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P2Y nucleotide receptors: promise of therapeutic applications

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Extracellular nucleotides, such as ATP and UTP, have distinct signaling roles through a class of G-protein-coupled receptors, termed P2Y. The receptor ligands are typically charged molecules of low bioavailability and stability *in vivo*. Recent progress in the development of selective agonists and antagonists for P2Y receptors and study of knockout mice have led to new drug concepts based on these receptors. The rapidly accelerating progress in this field has already resulted in drug candidates for cystic fibrosis, dry eye disease and thrombosis. On the horizon are novel treatments for cardiovascular diseases, inflammatory diseases and neurodegeneration.

Introduction

Although nucleotides (such as ATP **1** and UTP **2**) are mainly intracellular, they can be released in the extracellular fluids by various mechanisms. One is cell damage: both necrotic and apoptotic cells release ATP and other nucleotides that thus constitute 'danger signals' [1,2]. But they can also be released without cell lysis by specific mechanisms: exocytosis of secretory granules, vesicular transport and membrane channels, such as ABC transporters, pannexins and connexins [3,4]. Nucleotides are released by exocytosis during platelet aggregation and synaptic transmission. They are also released in response to various types of stress: mechanical stimulation (stretch and shear stress), hypoxia or pathogen invasion.

Once in the extracellular fluid, nucleotides can activate two families of receptors: metabotropic P2Y receptors that are coupled to G proteins and fast P2X ion channels. The P2X receptors are more structurally restrictive than P2Y in agonist selectivity. They respond principally to ATP as the active ligand, whereas the P2Y receptors are activated by a group of five or more naturally occurring nucleotides, including **1**, **2**, ADP **3**, UDP **4** and UDP-glucose **5**.

The P2Y family is composed of eight members encoded by distinct genes, which can be subdivided into two groups based on their coupling to specific G proteins [5]. The $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors couple to G_q, to activate PLCβ, and the $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors couple to G_i, to inhibit adenylyl cyclase (Table 1). The $P2Y_{11}$ receptor has the unique property to couple through both G_q and G_s. It is also the only P2Y receptor of which the coding sequence is interrupted by an intron [6]. Comparisons of the structural characteristics and functionally important amino acid residues within the family have been explored using mutagenesis and modeling [7–10]. Specific conserved cationic residues that interact with the negatively charged phosphate groups have been identified [10]; they differ between the two subfamilies of P2Y receptors mentioned earlier [11].

The specificity of nucleotides for the various P2Y receptors is presented in Table 1. Naturally occurring dinucleotide phosphates activate various P2Y receptors, such as the endothelium-derived vasoconstrictive factor Ap_4U **6** (pEC_{50} 5.32, 6.46 and 5.84 at $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors, respectively) [12–14]. Because many cells express multiple P2Y receptor subtypes (as well as receptors of adenosine, a metabolic product of the adenine nucleotides), there is a complex and time-dependent signaling process at the cell surface. Indeed, a large family of ectonucleotidase enzymes hydrolyzes the native nucleotides and, thus, there is a chronological progression of longer phosphate chains leading to shorter phosphate chains and to the nucleosides [5].

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1359-6446/06/$ - see front matter. Published by Elsevier B.V. doi:10.1016/j.drudis.2010.05.011

TABLE 1

Properties of P2Y receptors.[a]

Group	Receptor	Chromosome (human)	Native agonist (human, pEC_{50})	Selective agonist (pEC_{50})	Selective antagonist (pIC_{50})	G protein
A	$P2Y_1$	3q24-25	ADP (5.09)	MRS2365 (9.40)	MRS2500 (9.02), MRS2179 (6.48)	G_q
	$P2Y_2$	11q13.5	UTP (8.10), ATP (7.07)	MRS2698 (8.10), MRS2768 (5.72)	PSB-716 (5.01), AR-C126313 (6)	G_q (+G_i)
	$P2Y_4$	Xq13	UTP (5.60)[b]	N/A[c]	N/A[c]	G_q (+ G_i)
	$P2Y_6$	11q13.5	UDP (6.52)[d]	PSB-0474 (7.15), 5-iodo-UDP (7.83)	MRS2578 (7.43) [non-competitive]	G_q
	$P2Y_{11}$	19p31	ATP (4.77)	NF546 (6.27)	NF340 (7.14)	G_q + G_s
B	$P2Y_{12}$	3q21-25	ADP (7.22)	N/A[c]	AZD6140 (7.90), AR-C69931MX (9.40), PSB-0739 (9.8)	G_i
	$P2Y_{13}$	3q24-25	ADP (7.94)	N/A[c]	MRS2211 (5.97)	G_i
	$P2Y_{14}$	3q24-25	UDP-glucose (6.45), UDP (6.80)	MRS2690 (7.31), MRS2802 (7.20)	[e]	G_i

