Pharmacochemistry of the platelet purinergic receptors

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Abstract Platelets contain at least five purinergic G protein-coupled receptors, e.g., the pro-aggregatory P2Y1 and P2Y12 receptors, a P2Y14 receptor (GPR105) of unknown function, and anti-aggregatory A2A and A2B adenosine receptor (ARs), in addition to the ligand-gated P2X1 ion channel. Probing the structure–activity relationships (SARs) of the P2X and P2Y receptors for extracellular nucleotides has resulted in numerous new agonist and antagonist ligands. Selective agents derived from known ligands and novel chemotypes can be used to help define the subtypes pharmacologically. Some of these agents have entered into clinical trials in spite of the challenges of drug development for these classes of receptors. The functional architecture of P2 receptors was extensively explored using mutagenesis and molecular modeling, which are useful tools in drug discovery. In general, novel drug delivery methods, prodrug approaches, allosteric modulation, and biased agonism would be desirable to overcome side effects that tend to occur even with receptor subtype-selective ligands. Detailed SAR analyses have been constructed for nucleotide and non-nucleotide ligands at the P2Y1, P2Y12, and P2Y14 receptors. The thienopyridine antithrombotic drugs Clopidogrel and Prasugrel require enzymatic pre-activation in vivo and react irreversibly with the P2Y12 receptor. There is much pharmaceutical development activity aimed at identifying reversible P2Y12 receptor antagonists. The screening of chemically diverse compound libraries has identified novel chemotypes that act as competitive, non-nucleotide antagonists of the P2Y1 receptor or the P2Y12 receptor, and antithrombotic properties of the structurally optimized analogues were demonstrated. In silico screening at the A2A AR has identified antagonist molecules having novel chemotypes. Fluorescent and other reporter groups incorporated into ligands can enable new technology for receptor assays and imaging. The A2A agonist CGS21680 and the P2Y1 receptor antagonist MRS2500 were derivatized for covalent attachment to polyamidoamine dendrimeric carriers of MW 20,000, and the resulting multivalent conjugates inhibited ADP-promoted platelet aggregation. In conclusion, a wide range of new pharmacological tools is available to control platelet function by interacting with cell surface purine receptors.

Keywords Purines · GPCR · Ion channel · Structure–activity relationship · Molecular modeling · Mutagenesis

Introduction: subtypes of P2X and P2Y receptors

Extracellular nucleotides activate cell surface P2 receptors which are widely distributed and participate in the regulation of nearly every physiological process, including in the immune, inflammatory, cardiovascular, muscular, and central and peripheral nervous systems [1, 2]. Closely related to these processes are the adenosine receptors (ARs), all four subtypes of which are G protein-coupled receptors (GPCRs). The P2 receptors are divided into two distinct families: fast P2X receptors (ligand-gated ion channels) and P2Y receptors (GPCRs). The P2Y receptors...
respond to various naturally occurring adenine and uracil mono- and dinucleotides. The P2X receptors are more structurally restrictive in native ligand selectivity than P2Y receptors and are activated principally by ATP. These extracellular nucleotides are produced in response to tissue stress and cell damage during neurotransmitter release and as a result of hemichannel formation. The concentration of extracellular nucleotides that act as P2 receptor agonists can vary dramatically depending also on the aforementioned circumstances. Thus, the state of activation of these receptors can be highly dependent on the stress conditions or disease states affecting a given organ or tissue.

The P2X receptors consist of seven subtypes that are numbered P2X1 through P2X7. Activation of P2X receptors leads to an influx of cations such as sodium and calcium, which depolarize excitable cells and activate cytosolic enzymes, respectively. The P2X7 receptor, in addition to forming the usual cation channel, and upon prolonged agonist exposure, also opens a large pore which can pass organic cations and dye molecules. The active ligand-gated ion channels of the P2X receptors are composed of trimeric aggregates of subunits. Both heterotrimers and homotrimers have been characterized [3], and the homotrimeric and heterotrimeric of a given subtype may have entirely different structural requirements for agonists or antagonists. For example, the P2X1 receptor can form heteromers with the P2X2, P2X4, and P2X5 receptors [4–7].

The eight human P2Y receptor subtypes are numbered P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14, which leaves some gaps due to premature assignment of numbers to certain putative P2Y receptors that were later shown to be either species homologs or entirely different types of GPCRs. The structures of representative native adenine and uracil (1–4 and 23–25 in Fig. 1) nucleotides that activate P2Y receptors are shown, and each of the native nucleotides may activate one or more P2Y receptor subtypes. In some cases, the same nucleotide might activate one subtype and antagonize another. The adenine nucleotide ATP 2 is a full agonist at two human P2Y subtypes (P2Y2 and P2Y11 receptors), and the corresponding diphosphate ADP 1 activates three different subtypes (P2Y1, P2Y12, and P2Y13 receptors). The uracil nucleotide UTP 24 activates two subtypes (P2Y2 and P2Y4 receptors), while UDP 23, previously thought to activate only a single subtype (P2Y6 receptor), is also now known to activate P2Y14 receptors along with the originally designated native agonist UDP-glucose 25 [8]. The naturally occurring dinucleotide Ap3A 3 and its homologues, including uracil derivatives, also activate various P2 receptors [9, 10]. Efforts have been made to identify other naturally occurring nucleotides that interact with known P2Y receptors and to deorphanize related GPCR sequences that have not yet been assigned a native agonist [2, 11].

Platelets contain at least five purinergic GPCRs, e.g., the ADP-activated P2Y1 and P2Y12 receptors, the P2Y14 receptor (pyrimidine-selective), and A2A and A2B ARs, in addition to the ligand-gated P2X1 ion channel (Table 1). Co-activation of P2Y1 and P2Y12 receptors is required for the aggregatory effect of ADP [12]. The P2Y12 receptor is the site of action of the important thienopyridine antiplatelet drugs Clopidogrel 29 and Prasugrel 32 (Fig. 2). Activation of the P2X1 receptor by ATP is also pro-aggregatory, but only transiently and under high shear stress conditions [13]. Antagonists of the P2X1 receptor inhibit the platelet shape change induced by α,β-meATP 5 [80]. A P2Y14 receptor was also detected in platelets, but its role is undetermined [14].

The first purine receptor detected in platelets was the Gs-coupled A2A AR, at which adenosine has an anti-aggregatory function [15]. The SAR of AR ligands will be covered only briefly in this review. There are a number of excellent reviews of A2A AR ligands [16, 17]. Recently, a novel role in platelet aggregation was proposed for the Gs-coupled A2B AR [18], which is expressed at low levels in mouse platelets [19]. This subtype is upregulated in platelets under injury or stress conditions in vivo, and it downregulates the expression and function of the P2Y1 receptor by raising cyclic AMP.

