTGGCAGCCTACGGC	1980
661	A L G R A V L R S P G M T V A E K L R V	680
1981	GCCCTCGGGCGGGCCGTGCTACGCAGCCAGGCATGACTGTGGCCGAGAAGCTGCGTGTG	2040
681	A A L V A S G V A L Y A S S Y V P Y H I	700
2041	GCAGCGTTGGTGGCCAGTGGTGTGGCCCTCTACGCCAGCTCCTATGTGCCCTACCATC	2100
701	M R V L N V D A R R R W S T R C P S F A	720
2101	ATGCGGGTGCTCAACGTGGATGCTCGGCGGGCGCTGGAGCACCCCGCTGCCCGAGCTTTGCA	2160
721	D I A Q A T A A L E L G P Y V G Y Q V M	740
2161	GACATAGCCCGGGCCACAGCAGCCCTGGAGCTGGGGCCCTACGTGGGCTACGAGGTGATG	2220

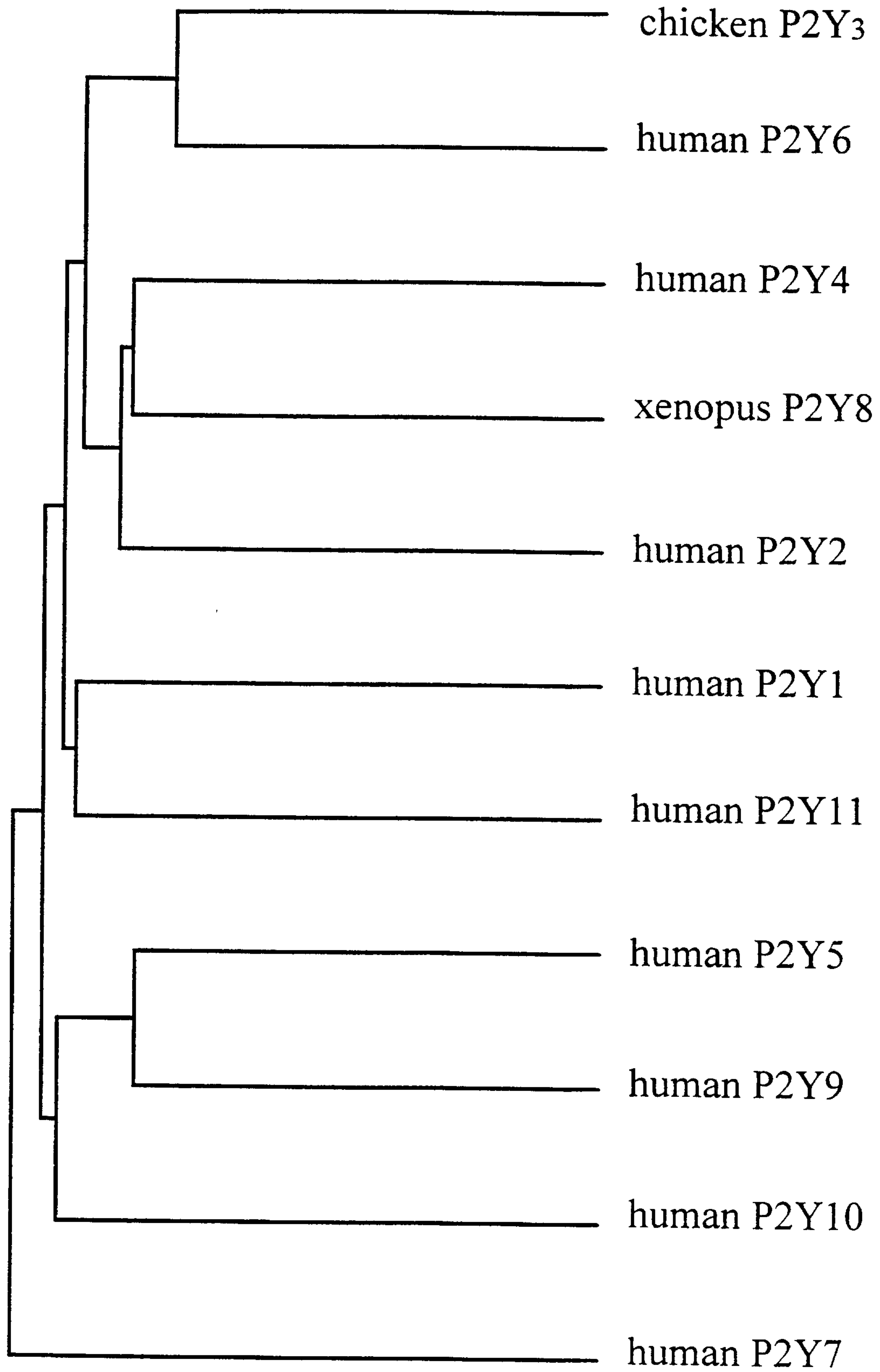
WO 99/02675

PCT/BE98/00108

3/6

741	R G L M P L A F C V H P L L Y M A A V P	760
2221	CGGGGCCTCATGCCCCTGGCCTTCTGTGTCCACCCTCTACTCTACATGGCCGCAGTGCCC	2280
761	S L G C C C R H C P G Y R D S W N P E D	780
2281	AGCCTGGGCTGCTGCTGCCGACACTGCCCCGGCTACAGGGACAGCTGGAACCCAGAGGAC	2340
781	A K S T G Q A L P L N A T A A P K P S E	800
2341	GCCAAGAGCACTGGCCAAGCCCTGCCCCTCAATGCCACAGCCGCCCTAAACCGTCAGAG	2400
801	P Q S R E L S Q *	
2401	CCCCAGTCCCGTGAGCTGAGCCAATGA	