[a] The missing numbers in the classification represent either nonmammalian orthologs or receptors having some sequence homology to P2Y receptors, but for which there is no functional evidence of responsiveness to nucleotides.
[b] The pharmacology of some P2Y receptors exhibits species differences: whereas the human $P2Y_4$ is a UTP receptor, the rat and mouse $P2Y_4$ receptors are activated equipotently by ATP and UTP.
[c] Selective ligands not yet available. Other useful nonselective agonists include (pEC_{50}): INS365 (7.00 at $P2Y_2$), 2′-azido-2′-deoxyUTP (7.14 at $P2Y_4$), INS48823 (6.90 at $P2Y_6$), AR-C67085 (5.05 at $P2Y_{11}$) and 2-MeSADP (7.85 at $P2Y_{12}$ and $P2Y_{13}$). Other useful nonselective antagonists include (pIC_{50}): PPADS (<5.0 at $P2Y_4$) and 2-MeSAMP (4.00 at $P2Y_{12}$).
[d] UTP is also an agonist of the $P2Y_6$ receptor [71,90].
[e] Non-nucleotide antagonists have been reported [27].

New ligand probes for P2Y receptors

As new clinical targets are revealed, there is an intense ongoing effort to design selective agonist and antagonist ligands for the P2Y receptors, both as pharmacological tools and as potential therapeutic agents [11,15] (Table 2). Many selective ligand probes, both agonists and antagonists of the P2Y receptors, are now available. Nevertheless, much more work is needed, and some subtypes, such as the $P2Y_4$ receptor, are entirely lacking such selective ligands. Detailed structure–activity relationships (SARs) have been constructed for $P2Y_1$ and $P2Y_{12}$ receptors. 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 [16–19]. Because of the difficulty of synthesizing and purifying nucleotide analogs and applying them in pharmacological models, in which stability and bioavailability might be limited, there is a quest for selective non-nucleotide antagonists, as already reported for all subtypes except $P2Y_4$ receptors [5,20–27]. The screening of chemically diverse compound libraries has resulted in competitive $P2Y_{12}$ receptor antagonists that are being tested as potential antithrombotic agents.

Few selective radioligands are available for the P2Y receptors. Previously, various radioactive nucleotides have been proposed for use as P2Y receptor radioligands, but currently only the $P2Y_1$ and $P2Y_{12}$ receptors have viable radioligands. Thus, improved and more versatile radioligands and other affinity probes, such as fluorescent probes, are still needed.

Some caution must be added in the use of the current selective ligand probes of the P2Y receptors. First, there can be cross-reactivity with other P2Y receptors or with P2X receptors (i.e. some of these probes are not altogether selective). There can also be interactions with nonreceptor proteins, such as G proteins. Second, the antagonists might be metabolized to other active species, such as the conversion of 5′-triphosphates to 5′-diphosphates, which could activate other subtypes. Third, P2 receptor agonists and antagonists are known to inhibit ectonucleotidases, which can

TABLE 2

New P2Y ligands currently in clinical development.

Molecule	Mechanism	Administration	Pathology	Phase	Company
Ticagrelor	$P2Y_{12}$ antagonist	Oral	Acute coronary syndrome	NDA submitted	AstraZeneca
Cangrelor	$P2Y_{12}$ antagonist	Iv	CABG	II	The Medicines Co.
Elinogrel	$P2Y_{12}$ antagonist	Oral or iv	PCI	II	Portola/Novartis
Diquafosol	$P2Y_2$ agonist	Local	Dry eye disease	III	Inspire
Denufosol	$P2Y_2$ agonist	Local	Cystic fibrosis	III	Inspire

Abbreviations: CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

introduce artifactual results by disturbing the balance of extracellular nucleotides.

