TOCRIS a bio-techne brand

P2X and P2Y Receptors

Kenneth A. Jacobson

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA. E-mail: kajacobs@helix.nih.gov

Kenneth Jacobson serves as Chief of the Laboratory of Bioorganic Chemistry and the Molecular Recognition Section at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health in Bethesda, Maryland, USA. Dr. Jacobson is a medicinal chemist with interests in the structure and pharmacology of G-protein-coupled receptors, in particular receptors for adenosine and for purine and pyrimidine nucleotides.

Contents

Subtypes and Structures of P2 Receptor Families

The P2 receptors for extracellular nucleotides are widely distributed in the body and participate in regulation of nearly every physiological process.[1,2] Of particular interest are nucleotide receptors in the immune, inflammatory, cardiovascular, muscular, and central and peripheral nervous systems. The ubiquitous signaling properties of extracellular nucleotides acting at two distinct families of P2 receptors – fast P2X ion channels and P2Y receptors (G-protein-coupled receptors) – are now well recognized. These extracellular nucleotides are produced in response to tissue stress and cell damage and in the processes of neurotransmitter release and channel formation. Their concentrations can vary dramatically depending on circumstances. Thus, the state of activation of these receptors can be highly dependent on the stress conditions or disease states affecting a given organ. The P2 receptors respond to various extracellular mono- and dinucleotides (Table 1). The P2X receptors are more structurally restrictive than P2Y receptors in agonist selectivity. P2X receptors are activated principally by ATPs, while the P2Y receptors are activated by a group of five or more naturally occurring nucleotides. The P2X receptors are distributed throughout the nervous system (autonomic, central, enteric and sensory neurons, cochlear and retinal cells), vascular system (cardiomyocytes, endothelium and smooth muscle), the pulmonary and digestive systems (epithelium and visceral smooth muscle), skeletal muscle, bone, and hematopoietic cells. The P2X receptors consist of trimeric ligand-gated ion channels. The subunits are numbered $P2X_1$ through $P2X_7$, and both heterotrimers and homotrimers occur. Activation of P2X receptors leads to influx of cations such as sodium and calcium, which depolarize excitable cells and activate cytosolic enzymes respectively. The $P2X_7$ receptor upon prolonged agonist exposure also opens a large pore, which can pass organic cations and dye molecules. The knowledge of P2X receptor structures was recently advanced with the X-ray crystallographic determination of the $P2X_4$ subunit.[3,4] However, this structure did not establish the precise ligand binding site within the protein. A major difficulty in designing new agonist and antagonist ligands for a given P2X receptor subtype is that the homotrimers and heterotrimers may have entirely different structural requirements.

The correspondence of the P2Y receptor subtypes with their native nucleotide ligands is shown in Table 1. The numbering of unique human P2Y receptors has some gaps – due to the fact that the assignment of numbers to certain putative P2Y receptors was later shown to be premature, with some of the previously designated sequences being P2Y species homologs and others being other types of receptors. Each of the native nucleotides may activate several P2Y receptor subtypes. The structures of representative adenine (Figure 1A, **1–10**) and uracil (Figure 1B,

11–28) nucleotides that activate P2 receptors are shown. The adenine nucleotide ATP is a full agonist at two human P2Y subtypes ($P2Y_2$ and $P2Y_{11}$ receptors), and the corresponding diphosphate ADP activates three different subtypes ($P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors). The uracil nucleotide UTP activates two subtypes ($P2Y_2$ and $P2Y_4$ receptors), while UDP, previously thought to activate only a single subtype ($P2Y_6$ receptors), is now known to also activate $P2Y_{14}$ receptors along with the originally designated native agonist UDP-glucose.[5] The naturally occurring dinucleotide Ap_4A and its homologs also activate various P2 receptors.

The structure, signaling, and regulation of P2Y receptors have been explored, and subfamilies of $P2Y_1$-like and $P2Y_{12}$-like receptors have been defined. These subfamilies constitute two pharmacologically distinct groups of P2Y receptors that also correlate with similarities in the function of key amino acid residues.[6] The preferential coupling of the first subfamily of $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors is to G_q, leading to activation of phospholipase Cβ (PLCβ), and the second subfamily of $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors couple to G_i resulting in the inhibition of adenylyl cyclase. $P2Y_{11}$ receptors also activate G_s to stimulate adenylyl cyclase. Comparisons of the structural characteristics and functionally important amino acid residues within the family have been described. Specific cationic residues and other residues in the TM region (e.g. Phe in TM3) and on the extracellular loops have conserved functions within the P2Y family. Molecular recognition in the $P2Y_1$, $P2Y_2$, $P2Y_{11}$, and $P2Y_{12}$ receptors has been extensively explored using mutagenesis.[7–10]

P2Y receptor regulation has also been studied. In platelets, which express two ADP-responsive P2Y subtypes, the $P2Y_1$ receptor is more rapidly desensitized than the $P2Y_{12}$ receptor.[11] The $P2Y_1$ receptor is desensitized mainly through PKC-dependent processes, and the $P2Y_{12}$ receptor is a good substrate for the GPCR kinases (GRKs) leading to arrestin binding. Residues on the cytosolic C-terminal domain involved in the regulation of the $P2Y_1$ receptor have been probed. The internalization of the $P2Y_{11}$ receptor is dependent on coexpression of the rapidly desensitizing $P2Y_1$ receptor, suggesting the occurrence of receptor dimers.[12] Various heterodimers of P2Y receptors with other P2Y and non-P2Y GPCRs have been proposed. For example, a dimer of A_1 adenosine receptors and $P2Y_1$ receptors was characterized.[13]

Recurrent issues in the use of typical P2 receptor ligands include cross-reactivity with multiple P2 receptors and low bioavailability, due to polyanions, such as phosphates and sulfonates, present in the molecules. Another drawback of many of the currently used ligands is lability in biological systems. A large family of ectonucleotidase enzymes hydrolyzes the native nucleotides

Table 1 | Subtypes of P2 receptors and their ligands (potency at the human homologs shown as pEC_{50}, unless noted r = rat)

Receptor	Main Distribution	Agonists (native in bold, pEC_{50})	Antagonists
$P2X_1$	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	BzATP *8.7* > **ATP** *7.3*, 2-MeSATP *7.3*, α,β-MeATP *6.7* (rapid desensitization) >> CTP *4.4*	NF 449 *9.5* > IP_5I *8.8* > TNP-ATP *8.2* > Ro 0437626 > NF 279 *7.7*
$P2X_2$	Smooth muscle, brain, pancreas, retina, chromaffin cells, autonomic and sensory ganglia	**ATP** *5.9*, 2-MeSATP *5.8* ≥ 2-MeSATP *5.8* >> α,β-MeATP *<4*	RB2 *6.4* (r), iso PPADS *6.4* (r) > PPADS *5.4* (r) > Suramin *4.5* (r)
$P2X_3$	Nociceptive sensory neurons, NTS, some sympathetic neurons	**ATP** *6.5*, 2-MeSATP *6.5* ≥ Ap_4A *6.3*, α,β-MeATP *6.1* (rapid desensitization)	TNP-ATP *9.0*, iso PPADS *7.1* > A317491 *7.6* > NF 110 *7.4* > PPADS *5.8*
$P2X_4$	Microglia, testis, colon, endothelial cells	**ATP** *6.3* >> α,β-MeATP *5.1* >> CTP *3.5*, Ivermectin (potentiates) *6.6*	5-BDBD >> TNP-ATP *4.8*, PPADS *4.6* > BBG *3.9* (r)
$P2X_5$	Proliferating cells in skin, gut, bladder, thymus, spinal cord, heart, adrenal medulla	ATPγS *6.2* (r), **ATP** *6.0* >> α,β-MeATP *<5.2*	BBG *6.3* > PPADS *5.6*, Suramin *5.4*
$P2X_6$	Brain, motor neurons in spinal cord	(no functional homomultimer)	–
$P2X_7$	Macrophages, mast cells, microglia, pancreas, skin, endocrine organs	BzATP *5.3* > **ATP** *4.0* ≥ 2-MeSATP *>4* >> α,β-MeATP *>4*	KN 62 *7.5*, BBG *8.0* (r)
$P2Y_1$	Brain, epithelial and endothelial cells, platelets, immune cells, osteoclasts	MRS 2365 *9.4* > 2-MeSADP *8.2* >> ADPβS *7.0* > **ADP** *5.1* > ATP	MRS 2500 *9.0* > MRS 2279 *7.3* > MRS 2179 *6.5*
$P2Y_2$	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	**UTP** *8.1*, MRS 2698 *8.1* ≥ **ATP** *7.1*, INS 365 *7.0* > INS 37217 *6.7*, UTPγS *6.6* > Ap_4A *6.1* > MRS 2768 *5.7*	AR-C 126313 *6* > Suramin *4.3* > RB2 *>4*
$P2Y_4$	Endothelial cells, placenta	2′-azido-dUTP *7.1* > UTPγS *5.8*, **UTP** *5.6* ≥ **ATP** *5.7* (rat), > Ap_4A *5.5* > CTP *5.2*, ITP *5.1*	ATP (human) *4.4* > RB2 *>4* > Suramin *>4*
$P2Y_6$	Airway and intestinal epithelial cells, spleen, placenta, T-cells, thymus	MRS 2693 *7.8* > UDPβS *7.3*, PBS 0474 *7.2* > INS 48823 *6.9*, Up_3U *6.6*, **UDP** *6.5* > UTP >> ATP	MRS 2578 *7.4* (noncompetitive) > RB2, PPADS
$P2Y_{11}$	Spleen, intestine, granulocytes	ATPγS *5.5* > ARC 67085MX *5.0* > BzATP *5.1* ≥ **ATP** *4.8*	NF 157 *7.4* > Suramin *4.8* > RB2 *>4*
$P2Y_{12}$	Platelets, brain (glial cells), microglial cells	2-MeSADP *7.9* ≥ **ADP** *7.2*	ARC 69931MX *8.4* > AZD 6140 *7.9*, INS 50589 *7.8* > RB2 *7.6* (r)> 2-MeSAMP *4.0*
$P2Y_{13}$	Spleen, brain, lymph nodes, bone marrow	**ADP** *7.9* = 2-MeSADP *7.9* > 2-MeSATP *7.1*, ATP *6.6*	ARC 69931MX *8.4* > ARC 67085 *6.7* > MRS 2211 *6.0*
$P2Y_{14}$	Placenta, mast cells, adipose tissue, stomach, intestine, discrete brain regions	MRS 2690 *7.3* > **UDP** *6.8*, **UDPglucose** *6.5* > UDP-galactose *6.2*	–

leading to complications in interpretation of biological results. Adenine nucleotides are progressively converted enzymatically, in the last step by the action of CD73/5′-nucleotidase on AMP, to form adenosine, which activates its own family of four receptors. Selective inhibitors of ectonucleotidases which can serve as modulators of receptor function are being explored.[14] Moreover, many P2 receptor agonists and antagonists are known to inhibit ectonucleotidases at comparable concentrations. Known P2 antagonists often interact intracellularly with other signaling mediators, such as G proteins.