FIG.1c

FIG. 2

5/6

FIG. 3

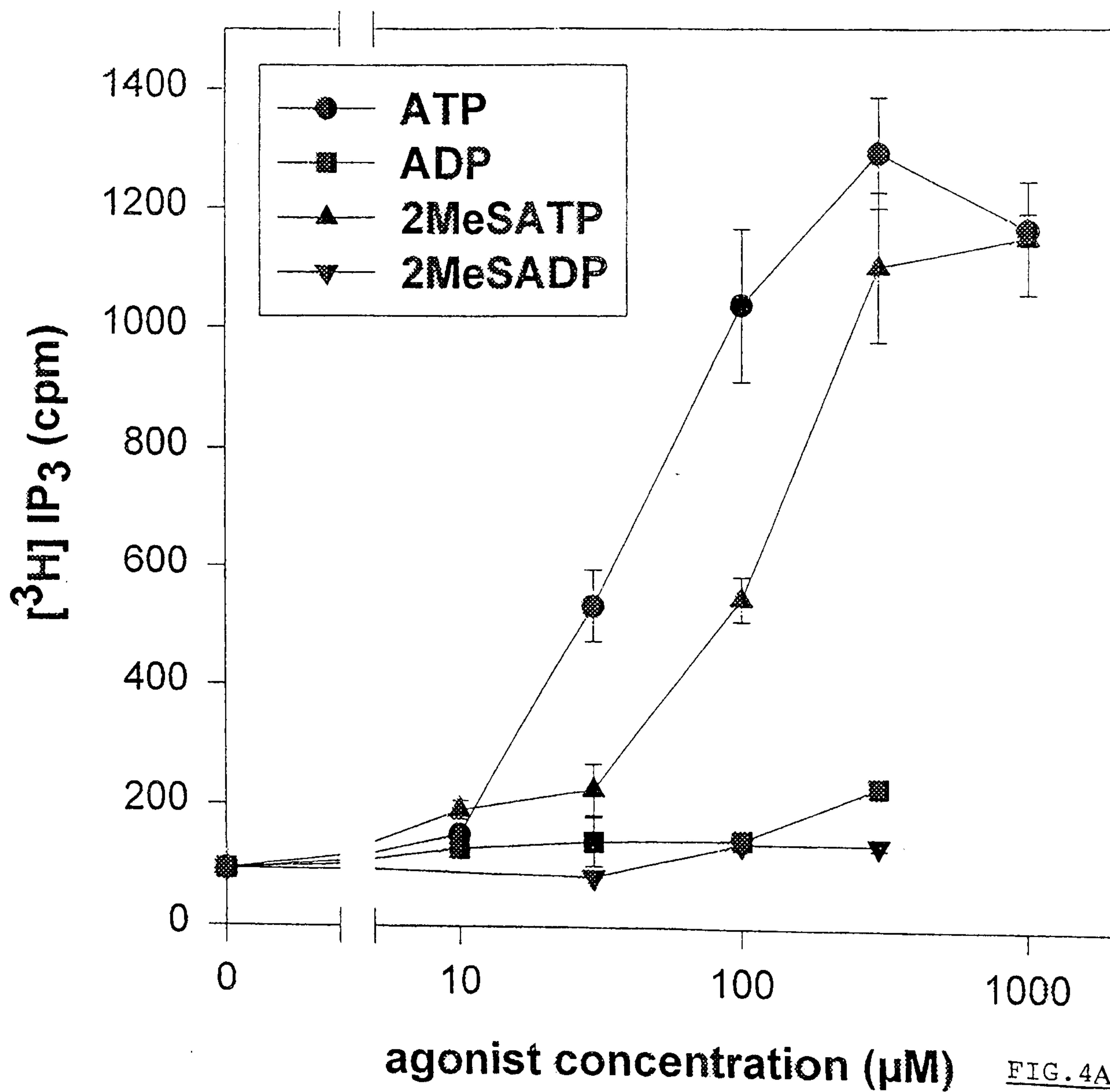