Structure and regulation of purine receptors that are expressed in platelets

The knowledge of P2X receptor structures was recently advanced with the X-ray crystallographic determination of the P2X4 subunit [20]. However, the crystal form did not contain a bound ligand, so predicting the orientation of ligands in the extracellular loop (EL) region of the P2X receptors is still subject to modeling. The X-ray structure of the P2X1 receptor in platelets has not yet been determined, but the structure–function relationships of various P2X subtypes have been probed using site-directed mutagenesis [21].

The structure, signaling, and regulation of P2Y receptors have been explored pharmacologically. Two subfamilies of P2Y1-like (five members: P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 receptors) and P2Y12-like receptors (three members: P2Y12, P2Y13, and P2Y14 receptors) have been defined. The P2Y1 receptor was first cloned from chick brain in 1994 [22], and the platelet P2Y12 receptor was first cloned in 2001 [23]. These subfamilies constitute two distinct groups based on signaling pathways and similarities in the function of key amino acid residues, but not on ligand structure [24, 25].

Fig. 1 Nucleotide derivatives that activate P2X and P2Y receptors, with emphasis on agonist ligands for studying these receptors in platelets. a Adenine nucleotides. b Uracil nucleotides. Phosphate derivatives would exist predominantly in an ionized form under physiological conditions
Table 1 Subtypes of platelet purine receptors and their representative ligands (potency at the human homologs, unless noted r = rat)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Main distribution</th>
<th>Agonists (native in bold, pEC50)</th>
<th>Antagonists (pIC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2Y1</td>
<td>Brain, epithelial and endothelial cells, platelets, immune cells, osteoclasts</td>
<td>MRS2365 9.4 &gt; 2-MeSADP 8.2 &gt;&gt; ADPβS 7.0 &gt; ADP 5.1 &gt; ATP</td>
<td>MRS2500 9.0 &gt; MRS2279 7.3 &gt; MRS2179 6.5</td>
</tr>
<tr>
<td>P2Y12</td>
<td>Platelets, brain (glial cells), microglial cells</td>
<td>2-MeSADP 7.9 ≥ ADP 7.2</td>
<td>AR-C69931MX 9.4 &gt; AZD 6140 7.9, INS 50589 7.8 &gt; RB2 7.6 (r) &gt; 2-MeSAMP 4.0</td>
</tr>
<tr>
<td>P2Y14</td>
<td>Placenta, mast cells, adipose tissue, stomach, intestine, discrete brain regions</td>
<td>MRS2905 8.7 &gt; MRS2690 7.3 &gt; UDP 6.8, UDP-glucose 6.5 &gt; UDP-galactose 6.2</td>
<td>Compound 80 8.7</td>
</tr>
<tr>
<td>P2X1</td>
<td>Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons</td>
<td>BzATP 8.7 &gt; ATP 7.3, 2-MeSATP 7.3, α,β-MeATP 6.7 (rapid desensitization) &gt;&gt; CTP 4.4</td>
<td>NF 449 9.5 &gt; Ip,i 8.8 &gt; TNP-ATP 8.2 &gt; Ro 0437626 &gt; NF 279 7.7</td>
</tr>
<tr>
<td>A2A</td>
<td>Striatum, platelets, lymphocytes</td>
<td>CGS21680 7.6 &gt; adenosine 6.5</td>
<td>ZM241385 8.8 &gt; SCH442416 8.4 &gt; CSC 7.3 &gt; theophylline 5.6 &gt; caffeine 4.6</td>
</tr>
<tr>
<td>A2B</td>
<td>Colon, fibroblasts, endothelial cells, astrocytes, bronchial smooth muscle, intestinal epithelial cells, mast cells, platelets</td>
<td>BAY 60-6583 8.0 &gt; adenosine 4.8</td>
<td>PSB603 9.3 &gt; MRS1754 8.7 &gt; MRE2029-F20 8.3 &gt; theophylline 5.0 &gt; caffeine 4.5</td>
</tr>
</tbody>
</table>

![A](image1.png) ![B](image2.png)

Fig. 2 Thienopyridines as non-nucleotide antagonists of the P2Y12 receptor that require activation in vivo. Enzymatic formation of the active metabolites precedes the formation of the disulfide bond with the target P2Y12 receptor on platelets. CYP cytochrome P450
Thus, both adenine and uracil nucleotides are found as agonists in each P2Y subfamily.

The signaling pathways of P2Y receptors have been extensively characterized. The preferential coupling of the first subfamily of P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 receptors is to Goq, leading to the activation of phospholipase Cβ and to a rise in intracellular calcium. The P2Y11 receptor alternately couples to Go θ. The second subfamily of P2Y12, P2Y13, and P2Y14 receptors couples to Go θ, resulting in the inhibition of adenyl cyclase to decrease production of cyclic AMP. Therefore, activation of the P2Y1-like receptors leads to a rise in intracellular calcium. The amino acid residues R333 and R334 in the carboxy terminal region of the human P2Y1 receptor were found using site-directed mutagenesis to be crucial for Goq coupling [26]. Activation of the P2Y1 receptor can inhibit ion channels, such as the M-type K+ current in hippocampal neurons [27].

Desensitization of P2Y receptors in response to prolonged agonist exposure has been studied pharmacologically [28, 29]. In platelets, which express two ADP-responsive P2Y subtypes, the P2Y1 receptor desensitizes more rapidly than the P2Y12 receptor. The P2Y1 receptor is desensitized mainly through protein kinase C-dependent processes, and the P2Y12 receptor is a good substrate for the GPCR kinases leading to arrestin binding.

Factors that affect the localization of P2Y receptors within the cell have been studied. In kidney cells, the role of residues on the cytosolic C-terminal domain of the P2Y1 receptor in basolateral sorting has been probed [30]. Deletion of this sorting signal that contains nine charged residues or alteration of the total number of charges caused a redirection of the receptor to the apical membrane. A P2Y1-like receptor response in calcium transport has been detected on mitochondrial membranes [31].

Dimerization of GPCRs is a general phenomenon that can lead to major effects on the pharmacology of a given receptor subtype. Homodimerization of the P2Y1 receptor expressed in human embryonic kidney (HEK)-293 cells was detected using fluorescence resonance energy transfer donor photobleaching analysis [32]. In membranes prior to agonist exposure, 44% of the P2Y1 receptors existed in the dimeric state; upon exposure to agonist, a reversible rise to 85–100% dimerization was observed. Both monomeric P2Y1 receptors and constitutive dimers were fully active. The terminal 19 amino acids of the cytosolic terminal region were essential for both dimerization and internalization, but activation by agonists was not reduced until >39 amino acids were removed from this region. Various heterodimers of P2Y receptors with other P2Y and non-P2Y GPCRs have been proposed. For example, a dimer of the A1 AR and the P2Y1 receptor was characterized [33]. The internalization of the P2Y11 receptor is dependent on the co-expression of the rapidly desensitizing P2Y1 receptor, suggesting the occurrence of P2Y1/P2Y11 receptor heterodimers [34].