One reason for the relatively slow progress in identifying competitive antagonists of the P2 receptors is that there are few selective radioligands available for either the P2X or P2Y receptors. Previously, various radioactive nucleotides have been suggested to bind to particular P2 receptors, but many of these reports were later questioned, and currently only the $P2Y_1$, $P2Y_{12}$, $P2X_1$ and $P2X_3$ receptors have viable radioligands.[15–17] Thus, improved and more versatile affinity probes for the P_2 receptors are still needed. New selective agonists and antagonists have recently been identified for some of the eight mammalian subtypes of P2Y receptors and for a few of the seven mammalian subtypes of P2X receptors. A careful probing of the structure activity relationships (SARs) at relevant P2 receptors has resulted in subtype-selective nucleotide agonists. Selective antagonist ligands for P2 receptors have been reported as a result of library screening, conversion of agonists into antagonists, and the careful structural modification of known non-selective ligands. The structures of representative nucleotide (Figure 2A, **29–42**) and non-nucleotide (Figure 2B, **43–73**) antagonists of the P2 receptors are shown.

Pharmacological Probes for P2X Receptors

The development of P2X receptor ligands for potential therapeutic application is underway. Selective P2X receptor antagonists are of interest in pain control, urinary incontinence, diabetic retinopathy, inflammatory diseases such as rheumatoid arthritis, and other conditions.

Non-Selective P2X Ligands

ATP activates all subtypes of P2X receptors, but at different concentrations varying from the low nanomolar to the high micromolar.[18] ADP and AMP, when purified, are inactive at P2X receptors. 2-Methylthioadenosine 5′-triphosphate (2-MeSATP) is a potent agonist at multiple P2X receptors, for example, $P2X_1$ (EC_{50} = 54 nM) and $P2X_3$ (EC_{50} = 350 nM) receptors. α,β-Methyleneadenosine 5′-triphosphate (Figure 1A **6**) activates and desensitizes the $P2X_1$ receptor and is inactive at the $P2X_2$ receptor. In tritiated form it serves as a radioligand of $P2X_1$ and $P2X_3$ receptors.

Older, non-selective and weak P2X antagonists (Figures 2A and 2B), such as organic dyes **43** and **45**, have been in use for decades. The antiparasitic drug polysulfonated Suramin and the pyridoxal phosphate derivatives PPADS and positional isomer iso-PPADS are relatively nonsubtype-selective P2X antagonists, that also block some P2Y subtypes.[19] The PPADS analog MRS 2159 is more potent than PPADS at the $P2X_1$ receptor and also antagonizes the $P2X_3$ receptor. The nucleotide derivative TNP-ATP is a potent P2X antagonist that is selective for several subtypes.[20] It antagonizes $P2X_1$, $P2X_3$ and heteromeric $P2X_{2/3}$ receptors with IC_{50} values of 6, 0.9 and 7 nM respectively, and displays 1000-fold selectivity for $P2X_3$ over $P2X_2$, $P2X_4$ and $P2X_7$ receptors. The polysulfonated biphenyl derivative Evans Blue acts as a P2X receptor antagonist, but it also affects other channels and amino acid binding sites.

$P2X_1$ and $P2X_2$ Receptors

$P2X_1$ antagonists have been reported in several compound classes. For example, the Suramin derivative NF 157 is a $P2X_1$ antagonist that also blocks the $P2Y_{11}$ receptor.[22] Other Suramin derivatives that act as selective $P2X_1$ antagonists include: PPNDS, NF 279, and the more highly selective $P2X_1$ antagonist NF 449.[23,24] The earlier-reported Suramin analog NF 023 is a moderately selective, competitive P2X antagonist with IC_{50} values of 0.21 and 28.9 μM at human $P2X_1$ and $P2X_3$ receptors respectively, and is inactive at $P2X_2$ and $P2X_4$ receptors.[25] In a separate chemical series, the benzimidazole-2-carboxamide derivative Ro 0437626 was recently reported to be a selective $P2X_1$ antagonist (IC_{50} = 3 μM) that displays > 30-fold selectivity over $P2X_2$, $P2X_3$ and $P2X_{2/3}$ receptors (IC_{50} > 100 μM).[26] The dinucleotide Ip_5I was shown to antagonize the $P2Y_1$ receptor.[42] The pyridoxal phosphate derivative MRS 2219 is a weak potentiator of $P2X_1$-mediated responses.[27]

There are no selective ligands for the $P2X_2$ receptor. The nonselective antagonists Suramin, TNP-ATP, RB-2, and isoPPADS have been used to study this receptor.

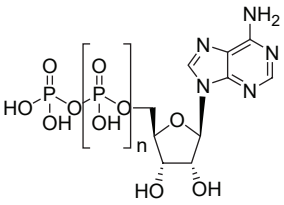
$P2X_3$ Receptor

The $P2X_3$ receptor may exist as a homotrimer or as a heterotrimer in combination with $P2X_2$ subunits. The Suramin derivative NF 110 is a high affinity $P2X_3$ receptor antagonist (K_i values are 36, 82 and 4140 nM for $P2X_3$, $P2X_1$ and $P2X_2$ receptors respectively) that is inactive at $P2Y_1$, $P2Y_2$ and $P2Y_{11}$ receptors (IC_{50} > 10 μM). A major advance was the introduction of the competitive antagonist by Abbott Laboratories, A 317491, which blocks $P2X_3$ (IC_{50} = 22 nM) and $P2X_{2/3}$ (IC_{50} = 92 nM) receptors and is roughly three orders of magnitude selective for $P2X_3$ in comparison to $P2X_1$ and $P2X_2$ receptors.[17] A 317491 is inactive at $P2X_4$ receptors and at all P2Y receptors. Due to the presence of three carboxylic acid groups, A 317491 is of limited bioavailability. Another potent $P2X_3$ antagonist is the pyrimidinediamine derivative RO-3, which is a selective antagonist of the homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors (pIC_{50} values are 7.0 and 5.9 nM respectively) and is inactive at $P2X_1$, $P2X_2$, $P2X_4$, $P2X_5$ and $P2X_7$ receptors (IC_{50} > 10 M).[29] The endogenous heptapeptide Spinorphin (LVVYPWT) was found to be a very potent $P2X_3$ antagonist (IC_{50} = 8.3 pM).[30]

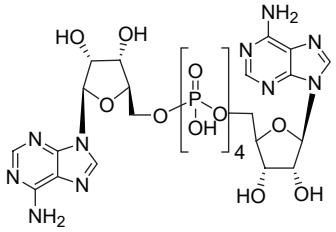
$P2X_4$ Receptor

Few of the known P2X antagonists act at the $P2X_4$ receptor. The benzofurodiazepinone derivative 5-BDBD is an antagonist of $P2X_4$-mediated currents (IC_{50} = 0.50 μM).[31] The bacteria-derived broad spectrum antiparasitic agent Ivermectin, which is a macrocyclic lactone, is a positive allosteric modulator for the $P2X_4$ receptor, but it also affects other ion channels, such as nicotinic acetylcholine receptors.[32]

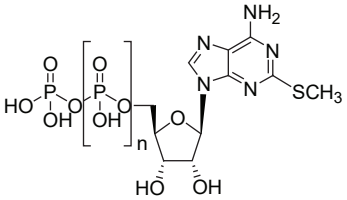
Figure 1A | Adenine derivatives that have been useful as antagonists in the study of P2 receptors



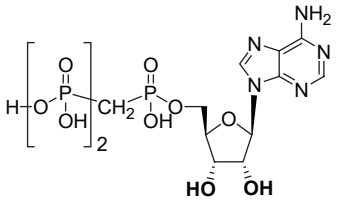
1 n=2, **ATP** (Cat. No. 3245)
2 n=1, ADP



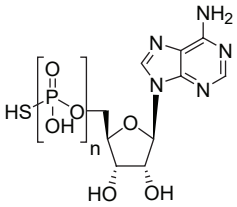
3 Ap_4A



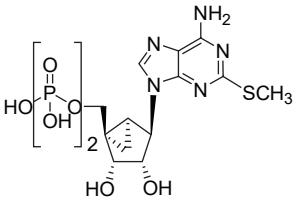
4 n=2, **2-MeSATP** (Cat. No. 1062)
5 n=1, **2-MeSADP** (Cat. No. 1624)



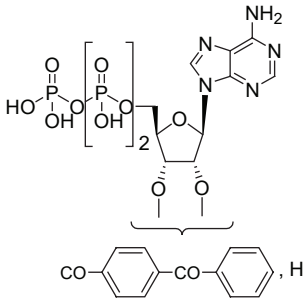
6 α,β-**Methyleneadenosine 5′-triphosphate** (Cat. No. 3209)



7 n=3, **ATPγS** :(Cat. No. 4080)
8 n=2



9 MRS 2365 (Cat. No. 2157)

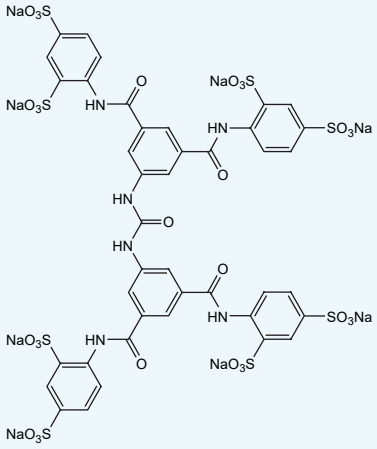


10 Bz-ATP (Cat. No. 3312)

(**Bold** text denotes compounds available from Tocris at time of publication)

$P2X_1$ Antagonist

NF 449 *Cat. No. 1391*

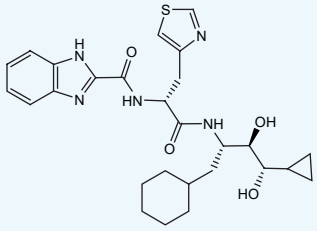


NF 449 is a potent purinergic receptor antagonist that displays high selectivity for $P2X_1$ (IC_{50} values are 0.28, 0.69, 120, 1820, 47,000 and > 300,000 nM for $rP2X_1$, $rP2X_{1+5}$, $rP2X_{2+3}$, $rP2X_3$, $rP2X_2$ and $P2X_4$ receptors respectively). The compound provides antithrombotic protection *in vivo*. NF 449 also acts as a $G_{s\alpha}$-selective antagonist.