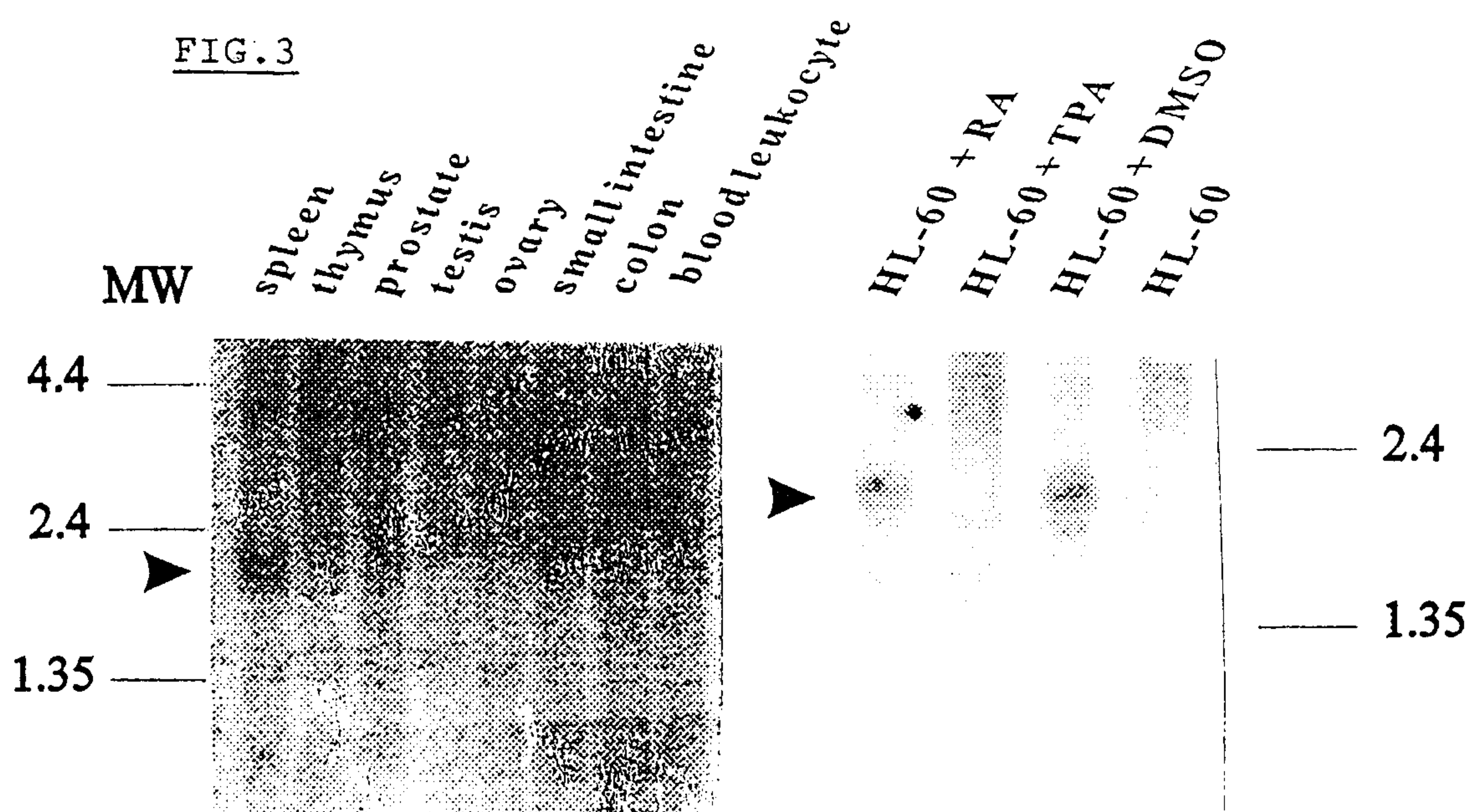