At the $P2Y_1$ receptor, 2-MeSADP **8** (Fig. 1) has been used widely for activation, but this compound also activates the $P2Y_{12}$ and $P2Y_{13}$ receptors. 2-MeSADP is preferred as a $P2Y_1$ receptor agonist over 2-MeSATP **9**, which can also activate $P2X_1$ (pEC_{50} 7.27), $P2X_3$ (pEC_{50} 6.46) and other receptors. Subsequent generations of ligands are more definitive for elucidating the action of the $P2Y_1$ receptor. Notably, the 2′-deoxy N^6-methyl derivative MRS2179 **12** (Fig. 2) is a protypical selective $P2Y_1$ antagonist (pK_B 6.99, throughout this review values at the human subtypes are given unless noted) [28], which was based on the discovery of receptor antagonism by various naturally occurring bisphosphate nucleotides, such as A3P5P [29].

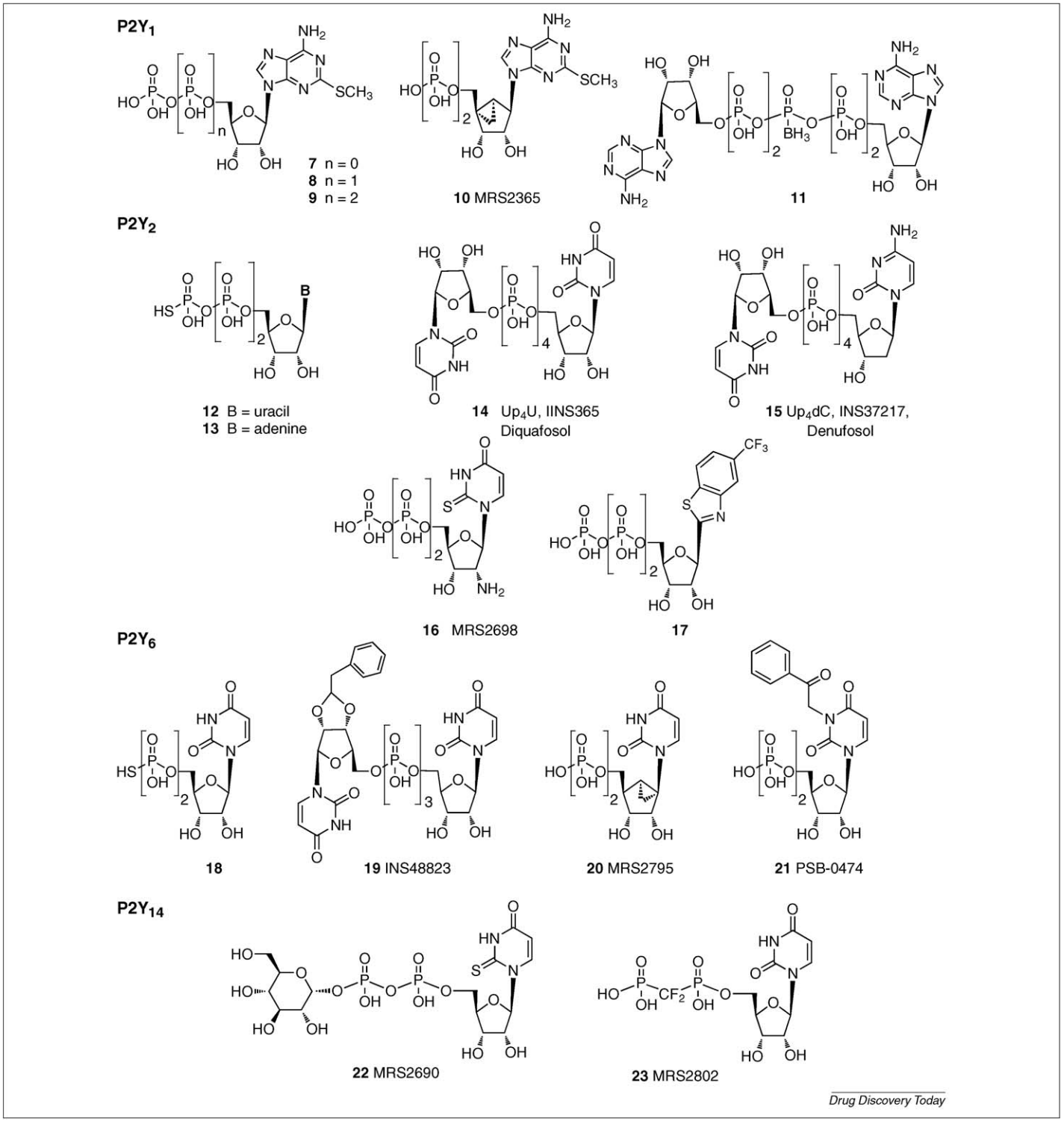

FIGURE 1

Nucleotide derivatives that have been useful as agonists in the study of the P2Y receptors.