Molecular recognition in the P2Y1 and P2Y12 receptors, i.e., ligand binding and activation functions, has been extensively explored using mutagenesis [35–37]. Homology modeling of the P2Y receptors based on bovine rhodopsin or one of the more recent template structures followed by small molecule docking has provided insight into the possible ligand-binding modes. Comparisons of the structural characteristics and functionally important amino acid residues within the family have been described. Molecular models of these two subtypes are compared in Fig. 3. In each of the subfamilies, specific cationic, anionic, aromatic, and other residues in the helical transmembrane (TM) region and on the ELs have conserved functions in coordinating the bound nucleotide [25].

The homology model of the P2Y1 receptor has been constructed using a two-template strategy, as described in de Castro et al. [38]. In particular, our experimentally supported rhodopsin-based P2Y1 homology model [24] was used for the construction of almost the entire receptor, while the newly reported A2A adenosine receptor structure (PDB ID: 3EML) [39] was used as the template for the conformation of the portion of second extracellular loop (EL2) downstream of C202, the conserved Cys that links EL2 to the third TM domain (TM3). The remaining portion of the loop, for which the published crystal structures of GPCRs suggest a greater variability among the receptors, was instead modeled without the use of a template. By using this approach, we obtained a model that closely resembles our previous one [24], but with a more solvent-exposed EL2. Receptor–ligand interactions were refined through Monte Carlo conformational searches, starting from our published experimentally supported binding modes [24].

Mutagenesis of the P2Y1 receptor has concentrated on the identification of residues involved in reversible binding of agonist and antagonist ligands and its regulation. Figure 3a, b shows the human P2Y1 receptor in complex with a selective antagonist (MRS2500) and a selective agonist ([N]-methanocarba-2-MeSADP, MRS2365), respectively. The residues shown are those that, when mutated, lead to a decrease of potency of 20 times or higher of nucleotide agonists. The main differences between models of the antagonist-bound and agonist-bound complexes are shown. When the agonist is bound, K280 rotates counterclockwise and the salt bridge between R128 and D204 breaks. The P2Y1 homology model indicated that the ribose moiety of nucleotide ligands was situated in a hydrophilic pocket between TM3 and TM6. The studies also indicated that the adenine ring of the ligands interacts with residues from TM7 and points in the direction of TM1. Q307(7.36) (using Ballesteros numbering for each TM) [40] was found
to form a critical H-bond with the \(N^\circ\)-amino group for both agonists and antagonists. The three critical cationic residues, i.e., R128(3.29), K280(6.55), and R310(7.39), appeared to form ionic interactions with both the bisphosphate chains of the antagonist and the diphosphate chain of the agonists. In particular, in the antagonist-bound complex, R128 interacted with the 5′-phosphate, K280 with both the 3′ and the 5′-phosphates, and R310 with the 3′-phosphate. Two of these interactions underwent significant rearrangement upon agonist docking. In particular, R128 interacted with the α- and β-phosphates, causing the disruption of the salt bridge with D204 (in EL2) that was found in the antagonist-bound complex, while K280 interacted with the β-phosphate, undergoing a significant rotation in the counterclockwise direction, when observed from the extracellular side. Instead, R310 underwent only a minor movement to interact with the α-phosphate group.

Mutagenesis of the P2Y\(_{12}\) receptor has concentrated on the identification of residues involved in binding of agonist and antagonist ligands, covalent binding of thiol-reactive ligands, and desensitization. The residues R256(6.55), Y259(6.58), and, possibly, H253(6.52), as well as K280(7.35), are required for the activation of the human P2Y\(_{12}\) receptor \[37\]. R256(6.55) is involved in the recognition of nucleotide
agonists and the non-nucleotide antagonist Reactive Blue-2, but not the nucleotide antagonist AR-C69931MX (Cangrelor). Hoffmann et al. [41] studied the recognition of the competitive non-nucleotide antagonist PSB-0739 at the human P2Y12 receptor. The residue R256 is involved in the interaction of this and other antagonists derived from Reactive Blue-2 with the human P2Y12 receptor.

The mutations F104S and S288P significantly increased agonist-induced receptor function without affecting the inhibition by AR-C69931MX [42]. R256 in TM6 and R265 in EL3 are more important for antagonist recognition than for agonist-induced activation. Compared to the wild-type P2Y12 receptor, R256T/Q and/or R265W mutations, which have been detected in a patient with congenital bleeding, significantly increased the sensitivity to AR-C69931MX. Both the cytosolic side of TM3 and the exofacial side of TM5 are critical for P2Y12 receptor function, which differs from the P2Y1 receptor. R256 in TM6 and R265 in EL3 appear to play a role in antagonist recognition rather than receptor activation.

Figure 3c, d shows the human P2Y12 receptor complexes with the agonist 2-MeSADP and the selective and competitive antagonist PSB-0739. Amino acid residues that were mutated corresponding to those in Fig. 3a, b are shown [43]. The three-dimensional structure of P2Y12 was built by means of comparative modeling using the crystal structure of the A2A adenosine receptor [39] as the template. The disulfide bridge between the N terminal and EL3, which is conserved in the P2Y family but is absent in the A2A receptor structure, was created on the P2Y12 model between C17 and C270. The extracellular loops were further refined. Molecular docking and Monte Carlo conformational studies have similar agonist activation profiles, the thiol-reactive activity [44].