Hohenegger *et al.* (1998) Gsa-selective G protein antagonists. *Proc.Natl. Acad. Sci.* **95** 346. **Hechler** *et al.* (2005) Inhibition of platelet functions and thrombosis through selective or non-selective inhibition of the platelet P2 receptors with increasing doses of NF449 [4,4′,4″,4‴-(carbonylbis(imino-5,1,3-benzenetriylbis-(carbonylimino)))tetrakis-benzene-1,3-disulfonic acid octasodium salt]. *J.Pharmacol.Exp.Ther.* **314** 232. **Rettinger** *et al.* (2005) Profiling at recombinant homomeric and heteromeric rat P2X receptors identifies the suramin analogue NF449 as a highly potent $P2X_1$ receptor antagonist. *Neuropharmacology*. **48** 461.

$P2X_1$ Antagonist

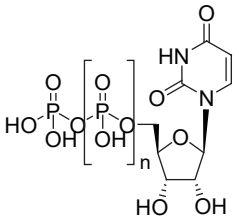
Ro 0437626 *Cat. No. 2188*



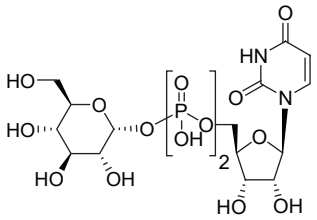
Ro 0437626 is a selective $P2X_1$ purinergic receptor antagonist (IC_{50} = 3 μM) that displays > 30-fold selectivity over $P2X_2$, $P2X_3$ and $P2X_{2/3}$ receptors (IC_{50} > 100 μM).

Jaime-Figueroa *et al.* (2005) Discovery and synthesis of a novel and selective drug-like $P2X_1$ antagonist. *Bioorg.Med.Chem.Lett.* **15** 3292. **King** *et al.* (2004) Investigation of the effects of P2 purinoceptor ligands on the micturition reflex in female urethane-anaesthetized rats. *Br.J.Pharmacol.* **142** 519. **Ford** *et al.* (2006) Purinoceptors as therapeutic targets for lower urinary tract dysfunction. *Br.J.Pharmacol.* **147** S132.

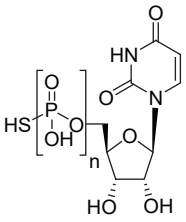
Figure 1B | Uracil derivatives that have been useful as antagonists in the study of P2 receptors



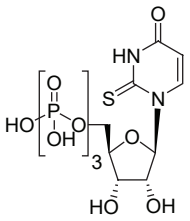
11 n=2, UTP
12 n=1, **UDP** (Cat. No. 3111)



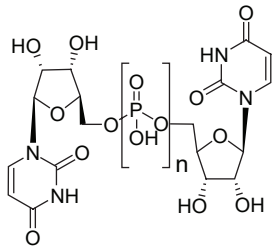
13 UDP-glucose



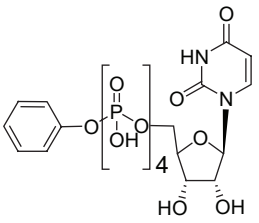
14 n=3, **UTPγS** (Cat. No. 3279)
15 n=2, UDPβS



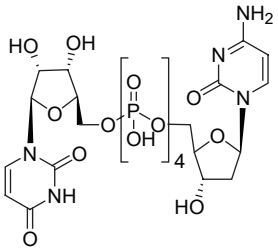
16 2-ThioUTP
(Cat. No. 3280)



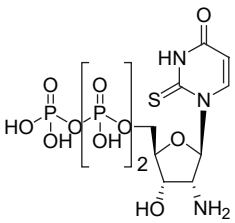
17 n=4, Up_4U, INS 365, Diquafosol
18 n=3, Up_3U



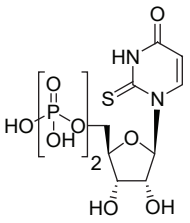
19 MRS 2768
(Cat. No. 3884)



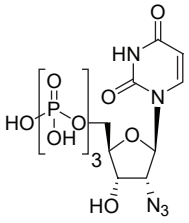
20 Up_4dC, INS 37217 Denufosol



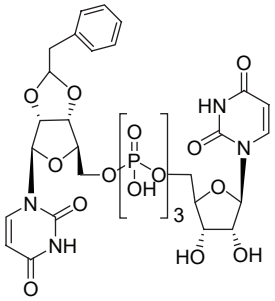
21 MRS 2698



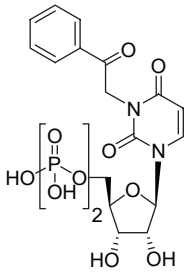
22



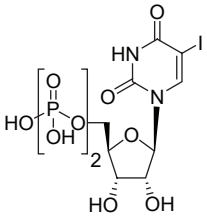
23 2′-azido-2′-deoxy-UTP



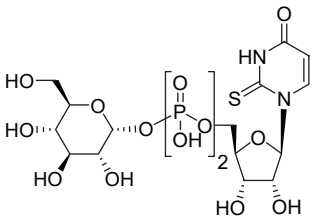
24 INS 48823



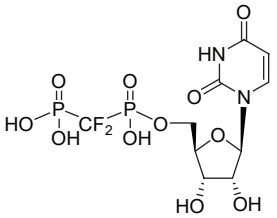
25 PBS 0474
(Cat. No. 2715)



26 MRS 2693
(Cat. No. 2502)



27 MRS 2690
(Cat. No. 2915)



28 MRS 2802

(**Bold** text denotes compounds available from Tocris at time of publication)

$P2X_5$ Receptor

There are no selective ligands for the $P2X_5$ and $P2X_6$ receptors. However, the dye Coomassie Brilliant blue G (BBG) has been used effectively to block $P2X_5$ receptor function (IC_{50} = 0.5 μM),[33] but this dye also blocks $P2X_4$ receptors (IC_{50} = 3 μM at human receptors) and $P2X_7$ receptors (IC_{50} values are 10 nM and 267 nM at rat and human receptors respectively).

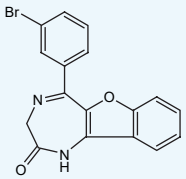
$P2X_7$ Receptor

2′(3′)-*O*-(4-Benzoylbenzoyl)adenosine-5′-triphosphate (BzATP) is a $P2X_7$ receptor agonist that exhibits an order of magnitude greater potency than ATP. It is also a partial agonist at $P2X_1$ (pEC_{50} = 8.7) and $P2Y_1$ receptors.[34] One of the first antagonists of the $P2X_7$ receptor identified was the tyrosine and isoquinoline derivative KN-62, but it acts in a non-competitive fashion and is inactive at the rat $P2X_7$ homolog. KN-62 is also an inhibitor of CaM kinase II.[35] Oxidized-ATP (o-ATP) has also been used extensively to antagonize $P2X_7$ receptors.

It has been a challenge to identify antagonists that block the $P2X_7$ receptor in a species-independent manner. The

$P2X_4$ Receptor Antagonist

5-BDBD *Cat. No. 3579*



5-BDBD is a potent $P2X_4$ receptor antagonist. The compound blocks $P2X_4$-mediated currents in Chinese hamster ovary cells (IC_{50} = 0.50 μM).

Donnelly-Roberts *et al.* (2008) Painful purinergic receptors. *J.Pharmacol.Exp. Ther.* **324** 409.

tetrazolylmethylpyridine derivative A 438079 is a potent, selective, and competitive $P2X_7$ antagonist (pIC_{50} = 6.9).[36] The quinolinamino derivative A 740003 is a potent and selective competitive $P2X_7$ receptor antagonist (IC_{50} values are 40 and 18 nM for human and rat $P2X_7$ receptors respectively) that is also highly selective in comparison to various P2X and P2Y receptors.[36,37] The adamantyl derivative from AstraZeneca AZ 10606120 antagonizes the $P2X_7$ receptor with K_D values of 1.4 and 19 nM at human and rat $P2X_7$ receptors respectively.[38] The biphenyl derivative AZ 11645373 potently antagonized the human $P2X_7$ receptor in a non-surmountable manner with K_B values ranging from 5–20 nM and was inactive at the rat $P2X_7$ receptor and at all other P2X subtypes.[39] The substituted glycyl anilide derivative GW 791343 is a positive allosteric modulator of the rat $P2X_7$ receptor and a negative allosteric modulator of the human $P2X_7$ receptor (pIC_{50} = 6.9–7.2).[40]

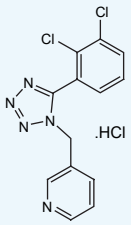
Pharmacological Probes for P2Y Receptors

There has been progress in the development of selective agonist and antagonist ligands for P2Y receptors for preclinical development.[2] Until recently, the only P2Y receptor ligand in pharmaceutical use was the antithrombotic $P2Y_{12}$ receptor antagonist Clopidogrel (Plavix).[41] Therefore, there is much activity to identify new agents to act at the $P2Y_{12}$ receptor and at other P2Y receptors for pharmaceutical development. The rapidly accelerating progress in this field has already resulted in new drug candidates for pulmonary diseases, dry eye disease, and thrombosis.

Many selective ligand probes, both agonists and antagonists of the P2Y receptors, are now available. However, some subtypes, such as the $P2Y_4$ receptor, are entirely lacking such selective ligands. Detailed SAR analyses have been constructed for $P2Y_1$ and $P2Y_{12}$ receptors, which are both proaggregatory in platelets. Nucleotide agonists selective for $P2Y_1$, $P2Y_2$, $P2Y_6$, and $P2Y_{14}$ receptors and nucleotide antagonists selective for $P2Y_1$ and $P2Y_{12}$ receptors have been described. Selective non-nucleotide antagonists are now sought to avoid issues of limited stability and bioavailability. Such antagonists have been reported for $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors. The screening of chemically diverse compound libraries has resulted in competitive $P2Y_{12}$ receptor antagonists that are being tested as potential antithrombotic agents.