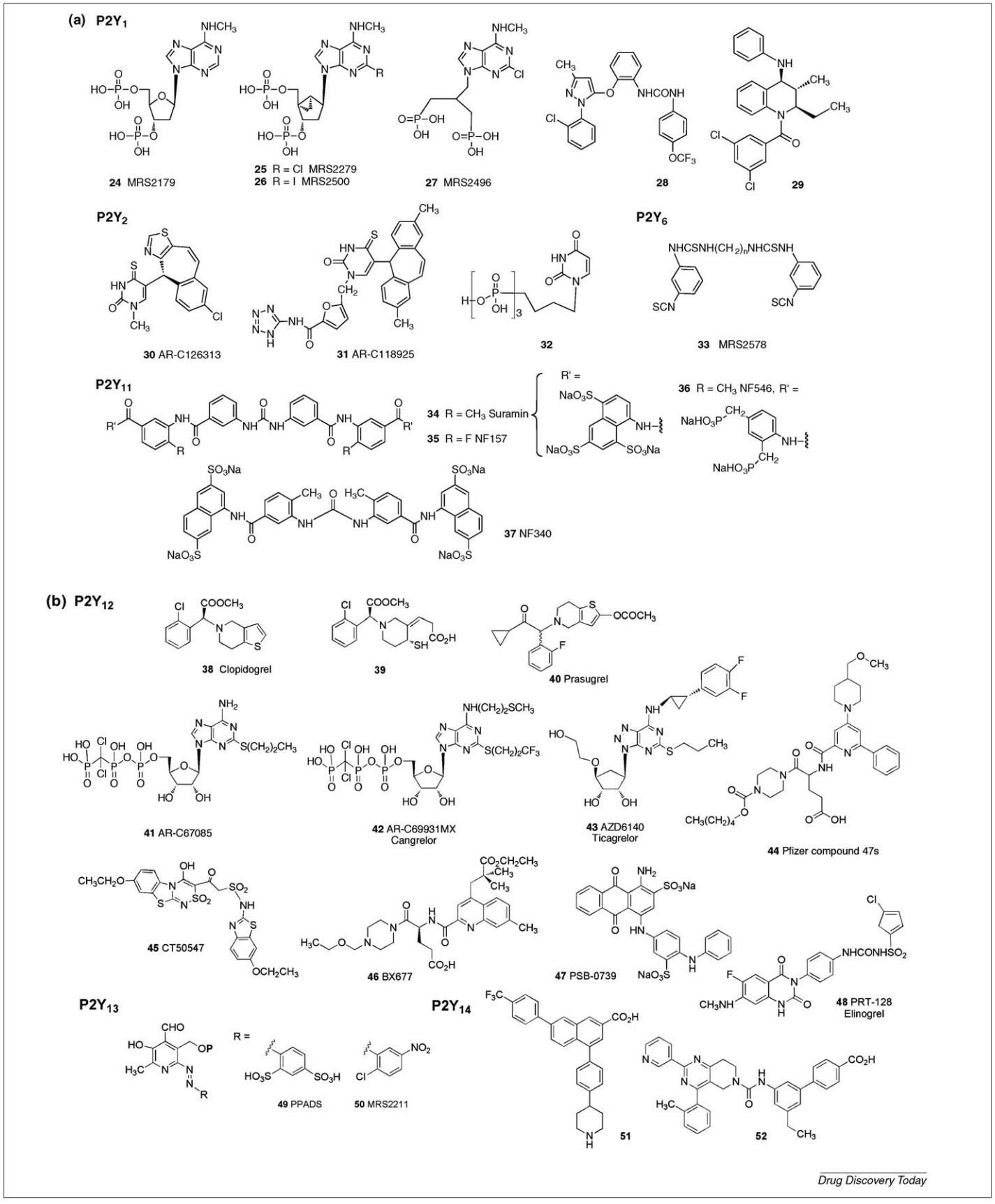

FIGURE 2

Nucleotides and non-nucleotides that have been useful antagonists in the study of P2Y receptors. **(a)** Antagonists of the Gq-coupled $P2Y_1$-like subfamily. **(b)** Antagonists of the Gi-coupled $P2Y_{12}$-like subfamily.

The favored ribose-ring conformation for each of the subtypes of the $P2Y_1$-like family has been established using conformationally restricted (i.e. rigid) ribose equivalents. The later generation of synthetic bisphosphate antagonists incorporates a rigid substitute for the normally flexible ribose ring. MRS2279 **25** (pK_B 8.10) and MRS2500 **26** are generally useful as selective, high-affinity antagonists of the $P2Y_1$ receptor in various species [30,31]. The presence of the (N)-methanocarba ring in these nucleotide analogs both enhances receptor affinity and improves stability toward nucleotidases. Antagonists of the $P2Y_1$ receptor of moderate affinity might also be derived from acyclic nucleotides (bisphosphates and bisphosphonates), such as MRS2496 **27** [32]. When the cyclic ribose-like ring is intact, either agonism or antagonism might result, whereas in the acyclic series, only antagonism has been observed.

The same conformational constraint of the ribose moiety that enhances antagonist action favors the potency and selectivity in nucleotide agonists. Evidently, the two series bind to the same site on the receptor in a similar mode [32]. The (N)-methanocarba analog of 2-MeSADP, MRS2365 **10**, is a selective, high-affinity agonist of the $P2Y_1$ receptor [16]. Another means of improving hydrolytic stability is the introduction of a borano group in the phosphate moiety of P2Y receptor agonists; compound **11** was equipotent to 2-MeSADP at the $P2Y_1$ receptor [33].

The screening of structurally diverse chemical libraries by the pharmaceutical industry has led to non-nucleotide antagonists of the $P2Y_1$ receptor [20,34,35]. Compound **28** is a selective antagonist that displays a K_i value of 90 nM at the human $P2Y_1$ receptor and oral bioavailability in rats with a $t_{1/2}$ of 2.8 h. A tetrahydro-4-quinolinamine derivative **29** inhibited the $P2Y_1$ receptor effects and platelet aggregation (pIC_{50} 6.30) [35].