Ding et al. [45] compared the differential reactivity of P2Y1 and P2Y12 receptors toward thiol reagents. Although both subtypes are encoded on the same chromosome and have similar agonist activation profiles, the thiol-reactive p-chloromercuribenzenzene sulfonic acid and the irreversibly binding metabolites (e.g., 31 and 33) of the antiplatelet drugs Clopidogrel (Plavix®) 29 and Prasugrel (Effient®) 32 (Fig. 2) inactivated the P2Y12, but not the P2Y1 receptor. The two enantiomers of Prasugrel are similar in activity and also rapidly racemize; therefore, it is used in its racemic form. The interaction of these thiol-reactive metabolites with specific Cys residues on the human P2Y12 receptor was probed by site-directed mutagenesis. There are four Cys residues in the extracellular region of the P2Y12 receptor—at positions 17, 97, 175, and 270—which presumably form two disulfide pairs. It was speculated that the active metabolites of the thienopyridines 31 and 33, which themselves are reactive thiols, formed disulfide bridges in this extracellular region, thus inactivating the receptor. The sites of covalent modification of the P2Y12 receptor were suggested to be at C17 and C270 in the N-terminal domain and in EL3, respectively [45]. Algaier et al. [46] reached different conclusions about which Cys residues of the ELs of the P2Y12 receptor are involved in the thiol reactivity of the active metabolite of Prasugrel, R-
Selective ligands for the P2Y and P2X receptors in platelets

New selective agonists and antagonists have recently been identified for the P2 receptors that occur in platelets. The structures of representative nucleotide (34–54 in Fig. 4) and non-nucleotide (55–81 in Figs. 5 and 6) antagonists of the platelet P2 receptors are shown. Selective antagonist ligands for these receptors have been reported as a result of the systematic conversion of agonists into antagonists, the careful structural modification of known non-selective ligands, and, more recently, the screening of structurally diverse chemical libraries.

A recurrent issue in the use of typical P2 receptor ligands is the lack of specificity for a single subtype among multiple P2 receptors. Often, the complete P2 receptor selectivity profile of a given ligand is unknown. Also, many of the ligands display low bioavailability due to high molecular weight or multiple charged groups, such as phosphates and sulfonates, present in the molecules. Another drawback of many of the currently used ligands is their lability in biological systems. The use of P2 receptor ligands is also complicated by the presence of ectonucleotidases that degrade both native agonists and, often, the synthetic nucleotides that are used as agonists or antagonists [58]. Adenine nucleotides are progressively converted enzymatically to AMP and finally to adenosine, which activates its own family of four ARs. Selective inhibitors of ectonucleotidases that can serve as modulators of receptor function are being explored [59, 60]. Moreover, many P2 receptor agonists and antagonists are known to inhibit ectonucleotidases at comparable concentrations. The transformation of nucleotides can also proceed in the other direction, for example in the conversion of 5′-diphosphates to 5′-triphosphates by extracellular nucleoside diphosphokinase [61]. The purity of commercial preparations of nucleotides isolated from natural sources is variable. Thus, ATP might contain ADP and other nucleotides as contaminants. The use of synthetic and rigorously purified nucleotides minimizes this ambiguity. Finally, many of the early P2 antagonists have been found to interact intracellularly with other signaling mediators, such as G proteins at concentrations similar to those needed at P2 receptors. For example, suramin and its analogues inhibit G proteins [62]. Recently, a known P2X1-selective antagonist (NF449, 64) was found to inhibit signaling from the fibroblast growth factor receptor 3 [63]. In general, novel drug delivery methods, produg approaches, allosteric modulation, and biased agonism would be desirable to overcome side effects that tend to occur even with receptor subtype-selective ligands.

Generally, radioligand binding serves as a primary research tool for the screening of new ligands at a given
GPCR, but most of the P2 receptors do not have viable radioligands yet. Fortunately, the platelet purine receptors, i.e., P2Y₁, P2Y₁₂, P2X₁, A₂A, and A₂B receptors, have such high-affinity radioligand tools [17, 64–67]. Nevertheless, much of the drug discovery at these subtypes has relied on functional assays, for example, the activation of phospholipase C by nucleotides acting at the P2Y₁ receptor expressed in 1321N1 astrocytoma cells [68].

Fig. 4 Nucleotide derivatives that have been useful as antagonists in the study of the P2X and P2Y receptors in platelets. Phosphate derivatives would exist predominantly in an ionized form under physiological conditions.
P2X1 receptors

Novel P2X receptor ligands have been introduced for use as pharmacological probes and as potential therapeutic agents. Selective P2X receptor antagonists are of interest in pain control, depression, urinary incontinence, rheumatoid arthritis, chronic inflammation, and other conditions [69].

Non-selective P2X ligands

ATP 2, but not UTP 24, activates P2X receptors, and its EC50 at the various subtypes varies from the low nanomolar to the high micromolar [70]. Purified ADP 1, AMP, and adenosine are inactive at P2X receptors. 2-Methylthioadenosine 5′-triphosphate (2-MeSATP, 9) is a potent agonist at multiple P2X receptors, for example, P2X1 (EC50 = 54 nM) and P2X3 (EC50 = 350 nM) receptors. α,β-Methyleneadenosine 5′-triphosphate (α,β-meATP, 5) activates the P2X1 receptor, but not the P2X2 receptor, and its ability to rapidly desensitize the P2X1 receptor allows it to be used in functional pharmacological experiments in place of an antagonist. A wide variety of ATP derivatives were compared in the inhibition of specific binding of [3H]α,β-meATP at the rat urinary bladder P2X1 receptor [66]. Among analogues with a substituted adenine ring, 2(6-cyanohexylthio)-ATP 21 displayed a potent pIC50 value of 7.24. Various nucleotide derivatives were assayed functionally at P2X receptors expressed in astrocytoma cells and oocytes [71, 72]. For example, 2′,3′-O-(4-benzoxybenzoyl) adenosine 5′-triphosphate (BzATP, 22), which is the most potent known agonist of the P2X7 receptor, was also found to potently activate the P2X1 receptor. The same nucleotide also acts as an antagonist of the P2Y12 receptor with an IC50 of 116 μM [45].

Until recently, the only antagonists of the P2X1 receptor were highly charged compounds. Various negatively charged organic dyes, such as Reactive Blue-2 59 (Fig. 5), act as non-selective and weak P2X antagonists. Reactive Blue-2 was also shown to be a potent antagonist of the P2Y12 receptor with a pK_B of 7.6 [73].
polysulfonated biphenyl derivative Evans Blue (structure not shown) acts as a P2X receptor antagonist, but it also affects other channels and amino acid binding sites [74]. Another polysulfonated derivative that has been widely used as a nonselective P2 receptor antagonist is the antiparasitic drug suramin 61. The aryldiazo-bridged pyridoxal phosphate derivative PPADS 55 was found to inhibit P2 receptors, and its potency and selectivity have been increased by chemical modification [75]. Typically, such compounds are relatively non-subtype-selective P2X antagonists that also block some P2Y subtypes [76]. Later, a positional isomer, iso-PPADS 56, was introduced and found to be more potent than PPADS at P2X receptors. The p-carboxylate analogue MRS2159 57 is somewhat selective for the P2X1 receptor, but also antagonizes the P2X3 receptor. The pyridoxal phosphate derivative lacking an aryldiazo moiety MRS2219 58 is a weak potentiator of P2X1-mediated responses [77].