$P2X_7$ Receptor Antagonist

A 438079 *Cat. No. 2972*

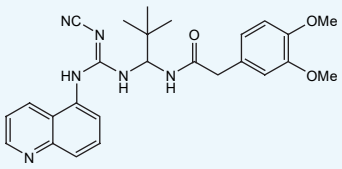


A 438079 is a competitive $P2X_7$ receptor antagonist (pIC_{50} = 6.9 for the inhibition of Ca^{2+} influx in a human recombinant cell line expressing $P2X_7$). The compound is devoid of activity at other P2 receptors (IC_{50} >> 10 μM). A 438079 possesses antinociceptive activity in models of neuropathic pain *in vivo*.

Nelson *et al.* (2006) Structure-activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole $P2X_7$ antagonists. *J.Med.Chem.* **49** 3659. **Donnelly-Roberts and Jarvis** (2007) Discovery of $P2X_7$ receptor-selective antagonists offers new insights into $P2X_7$ receptor function and indicates a role in chronic pain states. *Br.J.Pharmacol.* **151** 571. **McGaraughty** *et al.* (2007) $P2X_7$-related modulation of pathological nociception in rats. *Neuroscience* **146** 1817.

$P2X_7$ Receptor Antagonist

A 740003 *Cat. No. 3701*

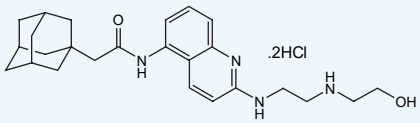


A 740003 is a potent, selective and competitive $P2X_7$ receptor antagonist (IC_{50} values are 18 and 40 nM for rat and human receptors respectively). The compound displays selectivity over a variety of P2X and P2Y receptors up to a concentration of 100 μM. A 740003 reduces nociception in animal models of persistent neuropathic and inflammatory pain.

Honore *et al.* (2006) A-740003 [N-(1-{[(cyanoimino)(5-quinolinylamino)methyl] amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective $P2X_7$ receptor antagonist, dose-dependently reduces neuropathic pain in the rat. *J.Pharmacol.Exp.Ther.* **319** 1376. **King** (2007) Novel $P2X_7$ receptor antagonists ease the pain. *Br.J.Pharmacol.* **151** 565. **Donnelly-Roberts** *et al.* (2009) Mammalian $P2X_7$ receptor pharmacology: comparison of recombinant mouse, rat and human $P2X_7$ receptors. *Br.J.Pharmacol.* **157** 1203.

$P2X_7$ Receptor Antagonist

AZ 10606120 *Cat. No. 3323*



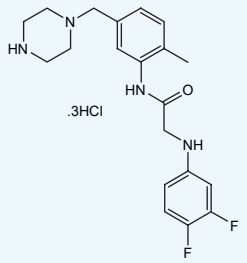
AZ 10606120 is a potent $P2X_7$ receptor antagonist (K_D values are 1.4 and 19 nM at human and rat $P2X_7$ receptors respectively). The compound binds in a positive cooperative manner to sites distinct from, but coupled to, the ATP binding site and acts as a negative allosteric modulator.

Michel *et al.* (2007) Direct labelling of the human $P2X_7$ receptor and identification of positive and negative cooperativity of binding. *Br.J.Pharmacol.* **151** 103. **Michel and Fonfria** (2007) Agonist potency at $P2X_7$ receptors is modulated by structurally diverse lipids. *Br.J.Pharmacol.* **152** 523. **Michel** *et al.* (2008) Negative and positive allosteric modulators of the $P2X_7$ receptor. *Br.J.Pharmacol.* **153** 737.

(Sold for research purposes under agreement from AstraZeneca)

$P2X_7$ Allosteric Modulator

GW 791343 *Cat. No. 3385*



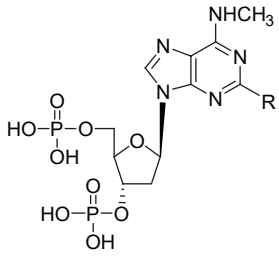
GW 791343 is a $P2X_7$ allosteric modulator. The compound exhibits species-specific activity and acts as a negative allosteric modulator of human $P2X_7$ (pIC_{50} = 6.9–7.2) and a positive allosteric modulator of rat $P2X_7$.

Michel *et al.* (2008) Negative and positive allosteric modulators of the $P2X_7$ receptor. *Br.J.Pharmacol.* **153** 737. **Michel** *et al.* (2008) Identification of regions of the $P2X_7$ receptor that contribute to human and rat species differences in antagonist effects. *Br.J.Pharmacol.* **155** 738.

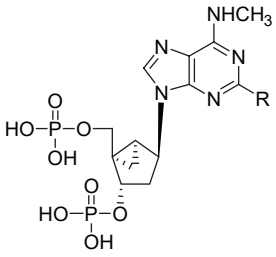
$P2Y_1$ Receptor

2-MeSADP, like ADP, activates the $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors. 2-MeSADP is a more potent agonist at the $P2Y_1$ receptor than 2-MeSATP. N^6-methyl nucleotides are tolerated at the $P2Y_1$ receptor, consistent with a small hydrophobic pocket in the $P2Y_1$ receptor binding site surrounding the N^6-position of adenine nucleotides. The favored ribose-ring conformation for each of the subtypes of the $P2Y_1$-like subfamily has been established using conformationally-restricted (i.e. rigid) ribose equivalents, which also improve stability of the phosphate esters toward nucleotidases. In particular, the methanocarba ring system consisting of fused cyclopropane and cyclopentane has been useful in exploring the biologically active conformations of nucleoside and nucleotide derivatives. The North (N)-methanocarba analog of 2-MeSADP, MRS 2365 (EC_{50} = 0.4 nM), is a selective, high affinity agonist of the $P2Y_1$ receptor.[11] Another means of improving hydrolytic stability is the introduction of a borano group within the phosphate moiety of P2Y receptor agonists.[43] Many nucleotide antagonists of the $P2Y_1$ receptor

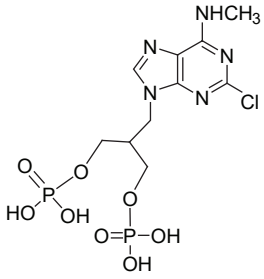
Figure 2A | Nucleotide derivatives useful as antagonists in the study of the P2 receptors



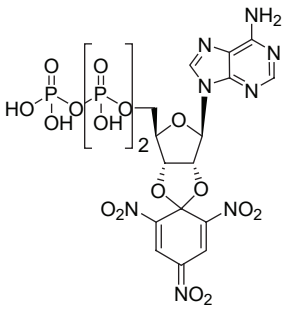
29 R=H, **MRS 2179**
(Cat. No. 0900)
30 R=Cl, MRS 2216



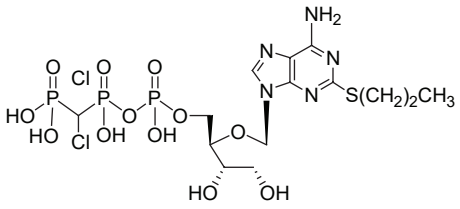
31 R=Cl, **MRS 2279**
(Cat. No. 2158)
32 R=I, **MRS 2500**
(Cat. No. 2159)



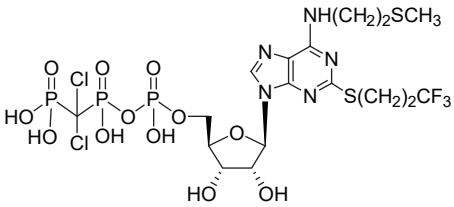
33 MRS 2298



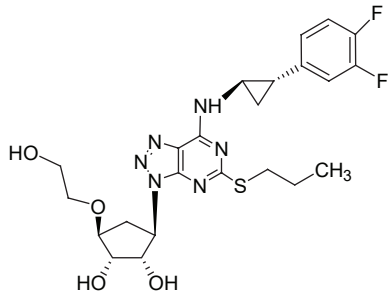
34 TNP-ATP
(Cat. No. 2464)



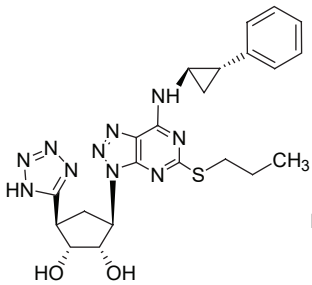
35 AR-C 67085



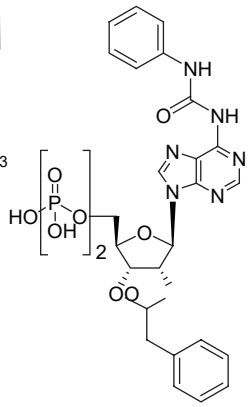
36 AR-C 69931MX
Cangrelor (Cat. No. 5720)



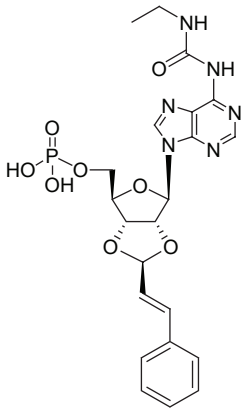
37 AZD 6140
Ticagrelor (Cat. No. 6864)



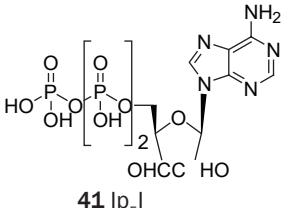
38



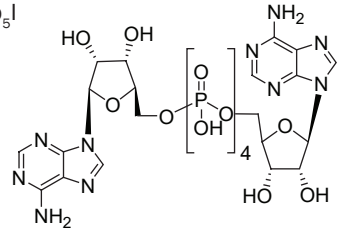
39 INS 49266



40 INS 50589



41 Ip_5I



42 o-ATP

(**Bold** text denotes compounds available from Tocris at time of publication)

have been introduced. Usually these are adenine nucleotides containing bisphosphate groups, for example a ribose 3′,5′-bisphosphate moiety. A *N*^{6}-methyl 2′-deoxyadenosine bisphosphate derivative MRS 2179 (pK_B = 6.99) and its 2-chloro analog MRS 2216 are selective P2Y_1 antagonists.[44] The same (N)-conformational constraint of the ribose moiety that enhances agonist action also favors the potency and selectivity in nucleotide antagonists. For example, the ring-constrained (N)-methanocarba nucleotide bisphosphates MRS 2279 (pK_B = 8.10) and MRS 2500 (pK_B = 9) are selective, high affinity antagonists of the P2Y_1 receptor.[45] The antithrombotic action of MRS 2500 (by blocking the P2Y_1 receptor selectively) has been demonstrated *in vivo* in the mouse and other species.[46,47] Antagonists of the P2Y_1 receptor of moderate affinity may also be derived from acyclic nucleotides, such as the bisphosphates derivative MRS 2298.[46]