At the $P2Y_2$ and $P2Y_4$ receptors, both of which are activated by UTP, there is a need for more definitive agonists and antagonists. UTP-γ-S **12** (Fig. 1) has been used as a more stable activator of these P2Y subtypes than UTP; however, this compound suffers from chemical instability. Dinucleoside tetraphosphates have the preferred phosphate chain length for activation of the $P2Y_2$ and $P2Y_4$ subtypes. Several INS compounds have been introduced to clinical trials as $P2Y_2$ receptor agonists, such as Up_4U **14** (INS365, Diquafosol, pEC_{50} 7.00) and Up_4dC **15** (INS37217, Denufosol, pEC_{50} 6.66), but these agonists are nonselective compared to the $P2Y_4$ receptor [14]. By virtue of being dinucleotides, they are more stable to enzymatic hydrolysis than nucleoside triphosphates. A selective $P2Y_2$ agonist, the 5′-triphosphate derivative MRS2698 **16**, is 300-fold selective in comparison to the $P2Y_4$ receptor [17]. Alternative nucleobases have been shown to be acceptable in $P2Y_2$ receptor agonists, such as the benzothiazole derivative **17**, which is twice as potent as UTP at the $P2Y_2$ receptor [36]. Definitive antagonists of the $P2Y_2$ receptor are not available. AR-C126313 **30** and its higher molecular-weight analog AR-C118925 **31** [21] were reported to selectively antagonize the $P2Y_2$ receptor; however, it seems that these compounds are only micromolar in affinity (Fig. 2). The uracil phosphonate derivative **32** antagonized the $P2Y_2$ receptor with a pIC_{50} of 4.04 [37]. The large polyanionic molecules Reactive Blue 2 (structure not shown) and suramin **34** are often used as slightly selective antagonists of the $P2Y_2$ and $P2Y_4$ receptors, respectively. Truly selective agonists and antagonists for the $P2Y_4$ receptor are needed to distinguish this subtype pharmacologically from the $P2Y_2$ receptor, which is also activated by UTP. The agonist 2′-azido-2′-deoxy-UTP displayed slight $P2Y_4$ selectivity [38].

UDP activates both the $P2Y_6$ and $P2Y_{14}$ receptors [91]; however, it is to be cautioned that the addition of UDP to tissue can generate UTP through the action of nucleoside diphosphokinase [40]. Thus, artifactual results might be obtained using UDP alone in pharmacological studies if multiple P2Y subtypes are present. UDP-β-S **18** is a more stable activator of these P2Y subtypes than UDP. The SAR of nucleotide derivatives in activating the $P2Y_6$ receptor has been explored [41,42]. Other UDP derivatives (e.g. **20** and **21**) are selective $P2Y_6$ agonists. Molecular modeling predicted that the South (S) conformation of the ribose ring is the $P2Y_6$-preferred conformation, which was then confirmed by synthesis of a rigid methanocarba analog of UDP **20** [43,44]. Dinucleotides have been explored as $P2Y_6$ receptor ligands with diuridine triphosphates such as INS48823 **19** (pEC_{50} 6.90) having the preferred phosphate chain length [18]. The only $P2Y_6$ receptor antagonist class reported includes MRS2578 **33** and its related chemically reactive diisothiocyanate derivatives, which have a spectrum of P2Y receptor interactions and suffer from aqueous instability and hydrophobicity [22].

Potent agonists at the $P2Y_{11}$ receptor are ATP-γ-S **13** (Fig. 1, pEC_{50} 4.87) and the $P2Y_{12}$ antagonist 2-propylthio-β,γ-dichloromethylene-ATP **41** (AR-C67085, Fig. 2, pEC_{50} 5.05) [45]. Thus, **41** must be used with caution in pharmacological studies in which both $P2Y_{11}$ and $P2Y_{12}$ subtypes might be present because of pharmacological ambiguity of an agonist and antagonist, respectively. A potent $P2Y_{11}$ receptor antagonist NF157 **35** (pK_i value of 7.35) derived from nonselective P2 antagonist suramin **34** [46] also antagonizes the $P2X_1$, $P2X_2$, and $P2X_3$ receptors. Related derivatives **36** and **37** were found to activate and antagonize the $P2Y_{11}$ receptor [47].

The medicinal chemistry of the $P2Y_{12}$ receptor has been extensively explored. The thienopyridines, such as clopidogrel **38** (Fig. 2), were serendipitously identified as inhibitors of platelet aggregation by ADP 20 years before the cloning and identification of their target, the $P2Y_{12}$ receptor. They act as liver-activated prodrugs, the active metabolites of which are irreversible inhibitors of the $P2Y_{12}$ receptor [48]. Thiol **39** is reported to be the active metabolite of clopidogrel [49]. It binds covalently to cysteine 97 in the first extracellular loop of $P2Y_{12}$ receptor. This results in the breakdown of $P2Y_{12}$ oligomers into monomers or dimers and their partitioning out of lipid rafts [50].