FIG. 4A

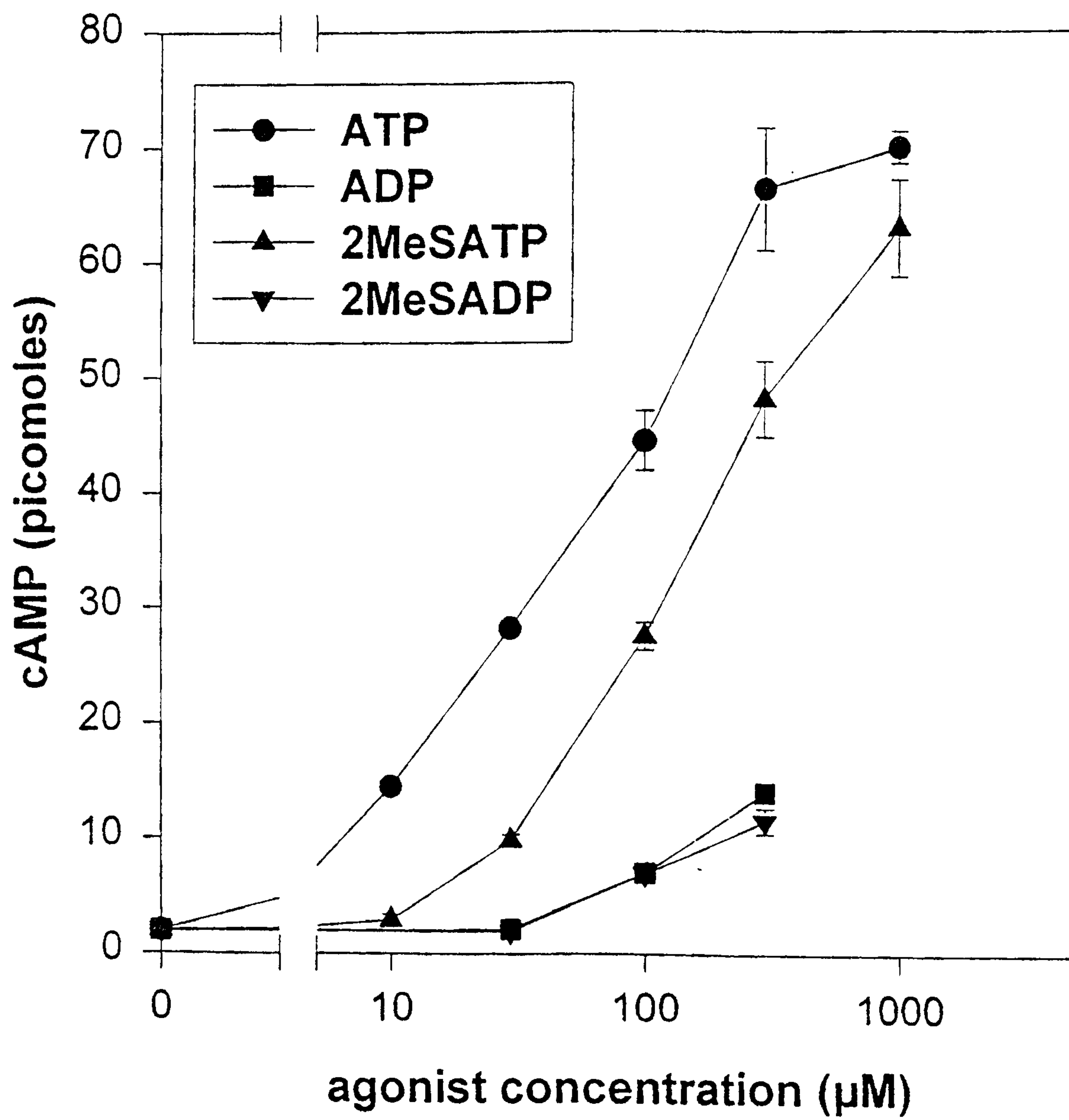


FIG.4B