A few nucleotide derivatives have been found to block P2X receptors. For example, the dinucleotide Ip 5I 53 potently antagonizes the P2X1 receptor [78]. TNP-ATP 54 is a potent P2X antagonist that is selective for several subtypes [79]. It antagonizes P2X1, P2X3, and heteromeric P2X2/3 receptors with IC50 values of 6, 0.9, and 7 nM, respectively, and displays 1,000-fold selectivity for homomeric P2X3 over P2X2, P2X4, and P2X7 receptors.

P2X1 antagonists of greater selectivity have been reported in compound classes both related to the known, nonselective antagonists, and to novel chemotypes. For example, the suramin analogue NF 023 62 is a moderately

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**Fig. 6** Selective non-nucleotides that have been useful antagonists in the study of P2Y receptors in platelets. See Fig. 2 for thienopyridine antagonists of the P2Y12 receptor. Sulfonate and carboxylate derivatives would exist predominantly in ionized forms under physiological conditions.
selective, competitive P2X antagonist with IC₅₀ values of 0.21 and 28.9 µM at human P2X1 and P2X3 receptors, respectively, and is inactive at the P2X2 and P2X4 receptors [80]. Another analogue NF 157 (not shown) was found to be a P2X1 antagonist, but it is not absolutely specific because it also blocks the P2Y₁₁ receptor [81]. The P2X1 potency selectivity in the suramin series of antagonists improved in later structural iterations. Other suramin derivatives that act as selective P2X1 antagonists include PPNDS, NF 279 63, and the more highly selective P2X1 agonist NF 449 64 [82, 83]. An additional high-affinity antagonist in this series is NF864 65 (8.8,8′,8″-(carbonylbis(imino-5,1,3-benzenetriyl-bis(carbonylimino))tetrakis-naphthalene-1,3,5-trisulfonic acid-dodecasodium salt. The ability to inhibit the platelet P2X1 receptor displayed the following order (pA₂ in shape change): NF864 (8.49) > NF449 (7.61) > NF110 (7.22) > NF023 (6.11) = MK-HU1 (5.98, structure not shown) = suramin (5.76) [84]. In the series of benzimidazole derivatives introduced by Roche, a 2-carboxamide derivative Ro 0437626 66 was found to be a selective P2X1 antagonist (IC₅₀=3 µM) that displays >30-fold selectivity over P2X2, P2X3, and P2X2/3 receptors (IC₅₀>100 µM) [85].

P₂Y₁, P₂Y₁₂, and P₂Y₁₄ receptors

Selective agonist and antagonist ligands for P₂Y receptors are in preclinical development for pulmonary diseases, thrombosis, and other conditions [86]. The rapidly accelerating progress in this field has already resulted in new drug candidates for pulmonary diseases, dry eye disease, and thrombosis. There is much activity in the pharmaceutical industry to identify novel antagonists of the P₂Y₁₂ receptor, with the success of the antithrombotic Clopidogrel 29, which acts as a prodrug of an irreversibly binding P₂Y₁₂ receptor antagonist [87]. The P₂Y₁ receptor might also prove to be a useful target for antithrombotic drugs [88]. Detailed analyses of SAR have been performed on the pro-aggregatory P₂Y₁ and P₂Y₁₂ receptors, for which selective nucleotide ligands have been reported. Because nucleotide analogues generally have limited bioavailability and stability in vivo, selective non-nucleotide antagonists of the P₂Y₁ and P₂Y₁₂ receptors are also being developed as potential antithrombotic agents. Structurally diverse chemical libraries have been screened to identify novel chemotypes to act as competitive, non-nucleotide antagonists of these subtypes, which may be optimized for selectivity and potency.

P₂Y₁ receptor

ADP activates the P₂Y₁, P₂Y₁₂, and P₂Y₁₃ receptors and therefore is of limited use in characterizing subtype-specific effects. 2-MeSADP 8 (EC₅₀=6 nM) is a more potent agonist at the P₂Y₁ receptor than ADP (EC₅₀=8 µM). 2-MeSATP 9 and its 2-thioether congeners have also been shown to activate the P₂Y₁ receptor, although depending on conditions where 5′-triphosphates might appear to be less than full agonists. ATP, itself, has been characterized as an agonist, a partial agonist, or an antagonist at the P₂Y₁ receptor, depending on the model used and the level of spare receptors expressed. N⁶-methyl adenine nucleotides also potently activate the P₂Y₁ receptor, but larger substituents on the exocyclic amine reduce potency, consistent with a small hydrophobic pocket surrounding the N⁶-position within the binding site of the P₂Y₁ receptor. For example, N⁶-(2-phenylethyl)-ATP is inactive at the P₂Y₁ receptor [89]. N⁶-Disubstituted ATP derivatives are inactive at the P₂Y₁ receptor and have found application as inhibitors of ectonucleotidases, such as ARL 67156 [59].

The conformation of the ribose moiety has been the focus of recent studies of P₂ receptor agonists. By comparing two isomeric conformationally restricted (i.e., rigid) equivalents of the ribose moiety in nucleotide derivatives (i.e., methanocarba ring system containing fused cyclopropane and cyclopentane rings), the favored ribose ring conformation at the P₂Y₁ receptor was established [90, 91]. The North (N)-methanocarba analog of 2-MeSADP (MRS2365, 14) displayed an EC₅₀ of 0.4 nM as an agonist of the P₂Y₁ receptor [92] and did not activate appreciably the P₂Y₁₂ and P₂Y₁₃ receptors. Addition of the (N)-methanocarba ring system to N⁶-methyl-ATP in 15 enhanced its potency at the turkey P₂Y₁ receptor by >200-fold [91]. The preference for the (N)-methanocarba over the isomeric (S)-methanocarba nucleotide analogues was also demonstrated for the activation of the P₂X1 receptor [93]. However, this conformational preference does not apply to all of the P₂Y receptors; the P₂Y₁₂ receptor is not activated by the (N)-methanocarba analogue of 2-MeSADP 14 [92].

Extension of the 2-methylithio ether on adenine nucleotides to longer alkyl and arylalkyl chains was one of the first classes of favorable modifications identified to preserve or enhance P₂Y₁ receptor potency. Extended groups such as the p-aminophenylethylthio ether were tolerated at platelet P₂Y receptors [94, 95]. Thio ethers (RS-) were found to be superior P₂Y₁ receptor agonists in comparison to the corresponding oxygen ethers (RO-) or amines (RNH-) at the 2 position [96].