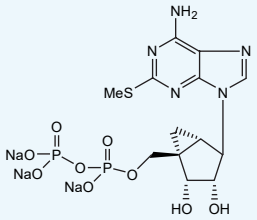
Non-nucleotide antagonists of the P2Y_1 receptor have been discovered through screening of structurally diverse chemical libraries. The first such compound to be reported was **63**, which is a selective and orally bioavailable antagonist of the human P2Y_1 receptor from Pfizer of novel chemotype with a K_i value of 90 nM.[48] Other structurally diverse antagonists of the P2Y_1 receptor have been reported. Pyridyl isatogen (PIT) is an allosteric modulator of the P2Y_1 receptor that displays mixed antagonism/potentiation.[49]

P2Y_2 and P2Y_4 Receptors

Both P2Y_2 and P2Y_4 receptors are activated by UTP, and simple modifications enhance selectivity for the P2Y_2 receptor. UTPγS is a more selective and stable agonist of the P2Y_2 receptor than UTP.[50] However, this compound is subject to chemical oxidation. 2-ThioUTP is also a selective agonist of the P2Y_2 receptor.[51] Combination of modifications of UTP in the selective P2Y_2 agonist MRS 2698 provided an EC_{50} of 8 nM and selectivity of 300-fold in comparison to the P2Y_4 receptor.[52] Certain dinucleoside tetraphosphates potently activate the P2Y_2 and P2Y_4 receptors. The uracil dinucleotides that have been in clinical trials are Up_4U (INS 365, Diquafosol, EC_{50} = 0.1 μM) and Up_4dC (INS 37217, Denufosol, EC_{50} = 0.22 μM).[53] Diquafosol was recently approved in Japan for use in treating dry eye. By virtue of being dinucleotides,

P2Y_1 Agonist

MRS 2365 *Cat. No. 2157*



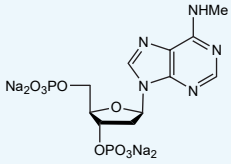
MRS 2365 is a highly potent, selective P2Y_1 receptor agonist (EC_{50} = 0.4 nM). The compound displays no activity at P2Y_{12} receptors and only very low agonist activity at P2Y_{13} receptors (at concentrations up to 1 μM).

Ravi *et al.* (2002) Adenine nucleotide analogues locked in a northern methanocarba conformation: enhanced stability and potency as P2Y_1 receptor agonists. *J.Med.Chem.* **45** 2090. **Chhatriwala** *et al.* (2004) Induction of novel agonist selectivity for the ADP-activated P2Y_1 receptor versus the ADP-activated P2Y_{12} and P2Y_{13} receptors by conformational constraint of an ADP analog. *J.Pharmacol.Exp.Ther.* **311** 1038.

(Sold under license from the NIH. US Patent 10/169975)

P2Y_1 Antagonist

MRS 2179 *Cat. No. 0900*

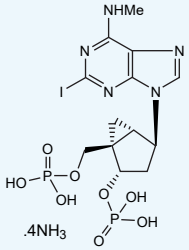


MRS 2179 is a competitive antagonist at P2Y_1 receptors (K_B = 100 nM). The compound is selective over P2X_1 (IC_{50} = 1.15 μM), P2X_3 (IC_{50} = 12.9 μM), P2X_2, P2X_4, P2Y_2, P2Y_4 and P2Y_6 receptors.

Boyer *et al.* (1998) Competitive and selective antagonism of P2Y_1 receptors by N^6-methyl 2′-deoxyadenosine 3′,5′-bisphosphate. *Br.J.Pharmacol.* **124** 1. **Moro** *et al.* (1998) Human P2Y_1 receptor molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. *J.Med. Chem.* **41** 1456. **Nandanan** *et al.* (2000) Synthesis, biological activity, and molecular modeling of ribose-modified deoxyadenosine bisphosphate analogues as P2Y_1 receptor ligands. *J.Med.Chem.* **43** 829. **Brown** *et al.* (2000) Activity of novel adenine nucleotide derivatives as agonists and antagonists at recombinant rat P2X receptors. *Drug Dev.Res.* **49** 253.

P2Y_1 Antagonist

MRS 2500 *Cat. No. 2159*



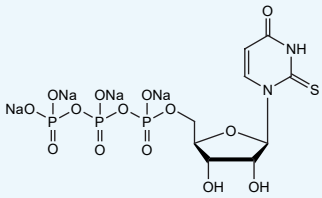
MRS 2500 is a highly potent and selective antagonist of the platelet P2Y_1 receptor (K_i = 0.78 nM). The compound inhibits ADP-induced aggregation of human platelets with an IC_{50} value of 0.95 nM. MRS 2500 prevents thrombus formation *in vivo*.

Kim *et al.* (2003) 2-Substitution of adenine nucleotide analogues containing a bicyclo[3.1.0]hexane ring system locked in a northern conformation: enhanced potency as P2Y_1 receptor antagonists. *J.Med.Chem* **46** 4974. **Cattaneo** *et al.* (2004) Antiaggregatory activity in human platelets of potent antagonists of the P2Y_1 receptor. *Biochem.Pharmacol.* **68** 1995. **Hechler** *et al.* (2006) MRS2500 [2-iodo-N^6-methyl-(N)-methanocarba-2′-deoxyadenosine-3′ ,5′-bisphosphate], a potent, selective, and stable antagonist of the platelet P2Y_1 receptor with strong antithrombotic activity in mice. *J.Pharmacol.Exp.Ther.* **316** 556.

(Sold under license from the NIH, US Patent 60/029,855.)

P2Y_2 Agonist

2-ThioUTP tetrasodium salt *Cat. No. 3280*

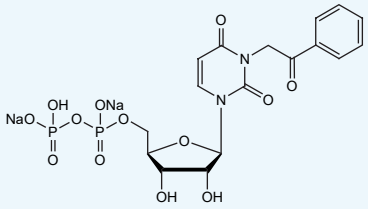


2-ThioUTP is a potent and selective P2Y_2 agonist. EC_{50} values are 0.035, 0.35 and 1.5 μM for hP2Y_2, hP2Y_4 and hP2Y_6 receptors respectively.

El-Tayeb *et al.* (2006) Synthesis and structure-activity relationships of uracil nucleotide derivatives and analogues as agonists at human P2Y_2, P2Y_4 and P2Y_6 receptors. *J.Med.Chem.* **49** 7076. **Ko** *et al.* (2008) Synthesis and potency of novel uracil nucleotides and derivatives as P2Y_2 and P2Y_6 receptor agonists. *Bioorg.Med.Chem.* **16** 6319.

$P2Y_6$ Agonist

PSB 0474 *Cat. No. 2715*



PSB 0474 is a potent and selective $P2Y_6$ receptor agonist. EC_{50} values are 70, > 1000 and > 10,000 nM for $P2Y_6$, $P2Y_2$ and $P2Y_4$ receptors respectively.

El-Tayeb *et al.* (2006) Synthesis and structure-activity relationships of uracil nucleotide derivatives and analogues as agonists at human $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors. *J.Med.Chem.* **49** 7076.

they are more stable to enzymatic hydrolysis than nucleoside triphosphates, but these agonists are non-selective compared to the $P2Y_4$ receptor. The 2′-deoxycytidine (dC) moiety of 20 serves to enhance the *in vivo* stability toward ectonucleotidases. The agonist MRS 2768 (uridine tetraphosphate δ-phenyl ester) is selective for the $P2Y_2$ receptor with moderate potency (EC_{50} = 1.89 μM).[54]

Definitive antagonists of the $P2Y_2$ receptor are not available. AR-C 126313 and its higher molecular weight analog AR-C 118925 were reported to selectively antagonize the $P2Y_2$ receptor, however it appears that these compounds are only micromolar in affinity (Figure 2B).[55] The large polyanionic molecules Reactive blue 2 (RB2, an anthraquinone dye) and Suramin are slightly selective antagonists of the $P2Y_2$ and $P2Y_4$ receptors, respectively. However, RB2 and Suramin also block various P2X receptors (Table 1).

There are no truly selective ligands for the $P2Y_4$ receptor. The agonist 2′-azido-2′-deoxy-UTP (Figure 1B **23**) displayed 5-fold $P2Y_4$ selectivity.[51] Thus, new agonists and antagonists are needed to distinguish this subtype pharmacologically from the $P2Y_2$ receptor, which is also activated by UTP. The other native agonist of the $P2Y_2$ receptor, ATP, acts as an antagonist at the human, but not the rat, $P2Y_4$ receptor.

$P2Y_6$ Receptors

UDP activates both the $P2Y_6$ and $P2Y_{14}$ receptors. It is worth noting, however, that extracellular UDP can serve as a substrate for the generation of UTP through the action of nucleoside diphosphokinase (NDPK), which may complicate pharmacological studies. The action of UPD at the $P2Y_{14}$ receptor has been controversial. UDP was initially described as inactive at the newly cloned $P2Y_{14}$ receptor, however later study found an antagonist action of UDP at the human but not rat $P2Y_{14}$ receptor. Finally the observed antagonist action of UDP was shown to occur in cells expressing an unnatural, engineered chimeric G protein. However, in HEK293 and other cells in which endogenous G_i proteins mediate the functional response, UDP acts as a potent agonist.[5] Thus, inaccurate results might be obtained using UDP alone in pharmacological studies if multiple P2Y subtypes are present. UDPβS (**15**) and Up_3U have been used as more stable activators of the $P2Y_6$ receptor subtype than UDP,[50,53] although UDPβS also activates the $P2Y_{14}$ receptor.

The SAR of nucleotide derivatives in activating the $P2Y_6$ receptor has been explored. Certain dinucleoside triphosphates have been explored as $P2Y_6$ receptor ligands, for example, INS 48823 (EC_{50} = 125 nM) potently activates the receptor.[56] Other UDP derivatives, e.g. 3-phenacyl UDP (PSB 0474) and 5-iodo-UDP (MRS 2693), are selective $P2Y_6$ agonists with EC_{50} values of 70 and 15 nM respectively.[57,58] Molecular modeling predicted that the South (S)-conformation of the ribose ring is the preferred conformation in receptor binding, which was then confirmed by synthesis of a conformationally constrained methanocarba analog of UDP. The noncompetitive $P2Y_6$ receptor antagonist MRS 2578 is a diisothiocyanate derivative, which has low stability and solubility in aqueous medium.[59] Competitive antagonists of the $P2Y_6$ receptor have not yet been reported.

$P2Y_{11}$ Receptors

ATPγS (Figure 1A) acts as a potent $P2Y_{11}$ receptor agonist. The $P2Y_{12}$ antagonist 2-propylthio-β,γ-dichloromethylene-ATP (AR-C 67085, Figure 2A) is also the most potent reported agonist of the $P2Y_{11}$ receptor (EC_{50} = 8.9 μM).[60] Thus, it must be used with caution in pharmacological studies in which both P2Y subtypes might be present.

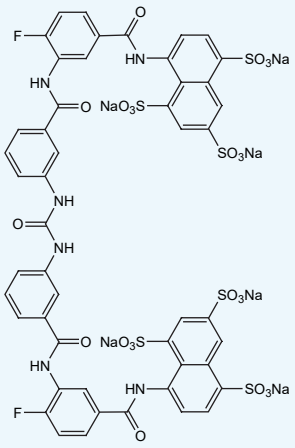
A potent antagonist NF 157, derived from nonselective P2 antagonist Suramin, has been reported to be a selective antagonist of the $P2Y_{11}$ receptor (pK_i = 7.35).[61] However, this compound also antagonizes the $P2X_1$, $P2X_2$, and $P2X_3$ receptors.

$P2Y_{12}$ Receptors

The medicinal chemistry of the $P2Y_{12}$ receptor has been extensively explored. The thienopyridines, such as Clopidogrel (Figure 2B), act as liver-activated prodrugs that are irreversible inhibitors of the $P2Y_{12}$ receptor.[62] This thienopyridine $P2Y_{12}$

$P2X_1$/$P2YX_{11}$ Antagonist

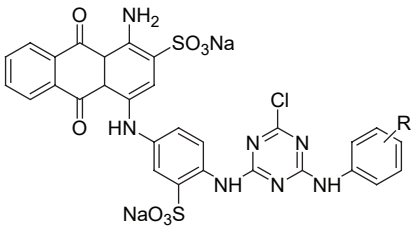
NF 157 *Cat. No. 2450*



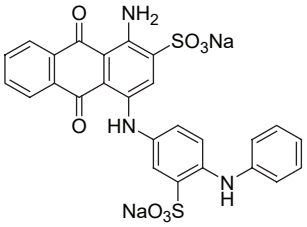
NF 157 is a purinergic receptor antagonist that potently inhibits $P2Y_{11}$ receptor activity (IC_{50} = 463 nM). The compound displays selectivity for $P2Y1_1$ and $P2X_1$ receptors over $P2Y_1$, $P2Y_2$, $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_7$ receptors. NF 157 inhibits NAD+-induced activation of human granulocytes.

Ullmann *et al.* (2005) Synthesis and structure-activity relationships of suramin-derived $P2Y_{11}$ receptor antagonists with nanomolar potency. *J.Med. Chem.* **48** 7040. **Moreschi** *et al.* (2006) Extracellular NAD+ is an agonist of the human $P2Y_{11}$ purinergic receptor in human granulocytes. *J.Biol.Chem.* **281** 31419.

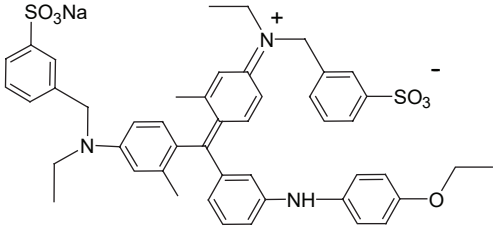
Figure 2B | Non-nucleotides that have been useful antagonists in the study of P2 receptors



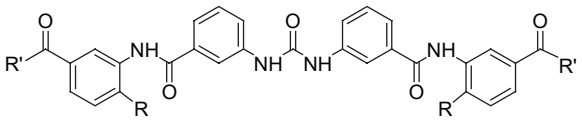
43 R=SO_3Na m- and p-mixture
Reactive Blue 2

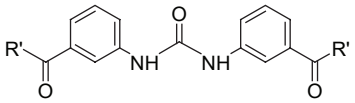


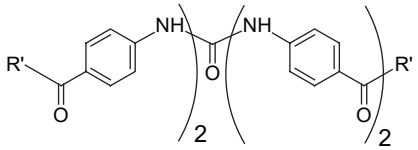
44 PSB 0739
(Cat. No. 3983)



45 Coomassie brilliant blue G (BBG)





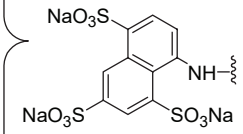


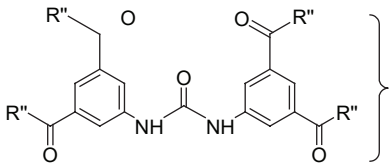
46 R = **CH_3 Suramin**
(Cat. No. 1472)
47 R = F **NF 157**
(Cat. No. 2450)

48 NF 023
(Cat. No. 1240)

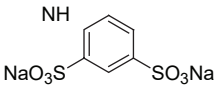
49 NF 279
(Cat. No. 1199)

R' =



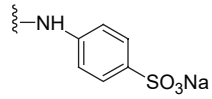


R'' =

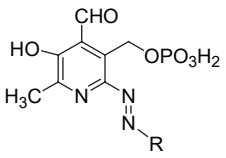


50 NF 449
(Cat. No. 1391)

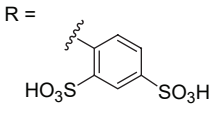
R'' =



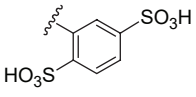
51 NF 110
(Cat. No. 2548)



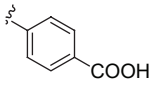
R =



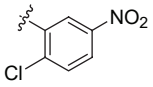
52 PPADS
(Cat. No. 0625)



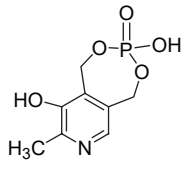
53 iso PPADS
(Cat. No. 0683)



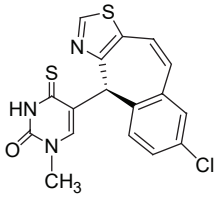
54 MRS 2159



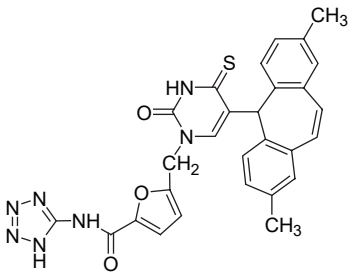
55 MRS 2211
(Cat. No. 2402)



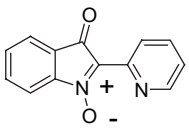
56 MRS 2219
(Cat. No. 1203)



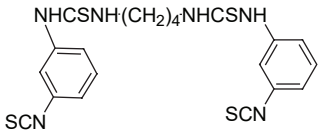
57 AR-C 126313



58 AR-C 118925
(Cat. No. 4890)



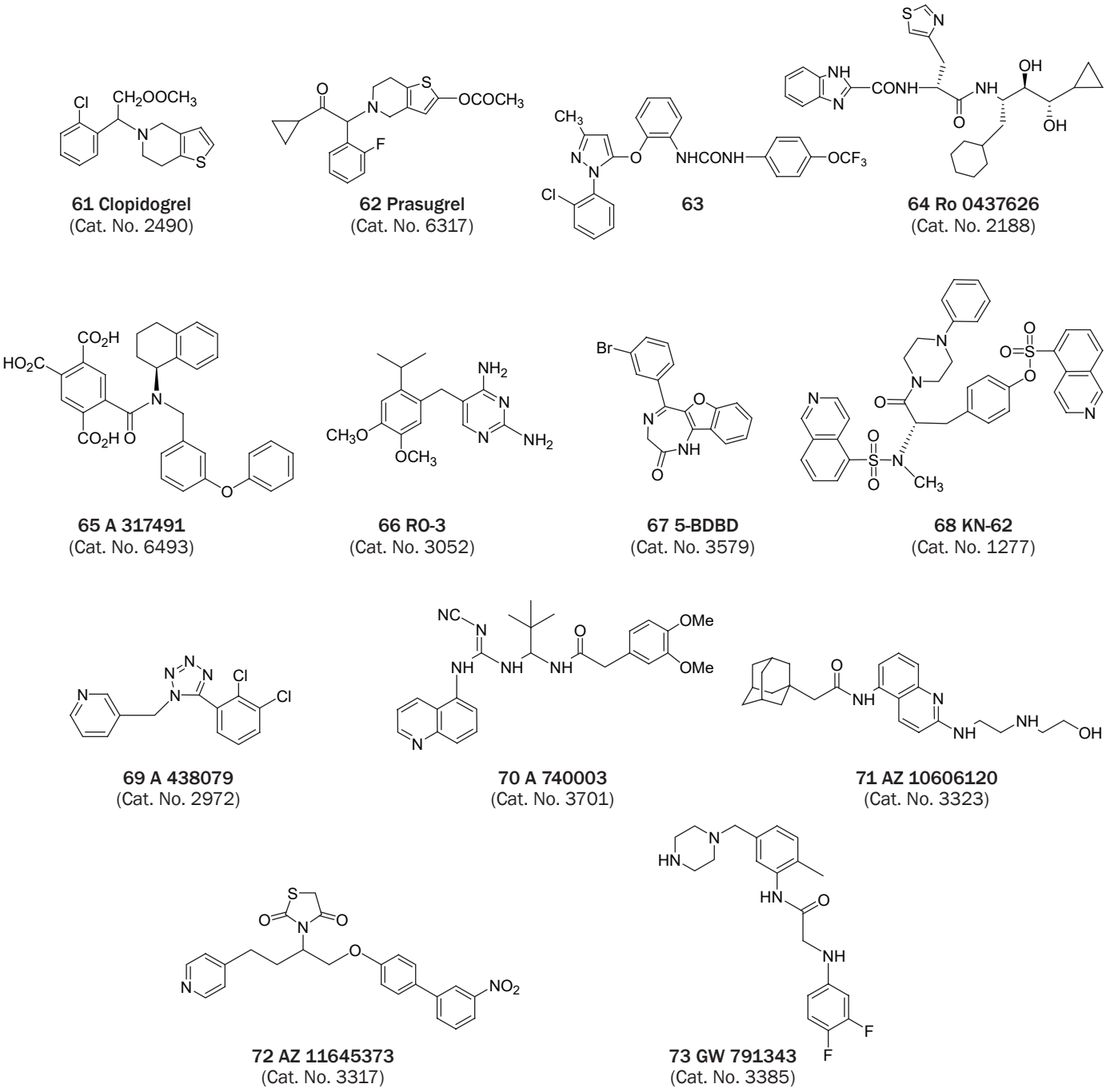
59 PIT (Cat. No. 1682)