The presence of a (N)-methanocarba ring system greatly improved the stability of the phosphate ester of the AMP derivative toward hydrolysis by an ectonucleotidase [91], and the phenomenon occurred to a lesser degree for ADP and ATP derivatives. Another means of improving hydrolytic stability is the introduction of a borano group within the phosphate moiety of P₂Y receptor agonists [97]. Phosphonate groups have been included in the phosphate chain to increase the stability of the nucleotide analogues. β,γ-methylene-ATP 5 itself is inactive at the human P₂Y₁ receptor, but when
combined with the conformationally constrained (N)-methanocarba modification, the resulting analogue 16 is a potent agonist at this subtype [91]. Analogues of 2-MeSAMP 9 that contain either a β,γ-methylene modification 10 or an α-cborano group 12 are more stable toward hydrolysis than 9 [98]. They activate the human P2Y1 receptor with EC50 values of 80 nM and 17.2 μM, respectively, and are inactive or only weakly active at the P2Y2, P2Y4, and P2Y6 receptors. 2-MeS-β,γ-CCl2-ATP 11 activates the P2Y1 receptor with an EC50 value of 80 nM and is resistant to a 30-min treatment with alkaline phosphatase [99].

Adenine dinucleotides, specifically diadenosine polyphosphates, are naturally occurring in secretory granules of nerve terminals. In a series of dinucleotides of varying lengths (two to six phosphates), Ap3A 3 was found to have the highest potency at the human P2Y1 receptor expressed in 1321N1 astrocytoma cells (pEC50=7.5), and the potency was similar to ADP [100]. The compound also displayed >1,000-fold selectivity over the human P2Y2 receptor. However, Ap3A was also reported to activate rat P2X1 and P2X3 receptors [9]. The homologue Ap4A 4, which is a constituent of platelet dense granules, was the most potent agonist in the series at the P2Y2 receptor, but was inactive as an agonist at the P2Y1 receptor. Chemically synthesized Ap4A of high purity was recently found to inhibit human platelet aggregation by antagonizing the P2Y1 receptor (fully) and the P2Y12 receptor (partially) [101]. Ap4A was also found to be a full agonist of the P2X1 receptor and a partial agonist of the P2Y12 receptor. However, a modified analogue, di-(2-MeS)-adenosine-5′,5′′-P1,P4,α,β-methylene-tetraphosphate 13 was found to activate the human P2Y1 receptor with an EC50 value of 0.42 μM and was 2.5-fold more stable in human blood serum than ATP, with a t1/2 of 12.1 h [102].

Other naturally occurring nucleotides have been found to interact with the P2Y1 receptor. For example, ADP-ribose at micromolar concentrations can act as an endogenous agonist the P2Y1 receptor [103]. Also, extracellular nicotinamide adenine dinucleotide (NAD⁺) 18 was shown to activate the P2Y1 receptor to induce a rise in intracellular calcium ions in transfected astrocytoma cells (EC50=743 nM, efficacy of 77%, compared to ADP) [104]. NAD⁺ contains a β-blocked diphosphate group. Beta-NAD has been shown to be an inhibitory neurotransmitter that activates P2Y1 receptors in gastrointestinal smooth muscle [105]. Curiously, another blocked diphosphate derivative 19, in which the β-phosphate of 2-MeSADP was masked as photoreversible 2-nitroveratryl ester, did not activate the P2Y1 receptor until irradiated to free the 5′-diphosphate. This compound acted as a caged agonist of the P2Y1 and P2Y12 receptors for use as a tool for the light-directed facilitation of platelet aggregation [106].

Many nucleotide antagonists of the P2Y1 receptor have been introduced (Fig. 4). The initial observation was that adenosine 3′,5′-bisphosphate (A3P5P, 34) and its naturally occurring congeners acted as partial agonists or antagonists, respectively, at the turkey and human P2Y1 receptors [107]. Various chemical modifications of adenosine nucleotides containing bisphosphate groups, for example N6-methyl 2′-deoxygenoadenosine 3′, 5′-bisphosphate MRS2179 35 (pKb=6.99), and its 2-chloro analog MRS2216 36 (pKb=6.69), provided potent and selective P2Y1 antagonists [108]. [33P] MRS2179 was studied as a radioligand of the P2Y1 receptor [109]. C-nucleoside pyrazolo[1,5-a]-1,3,5-triazines were prepared, and their 3′,5′-bisphosphate C-nucleotide analogues are stable in vivo as P2Y1 receptor antagonists [110].

The same conformationally constrained (N)-methanocarba modification of the ribose moiety that enhanced agonist action in MRS2365 14 also favored antagonist action in nucleotide bisphosphate derivatives. For example, the 2-chloro analogue MRS2279 37 (pKb=8.10) and the 2-iodo analogue MRS2500 38 (pKb=9) were selective, high-affinity antagonists of the P2Y1 receptor [111]. MRS2500 effectively inhibited platelet aggregation in vivo in the mouse and other species [112, 113]. The (N)-methanocarba antagonist [3H] MRS2279 37 was introduced as a radioligand for the P2Y1 receptor [114]. The higher affinity antagonist MRS2500 38 has been prepared as a radioligand for the P2Y1 receptor both as a 32P form and as a 125I form [64, 115].

Although the steric constraint of the (N)-methanocarba ring greatly enhanced affinity at the P2Y1 receptor, a cyclic form was not essential for P2Y1 receptor antagonism [116]. The acyclic bisphosphate derivative MRS2298 39 (IC50=62.8 nM) and bisphosphonate derivative MRS2496 40 (IC50=1.5 μM) were effective inhibitors of ADP-promoted platelet aggregation with intermediate potency [112].

Costanzi et al. [117] studied QSAR of antagonists of the P2Y1 receptor based on ligand docking models and focusing on halo and alkynyl groups at the 2 position. Other alkynyl nucleotides were evaluated at the platelet P2Y receptors. The 5′-diphosphate derivative of 2-phenylethenyladenosine (PEADP, 42) was found to interact mainly with the platelet P2Y1 receptor as an antagonist, while the corresponding 2-hexynyladenosine derivative (HEADP, 17) activated the platelet P2Y12 receptor, but not the P2Y1 receptor [118].