60 MRS 2578
(Cat. No. 2146)

(**Bold** text denotes compounds available from Tocris at time of publication)

Figure 2B | Non-nucleotides that have been useful antagonists in the study of P2 receptors



61 Clopidogrel
(Cat. No. 2490)

62 Prasugrel
(Cat. No. 6317)

63

64 Ro 0437626
(Cat. No. 2188)

65 A 317491
(Cat. No. 6493)

66 RO-3
(Cat. No. 3052)

67 5-BDBD
(Cat. No. 3579)

68 KN-62
(Cat. No. 1277)

69 A 438079
(Cat. No. 2972)

70 A 740003
(Cat. No. 3701)

71 AZ 10606120
(Cat. No. 3323)

72 AZ 11645373
(Cat. No. 3317)

73 GW 791343
(Cat. No. 3385)

(**Bold** text denotes compounds available from Tocris at time of publication)

receptor antagonist requires a two-step preactivation *in vivo* and therefore has a delayed onset of action and long reversal of the platelet effect after drug administration is stopped. Another thienopyridine antagonist that has been in clinical trials, Prasugrel is a more potent $P2Y_{12}$ antagonist, but displays a longer bleeding time. Prasugrel only requires one step of preactivation *in vivo*.[63]

Directly-acting $P2Y_{12}$ receptor antagonists have also been reported. The observation that ATP acts as an antagonist at this ADP-activated subtype has enabled the introduction of various 5′-triphosphate analogs as selective receptor probes and clinical candidates. Thus, the antithrombotic nucleotide derivatives from AstraZeneca AR-C 67085 (EC_{50} = 30 μM) and ARC 69931MX (Cangrelor, EC_{50} = 0.4 nM) have been tested clinically as antithrombotic agents.[64] A 5′-triphosphate group in adenine nucleotides is not strictly required for $P2Y_{12}$ receptor antagonism, as in the case of compound **38** and the potent antagonist and approved medicine ticagrelor (AZD 6140 pIC_{50} = 7.9).[64,65] Other nucleotide antagonists of the $P2Y_{12}$ receptor that have been reported are nucleotide derivatives from Inspire Pharmaceuticals, INS 49266 (an ADP derivative with EC_{50} of 52 nM) and INS 50589 (an AMP derivative with EC_{50} of 11 nM).[66] Library screening has aided greatly in the identification of novel chemotypes that act as

$P2Y_{12}$ receptor antagonists, and several of these compounds are being developed by the pharmaceutical industry. One very potent and selective competitive antagonist of the $P2Y_{12}$ receptor, PSB 0739, derived from RB2 was recently reported.

$P2Y_{13}$ Receptors

ADP is also the preferred agonist ligand at the $P2Y_{13}$ receptor, and ATP is less potent. In the rat, ADP is 3-5-fold more potent than 2-MeSADP. A selective $P2Y_{13}$ receptor antagonist, MRS 2211, a derivative of PPADS, has a pK_i of 6.0 at this receptor.[67]

$P2Y_{14}$ Receptors

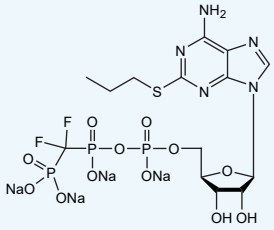
The SAR of analogs of UDP-glucose (EC_{50} = 0.35 μM) and UDP at the $P2Y_{14}$ receptor was recently systematically explored.[68] Other naturally occurring UDP-sugars activate this receptor less potently. The 2-thio analog of UDP-glucose, MRS 2690, is a 6-fold more potent agonist for the $P2Y_{14}$ receptor and, unlike UDP-glucose, is inactive at the $P2Y_2$ receptor. The $P2Y_{14}$ receptor is structurally restrictive with respect to modification of the nucleobase, ribose, and phosphate moieties of agonist ligands. However, the glucose moiety may be deleted in UDP analogs, some of which still are very potent in receptor activation. For example, α,β-difluoromethylene-UDP, MRS 2802, is inactive at the $P2Y_6$ receptor and fully activates the human $P2Y_{14}$ receptor with an EC_{50} of 63 nM.

Conclusion

Novel ligands for the P2X and P2Y receptor families are now available for use as tools in pharmacological studies. Selective nucleotide agonist ligands, although typically of low bioavailability and stability *in vivo*, have been designed. Recently, selective antagonist ligands for P2 receptors have been reported as a result of library screening, conversion of agonists into antagonist, and the careful structural modification of known non-selective ligands.

$P2Y_{12}$ Antagonist

AR-C 66096 tetrasodium salt *Cat. No. 3321*



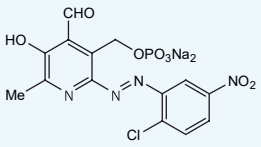
AR-C 66096 is a potent and selective $P2Y_{12}$ receptor antagonist. The compound blocks ADP-induced inhibition of adenylyl cyclase *in vitro* (pK_B =7.6) and inhibits ADP-induced aggregation of washed human platelets (pIC_{50} = 8.16).

Humphries *et al.* (1994) FPL 66094: a novel, highly potent and selective antagonist at human platelet P_{2T}-purinoceptors. *Br.J.Pharmacol.* **113** 1057. **Ingall** *et al.* (1999) Antagonists of the platelet P receptor: a novel approach to antithrombotic therapy. *J.Med.Chem.* **42** 213. **Simon** *et al.* (2001) Activity of adenosine diphosphates and triphosphates on a $P2Y_T$-type receptor in brain capillary endothelial cells. *Br.J.Pharmacol.* **132** 173.

(Sold for research purposes under agreement from AstraZeneca)

$P2Y_{13}$ Antagonist

MRS 2211 *Cat. No. 2402*

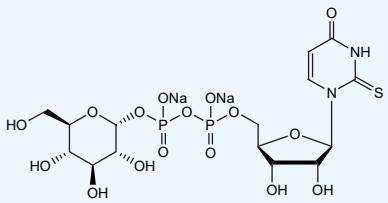


MRS 2211 is a competitive $P2Y_{13}$ receptor antagonist (pIC_{50} = 5.97). The compound displays > 20-fold selectivity over $P2Y_1$ and $P2Y_{12}$ receptors.

Kim *et al.* (2005) Synthesis of pyridoxal phosphate derivatives with antagonist activity at the $P2Y_{13}$ receptor. *Biochem.Pharmacol.* **70** 266. **von Kugelgen** (2006) Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. *Pharmacol.Ther.* **110** 415.

$P2Y_{14}$ Agonist

MRS 2690 *Cat. No. 2915*



MRS 2690 is a potent $P2Y_{14}$ receptor agonist (EC_{50} = 49 nM). The compound displays 7-fold higher potency than UDP-glucose.

Ko *et al.* (2007) Structure-activity relationship of uridine 5′-diphosphoglucose analogues as agonists of the human $P2Y_{14}$ receptor. *J.Med.Chem.* **50** 2030.

References

1. **Surprenant and North** (2009) *Ann.Rev.Physiol.* **71** 333.
2. **Abbracchio** *et al.* (2006) *Pharmacol. Rev.* **58** 281.
3. **Kawate** *et al.* (2009) *Nature* **460** 592.
4. **Young** (2009) *Trends Biochem.Sci.* **35** 83.
5. **Carter** *et al.* (2009) *Mol.Pharmacol.* **76** 1341.
6. **Ivanov** *et al.* (2006) *J.Comput.Aided Mol.Des.* **20** 417.
7. **Hoffmann** *et al.* (1999) *J.Biol.Chem.* **274** 14639.
8. **Hillmann** *et al.* (2009) *J.Med.Chem.* **52** 2762.
9. **Zylberg** *et al.* (2007) *Biochem.J.* **405** 277.
10. **Hoffmann** *et al.* (2009) *J.Pharmacol.Exp.Ther.* **331** 648.
11. **Bourdon** *et al.* (2006) *J.Thromb.Haemost.* **4** 861.
12. **Ecke** *et al.* (2008) *Biochem.J.* **409** 107.
13. **Nakata** *et al.* (2003) *Drug Dev. Res.* **58** 340.
14. **Baqi** *et al.* (2010) *J.Med.Chem.* **53** 2076.
15. **Houston** *et al.* (2006) *Br.J.Pharmacol.* **147** 459.
16. **El-Tayeb** *et al.* (2005) *Bioorg.Med.Chem.Lett.* **15** 5450.
17. **Jarvis** *et al.* (2004) *J.Pharmacol.Exp.Ther.* **310** 407.
18. **Jacobson** *et al.* (2002) *J.Med.Chem.* **45** 4057.
19. **Kim** *et al.* (2001) *J.Med.Chem.* **44** 340.
20. **Virginio** *et al.* (1998) *Mol.Pharmacol.* **53** 969.
21. **Whittenburg** *et al.* (1996) *Naunyn-Schmiedebergs.Arch.Pharmacol.* **354** 491.
22. **Ullmann** *et al.* (2005) *J.Med.Chem.* **48** 7040.
23. **Rettinger** *et al.* (2000) *Neuropharmacology* **39** 2044.
24. **Rettinger** *et al.* (2005) *Neuropharmacology* **48** 461.
25. **Soto** *et al.* (1999) *Neuropharmacology* **38** 141.
26. **Jaime-Figueroa** *et al.* (2005) *Bioorg.Med.Chem.Lett.* **15** 3292.
27. **Jacobson** *et al.* (1998) *J.Med.Chem.* **41** 2201.
28. **Hausmann** *et al.* (2006) *Mol.Pharmacol.* **69** 2058.
29. **Carter** *et al.* (2009) *Bioorg.Med.Chem.Lett.* **19** 1628.
30. **Jung** *et al.* (2007) *J.Med.Chem.* **50** 4543.
31. **Donnelly-Roberts** *et al.* (2008) *J.Pharmacol.Exp.Ther.* **324** 409.
32. **Silberberg** *et al.* (2007) *Neuron* **54** 263.
33. **Bo** *et al.* (2003) *Mol.Pharmacol.* **63** 1407.
34. **Bianchi** *et al.* (1999) *Eur.J.Pharmacol.* **376** 127.
35. **Chessell** *et al.* (1998) *Br.J.Pharmacol.* **124** 1314.
36. **Donnelly-Roberts** *et al.* (2009) *Br.J.Pharmacol.* **157** 1203.
37. **Michel** *et al.* (2008) *Br.J.Pharmacol.* **153** 737.
38. **Stokes** *et al.* (2006) *Br.J.Pharmacol.* **149** 880.
39. **Michel** *et al.* (2008) *Br.J.Pharmacol.* **155** 738.
40. **Cattaneo** (2010) *Circulation* **121** 171.
41. **King** *et al.* (1999) *Br.J.Pharmacol.* **128** 981.
42. **Eliahu** *et al.* (2009) *Eur.J.Med.Chem.* **44** 1525.
43. **Nandanan** *et al.* (1999). *J.Med.Chem.* **42** 1625.
44. **Kim** *et al.* (2003) *J.Med. Chem.* **46** 4974.
45. **Cattaneo** *et al.* (2004) *Biochem.Pharmacol.* **68** 1995.
46. **Hechler** *et al.*. (2006) *J.Pharm.Exp.Therap.* **316** 556.
47. **Pfefferkorn** *et al.* (2008) *Bioorg.Med.Chem.Lett* **18** 3338.
48. **Gao** *et al.* (2004) *Biochem.Pharmacol.* **68** 231.
49. **Hou** *et al.* (2002) *Am.J.Phys.Heart and Circ.Phys.* **282** 784.
50. **Jacobson** *et al.* (2006) *Biochem.Pharmacol.* **71** 540.
51. **Ivanov** *et al.* (2007) *J.Med.Chem.* **50** 1166.
52. **Shaver** *et al.* (2005) *Purinerg. Sig.* **1** 183.
53. **Ko** *et al.* (2008) *Bioorg.Med.Chem.* **16** 6319.
54. **Kindon** *et al.* (1998) *World Pat.* **98** 54180.
55. **Korcok** *et al.* (2005) *J.Biol.Chem.* **280** 16909.
56. **El-Tayeb** *et al.* (2006) *J.Med.Chem.* **49** 7076.
57. **Besada** *et al.* (2006) *J.Med.Chem.* **49** 5532-5543.
58. **Mamedova** *et al.* (2004) *Biochem. Pharmacol.* **67** 1763.
59. **Communi** *et al.* (1999) *Br.J.Pharmacol.* **128** 1199.
60. **Ullmann** *et al.* (2005) *J.Med.Chem.* **48** 7040.
61. **Savi** *et al.* (2002) *Throm.Haemostas.* **84** 891.
62. **Jakubowski** *et al.* (2007) *Br.J.Clin.Pharmacol.* **63** 421.
63. **Springthorpe** *et al.* (2007) *Bioorg.Med.Chem.Lett.* **17** 6013.
64. **Ye** *et al.* (2008) *ChemMedChem* **3** 732.
65. **Douglass** *et al.* (2008) *J.Med.Chem.* **51** 1007.
66. **Kim** *et al.* (2005) *Biochem.Pharmacol.* **70** 266.
67. **Das** *et al.* (2010) *J.Med.Chem.* **53** 471.

P2X and P2Y Receptor Compounds Available from Tocris

Cat. No.	Product Name	Primary Action
Non-selective P2 Receptor		
Agonists		
3245	ATP	P2 agonist
4080	ATPγS	Non-selective P2 agonist; analog of ATP (Cat. No. 3245)
3312	BzATP	$P2X_7$ agonist; also $P2X_1$ and $P2Y_1$ partial agonist; Photoaffinity label for ATPase
5157	DMNPE-caged ATP	Caged ATP
3209	α,β-Methyleneadenosine 5′-triphosphate	Non-selective P2 agonist
1062	2-Methylthioadenosine triphosphate (2-MeSATP)	Non-selective P2 agonist
Antagonists		
2450	NF 157	Selective $P2Y_{11}$ and $P2X_1$ antagonist
0625	PPADS tetrasodium salt	Non-selective P2 antagonist
1472	Suramin hexasodium salt	Non-selective P2 antagonist
P2X Receptor		
Antagonists		
6493	A 317491 sodium salt	Selective, high affinity $P2X_3$ and $P2X_{2/3}$ antagonist; antinociceptive
2972	A 438079	Competitive $P2X_7$ antagonist
3701	A 740003	Potent and selective $P2X_7$ antagonist
4473	A 804598	Potent and selective $P2X_7$ antagonist
4232	A 839977	Potent $P2X_7$ antagonist
3323	AZ 10606120	Potent $P2X_7$ antagonist
3317	AZ 11645373	Potent and selective human $P2X_7$ antagonist
3579	5-BDBD	Potent and selective $P2X_4$ antagonist
5545	BX 430	Selective $P2X_4$ allosteric agonist
0845	Evans Blue tetrasodium salt	Selective P2X antagonist; also non-NMDA iGluR antagonist
5299	JNJ 47965567	Potent and selective $P2X_7$ antagonist; brain penetrant
1277	KN-62	Non-competitive $P2X_7$ antagonist
1240	NF 023	Selective, competitive P2X antagonist
2548	NF 110	Potent $P2X_3$ antagonist
1199	NF 279	Potent and selective $P2X_1$ antagonist
1391	NF 449	Highly selective $P2X_1$ antagonist
0683	*iso*-PPADS tetrasodium salt	P2X antagonist
1309	PPNDS	Potent and selective $P2X_1$ antagonist
2188	Ro 0437626	Selective $P2X_1$ anagonist
4391	Ro 51	Potent $P2X_3$ and $P2X_{2/3}$ antagonist
3052	RO-3	Selective $P2X_3$ and $P2X_{2/3}$ antagonist
2464	TNP-ATP	Potent and selective P2X antagonist
Modulators		
3385	GW 791343	$P2X_7$ allosteric modulator
1260	Ivermectin	Positive allosteric modulator of $P2X_4$ receptor; also positive allosteric modulator of α7 nAChRs
5156	Ivermectin B1a-d_2	Deuterated Ivermectin (Cat. No. 1260)
Other		
1203	MRS 2219	Potentiates $P2X_1$-mediated responses
P2Y Receptors		
Agonists		
1624	2-Methylthioadenosine diphosphate (2-MeSADP)	Potent agonist at $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$
2157	MRS 2365	Highly potent and selective $P2Y_1$ agonist
2915	MRS 2690	Potent $P2Y_{14}$ agonist
2502	MRS 2693 trisodium salt	Selective $P2Y_8$ agonist

Cat. No.	Product Name	Primary Action
3884	MRS 2768 tetrasodium salt	Selective $P2Y_2$ agonist
4260	MRS 2957	Potent and selective $P2Y_6$ agonist
4261	MRS 4062	Selective $P2Y_4$ agonist
3892	NF 546	Selective $P2Y_{11}$ agonist
2715	PSB 0474	Potent and selective $P2Y_6$ agonist
4333	PSB 1114	Potent and selective $P2Y_2$ agonist
3279	UTPγS trisodium salt	Selective $P2Y_{2/4}$ agonist
Antagonists		
4890	AR-C 118925XX	Selective and competitive $P2Y_2$ antagonist
3321	AR-C 66096 tetrasodium salt	Potent and selective $P2Y_{12}$ antagonist
5720	AR-C 69931	Highly potent $P2Y_{12}$ antagonist
6085	AZD 1283	High affinity $P2Y_{12}$ antagonist
6078	BPTU	$P2Y_1$ allosteric antagonist
1820	(+)-Clopidogrel hydrogen sulfate	Selective $P2Y_{12}$ antagonist; active enantiomer of (±)-clopidogrel hydrochloride (Cat. No. 2490)
5316	Elinogrel	$P2Y_{12}$ antagonist
0900	MRS 2179 tetrasodium salt	Selective $P2Y_1$ antagonist
2402	MRS 2211	Competitive $P2Y_{13}$ antagonist
2158	MRS 2279	Selective, high affinity $P2Y_1$ antagonist
2159	MRS 2500	Highly potent and selective $P2Y_1$ antagonist
2146	MRS 2578	Selective $P2Y_6$ antagonist
4862	PPTN	High affinity and selective $P2Y_{14}$ antagonist
6317	Prasugrel	Irreversible $P2Y_{12}$ antagonist; antiplatelet and orally active
3983	PSB 0739	Highly potent $P2Y_{12}$ antagonist
5650	SAR 216471	Potent $P2Y_{12}$ antagonist; orally available
6864	Ticagrelor	High affinity and reversible $P2Y_{12}$ antagonist; displays inverse agonism *in vitro*; also inhibits ENT1
3931	Ticlopidine	Selective $P2Y_{12}$ antagonist
3111	UDP disodium salt	Competitive antagonist at $P2Y_{14}$ receptors; endogenous ligand
Ectonucleotidases (NTDPase)		
Inhibitors		
3633	Adenosine 5'-(α,β-methylene)diphosphate sodium salt	Ecto-5'-nucleotidase (CD73) inhibitor
1283	ARL 37156 trisodium salt	NTPDase inhibitor
2689	POM 1	Inhibitor of E-NTPDases
2574	PSB 06126	NTPDase 3 inhibitor
2573	PSB 069	Non-selective NTPDase inhibitor
Nucleoside Transporters		
Inhibitors		
1745	5-Iodoturbercidin	Nucleoside transporter inhibitor; also a broad spectrum kinase inhibitor
4588	8MDP	Potent equilibrative nucleoside transporter (ENT) inhibitor
2924	NBMPR	Equilibrative nucleoside transporter 1 (ENT1) inhibitor
5100	TC-T 6000	Potent elquilibrative nucleoside transporter 4 (ENT4) inhibitor

For a complete and up-to-date product listing please visit | tocris.com

Global info@bio-techne.com bio-techne.com/find-us/distributors TEL +1 612 379 2956
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373

bio-techne.com

RV_P2X-P2Y_29045