Thiol Coenzyme A (CoA-SH) and various drug-derived CoA derivatives antagonized the human P2Y1, but not the P2Y2, receptor expressed in Xenopus laevis oocytes [119]. Palmitoyl-CoA (16:0) 43 and CoA thioester derivatives of nafenopin and ciprofibrate, two clinically relevant hypolipidemic drugs, were more potent than CoA-SH as antagonists. This phenomenon was further studied using CoA derivatives with saturated acyl groups containing 16–18 carbons to influence the platelet aggregation and Ca2+ mobilization induced by various P2Y agonists [120]. Palmitoyl-CoA 43 was shown to act mainly as an antagonist of the P2Y1.
receptor but also as a partial antagonist at the P2Y\textsubscript{12} receptor. Not all inhibitors of the P2Y\textsubscript{1} receptor are competitive with the binding of nucleotides at the receptor. For example, pyridyl isatogen (PIT)\textsuperscript{67} was found to be an allosteric modulator of the P2Y\textsubscript{1} receptor\textsuperscript{[121]}. The screening of structurally diverse chemical libraries has helped identify lead compounds for the development of non-nucleotide antagonists of the P2Y\textsubscript{1} receptor (Fig. 6). For example, the urea derivative \textsuperscript{68} is a selective and orally bioavailable antagonist of the human P2Y\textsubscript{1} receptor of novel chemotype with a \(K_i\) value of 90 nM\textsuperscript{[122]}. Aminobenzazole derivatives from Bristol–Myers Squibb were reported as P2Y\textsubscript{1} receptor antagonists\textsuperscript{[123]}. Other structurally diverse antagonists of the P2Y\textsubscript{1} receptor have been reported. Tetrahydro-4-quinolinamines such as \textsuperscript{69} (\(K_i=70\) nM) were found to be novel P2Y\textsubscript{1} receptor antagonists\textsuperscript{[124]}. Recently, benzofuran-substituted uracil derivatives such as \textsuperscript{70} (\(K_i=140\) nM) were reported as novel P2Y\textsubscript{1} receptor antagonists\textsuperscript{[125]}

\textbf{P2Y\textsubscript{12} receptors}

ADP (EC\textsubscript{50}=69 nM) and 2-MeSADP (EC\textsubscript{50} =0.3 nM) are potent non-selective agonists at the platelet P2Y\textsubscript{12} receptor. \textsuperscript{[\textsuperscript{33}P]}2-MeSADP was utilized as a radioligand of the P2Y\textsubscript{1} receptor\textsuperscript{[126]}. Adenine nucleotides, including 5’-monophosphates, with extended 2-alkylthio groups were found to preserve or enhance the potency as agonists at the rat C6 glioma cell P2Y\textsubscript{12} receptor\textsuperscript{[43, 127]}. For example, 2-(hexenylthio)-ADP\textsuperscript{20} displayed a pEC\textsubscript{50} value of 83 nM and selectivity over the P2Y\textsubscript{1} receptor of 80-fold.

The SAR of antagonists of the P2Y\textsubscript{12} receptor has been extensively explored, resulting in clinical agents. Thienopyridines, notably the blockbuster antiplatelet drug Clopidogrel\textsuperscript{29} (Fig. 2), act as liver-activated prodrugs that are irreversible inhibitors of the P2Y\textsubscript{12} receptor\textsuperscript{[128]}. In order to form the P2Y\textsubscript{12} receptor antagonist species, a two-step pre-activation in vivo is required, which delays onset of action of the drug and the time required for reversal of the platelet effect after drug administration is ceased. This pre-activation process also is subject to pharmacogenomic factors, which explain the variability of the population to be effectively treated by Clopidogrel. A patient’s poor clinical response to Clopidogrel depends partly on factors that affect its metabolism through cytochrome P450 in the liver. The presence of a reduced-function CYP2C19*2 allele or the co-administration of the proton pump inhibitor omeprazole decreases the effectiveness of Clopidogrel, and cigarette smoking increases its pre-activation. Another thienopyridine antagonist that has been in clinical trials, Prasugrel\textsuperscript{32} (CS-747, LY640315), is a more potent P2Y\textsubscript{12} antagonist than Clopidogrel and leads to a more complete inhibition of platelet function, but it also displays a longer bleeding time. Prasugrel, with less genetic variability than Clopidogrel, requires only one step of pre-activation in vivo\textsuperscript{[130]} to the active metabolite R-138727\textsuperscript{33}.

As discussed above, the action of the thienopyridines depends on the covalent reaction of an active metabolite with a thiol on the P2Y\textsubscript{12} receptor. However, directly acting and reversible P2Y\textsubscript{12} receptor antagonists, both nucleotides and non-nucleotides, have also been reported. ATP, itself, has been characterized as an antagonist at the P2Y\textsubscript{12} receptor, which has enabled the introduction of various 5’-triphosphate analogs as selective receptor probes and clinical candidates. Thus, the antithrombotic nucleotide derivatives from AstraZeneca, AR-C67085\textsuperscript{45} and 5’-adenylic acid, N-[2-(methylthio)ethyl]-2-[(3,3,3-trifluoropropyl)thio]-, monoanhydrhidride with (dichloromethyle)bis [phosphonic acid] (AR-C69931MX, Cangrelor, \textsuperscript{46}), have been tested clinically as antithrombotic agents\textsuperscript{[131, 132]}. The EC\textsubscript{50} values of these P2Y\textsubscript{12} receptor antagonists are 30 μM and 0.4 nM, respectively. Note that these two P2Y\textsubscript{12} receptor antagonists, AR-C67085MX\textsuperscript{45} and AR-C69931MX\textsuperscript{46}, also activate the P2Y\textsubscript{11} receptor. Ding et al.\textsuperscript{[44]} reported that (\textit{E})-N\textsubscript{1}-[\textit{7}-(hexylamino)-5-(propylthio)-3H-1,2,3-triazolo-[4,5-d]-pyrimidin-3-yl]-1,5,6-trideoxy-beta-D-ribo-hept-5-eno-furanuronoyl]-L-aspartic acid (AR-C78511, \textsuperscript{47}) is an inverse agonist of the P2Y\textsubscript{12} receptor, but AR-C69931MX is a neutral antagonist. \textsuperscript{[\textsuperscript{3}H]2-Propylthiophenoadoxinosine-5’-adenylic acid (1,1-dichloro-1-phosphonomethyl-1-phosphoryl) anhydrhidride ([\textsuperscript{3}H]PSB-0413, which is the tritiated equivalent of AR-C67085MX), is a high-affinity antagonist radioligand of the P2Y\textsubscript{12} receptor\textsuperscript{[65]}.

A 5’-triphosphate group in adenine nucleotides is not strictly required for P2Y\textsubscript{12} receptor antagonists. The P2Y\textsubscript{12} receptor antagonists from Inspire Pharmaceuticals, INS 49266\textsuperscript{51} (an ADP derivative with EC\textsubscript{50} of 52 nM) and INS 50589\textsuperscript{52} (an AMP derivative with EC\textsubscript{50} of 11 nM)\textsuperscript{[133, 134]}, could be considered truncated derivatives of the proven 5’-triphosphate antagonists. The potent P2Y\textsubscript{12} receptor antagonist and clinical candidate AZD 6140\textsuperscript{48b} (Brilinta, Ticagrelor, pIC\textsubscript{50}=7.9) is an uncharged cyclo-pentyltetrazolopyrimidine analogue that was developed in an extensive SAR exploration by AstraZeneca\textsuperscript{[132, 135]}. Like\textsuperscript{47}, this nucleoside derivative contains a modified base, i.e., 8-azaadenine. The antithrombotic effects of\textsuperscript{48b} compare favorably with the thienopyridines in rat and dog models and with less risk of bleeding, possibly due to its reversible receptor binding\textsuperscript{[136]}. The receptor-binding properties and functional antagonism of\textsuperscript{48b} are complex (competitive toward 2-MeSADP, but not ADP)\textsuperscript{[67]}. A related carbocyclic nucleoside derivative\textsuperscript{[125I]}(1S,2R,3S,4R)-2,3-dihydroxy-4-[(2E)-3-iodoprop-2-en-1-yl]amino]-5-(propylthio)3H-[\textit{1}–3]triazolo-[4,5-d]-pyrimidin-3-yl]cyclopentane-carboxylic acid ([\textsuperscript{125I}]AZ11931285)\textsuperscript{48a} has been proven
useful as a high-affinity antagonist radioligand of the P2Y_{12} receptor [67]. Other uncharged analogues of nucleotides that act as potent antagonists of the P2Y_{12} receptor are carbocyclic nucleoside tetrazole derivatives, such as 50 [137]. An uncharged acyclic adenine diester derivative MRS2395 41 acted as a weak but selective antagonist of the P2Y_{12} receptor (IC_{50}=3.6 μM, rat) [116]. This is in contrast to 39 and 40 which contain the same acyclic 9-alkyladenine scaffold and interact only with the P2Y_{1} receptor as antagonists.

Library screening has identified novel chemotypes as antagonists of the P2Y_{12} receptor (Fig. 6). For example, Elinogrel (PRT-128, 77) [138], a competitive and reversible antagonist with an IC_{50} value of 20 nM at the P2Y_{12} receptor, is being developed as an antithrombotic agent by Portola Pharmaceuticals. Earlier, P2Y_{12} receptor antagonists consisting of pyrazolidine-3,5-dione derivatives including 71 were reported in an abstract [139]. Tricyclic benzothiazolo[2,3-e]thiadiazine antagonists of the P2Y_{12} such as CT50547 72 (pEC_{50}=6.74, also called C1330-7) were reported [140]. An ester derivative BX 667 73 and the corresponding free carboxylic acid BX 048 74 (binding IC_{50} values of 29 and 5.3 nM, respectively), which are derivatives of L-glutamic acid, reversibly inhibit the binding and functional effects of 2-MeSADP in platelets of several species [141, 142]. The functional selectivity of BX 667 and BX 048 for the P2Y_{12} receptor in comparison to P2Y_{1} and P2Y_{6} receptors was demonstrated. Parlow et al. [143–145] reported piperazinyl–glutamate–pyridine derivatives such as 76 (pEC_{50}=7.82) as potent orally bioavailable P2Y_{12} antagonists. 6-Amino-2-mercapto-3H-pyrimidin-4-one derivatives such as 78 (IC_{50}=8.1 μM) appear to antagonize the P2Y_{12} receptor [146]. One very potent and selective competitive antagonist of the P2Y_{12} receptor, the disulphonate derivative PSB 0739 75, which was derived from RB2, was recently introduced as a research tool [41, 147]. PSB-0739 was reported as the most potent competitive non-nucleotide antagonist at the human P2Y_{12} receptor described so far (K_{i}=24.9 nM). BF0801 79 is an uncharged adenine derivative that antagonizes the P2Y_{12} receptor in platelets to inhibit aggregation with an IC_{50} of 63.3 μM and also inhibits a phosphodiesterase [148].

P2Y_{14} receptors

The P2Y_{14} receptor is structurally restrictive with respect to the modification of the nucleobase, ribose, and phosphate moieties of agonist ligands. UDP-glucose 25 (EC_{50}=0.35 μM) and UDP 23 are nearly equipotent as agonists of the human P2Y_{14} receptor. Other naturally occurring UDP-sugars activate this receptor less potently. The SAR of analogues of both endogenous agonists was recently explored in a systematic fashion [149]. When the glucose moiety is present, there is a requirement for specific hydroxyl groups in order to potently activate the P2Y_{14} receptor. When the distal hexose moiety is entirely absent, very high potencies can be obtained, suggesting partly different modes of binding of the two ligand series. The 2-thiouracil modification enhances potency in both series. Thus, the 2-thio analog of UDP-glucose MRS2690 26 is a sixfold more potent agonist for the P2Y_{14} receptor and, unlike UDP-glucose, is inactive at the P2Y_{2} receptor. Because UDP activates both the P2Y_{6} and P2Y_{14} receptors, there is a need for agonist ligands that can distinguish between these two subtypes. Stabilizing phosphate groups have facilitated this selectivity. For example, α,β-difluoromethylen-UDP, MRS2802 27, is inactive at the P2Y_{6} receptor and fully activates the human P2Y_{14} receptor with an EC_{50} of 63 nM. MRS2905 28 displays an EC_{50} of 2 nM at the human P2Y_{14} receptor with a selectivity of >2,000 in comparison to the P2Y_{6} receptor.

The heterocyclic antagonists of the P2Y_{14} receptor 80 (K_{i}=2.2 nM) and 81 (K_{i}=4.0 nM) were disclosed in patents from Merck, but the full pharmacological characterization has not yet appeared in the literature [150, 151]. A prodrug approach in the structural series of compound 80 was recently reported [152].

Conclusions

Novel ligands for the purine receptor families in platelets, including both selective agents derived from known ligands and new chemotypes, are now available for use as tools in pharmacological studies. Some of these agents have entered into clinical trials. Functional properties of the P2Y_{1} and P2Y_{12} receptors were extensively explored using mutagenesis and molecular modeling, which are useful tools in drug discovery. Detailed SAR analyses have been constructed for nucleotide and non-nucleotide ligands at the P2X_{1}, P2Y_{1}, P2Y_{12}, and P2Y_{14} receptors. There is much pharmaceutical development activity aimed at identifying newer agents to act at the P2Y_{12} receptor that do not require enzymatic pre-activation in vivo. The screening of chemically diverse compound libraries has identified novel chemotypes that act as competitive non-nucleotide antagonists of the P2Y_{1} receptor or the P2Y_{12} receptor, and antithrombotic properties of the structurally optimized analogues were demonstrated. New tools have been developed for the discovery of ligands at platelet purine receptors, including in silico screening to identify novel antagonist chemotypes, fluorescent probes, and covalent attachment to dendrimeric carriers to produce multivalent conjugates that inhibit platelet aggregation. In conclusion, a wide range of new pharmacological tools is available to control platelet function by interacting with cell surface purine receptors.
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References


Purinergic Signalling