A drug development program by AstraZeneca to design $P2Y_{12}$ receptor antagonists has introduced numerous directly acting $P2Y_{12}$ receptor antagonists. The observation that ATP acts as an antagonist at this ADP-activated subtype has enabled the introduction of various 5′-triphosphate analogs as selective receptors probes, and one of them, AR-C69931MX **42** (Cangrelor), has been tested clinically as an antithrombotic agent. Curiously, the requirement of having a 5′-triphosphate group in adenine nucleotides that serve as $P2Y_{12}$ receptor antagonists can be circumvented. One of the products of this effort is ticagrelor **43** (AZD6140), an uncharged nucleoside derivative with a high affinity at the $P2Y_{12}$ receptor, which is currently in clinical trials [39,51,52].

The search for selective non-nucleotide antagonists of the $P2Y_{12}$ receptor, for potential use as antithrombotic agents, is continuing.

That the phosphate groups might be eliminated entirely, at least for the $P2Y_{12}$ receptor, has fueled this effort. Library screening has aided in this effort, leading to other molecules with promise as competitive $P2Y_{12}$ receptor antagonists, such as piperazinyl glutamate pyridine derivative **44** (pEC_{50} 7.82) [53], tricyclic benzothiazolo[2,3-c]thiadiazine derivative CT50547 **45** (pEC_{50} 6.74) [54] and BX677 **46** [55]. The highly potent $P2Y_{12}$ receptor antagonist PSB-0739 **47** is an analog of the known antagonist Reactive Blue 2 [8].

The agonist potency at the $P2Y_{13}$ receptor is ADP > 2-MeSADP > ATP. A selective $P2Y_{13}$ receptor antagonist MRS2211 **50** is a derivative of PPADS **49**, a nonselective P2Y and P2X receptor antagonist derived from pyridoxal phosphate [26]. MRS2211 has the disadvantage of containing a phosphate ester group and an aryl diazo linkage, both of which are subject to instability in tissue systems.

Recently, the SAR of analogs of the native ligand UDP-glucose **5** at the $P2Y_{14}$ receptor was systematically explored [19]. The $P2Y_{14}$ receptor seems to be the most structurally restrictive member of the P2Y family, at least with respect to modification of the nucleobase, ribose and phosphate moieties of agonist ligands. The glucose moiety of UDPG, however, can be modified. Other naturally occurring UDP sugars activate this receptor less potently, such as UDP-galactose (EC_{50} 0.67 μM) and UDP-*N*-acetylglucosamine (EC_{50} 4.38 μM). The 2-thio analog MRS2690 **22** (Fig. 1) is a sixfold more potent agonist for $P2Y_{14}$ receptor and, unlike UDPG, is inactive at the $P2Y_2$ receptor. Recently, α,β-difluoromethylene-UDP **23** (MRS2802), which is inactive at the $P2Y_6$ receptor, was found to fully activate the human $P2Y_{14}$ receptor [44]. The glucose moiety, therefore, is amenable to modification and is not required for activation of the $P2Y_{14}$ receptor. Non-nucleotide antagonists of the $P2Y_{14}$ receptors **51** and **52** have been reported with pIC_{50} values of 8.66 and 8.40, respectively [27].

In conclusion, most of the ligands of P2Y receptors identified so far are polyanionic molecules that do not readily cross the cell membrane. This raises a major problem of bioavailability; however, this lack of systemic action can be an asset in case of topical applications, such as spray or eye drops. The hydrolysis of nucleotide compounds by ectonucleotidases constitutes another limiting factor partially overcome by the development of more stable dinucleotide compounds. Orally active uncharged antagonists (e.g. thienopyridines and ticagrelor) are available only for the $P2Y_{12}$ receptor.

Current and potential therapeutic application of P2Y agonists and antagonists

The most highly developed therapeutic application of P2Y receptor ligands is that of $P2Y_{12}$ antagonists for thrombosis [56]. Indeed, the only P2Y ligands currently in pharmaceutical use are the thienopyridine compounds: ticlopidine (Ticlid) and clopidogrel (Plavix) have been on the market for years. Plavix is a blockbuster with sales of roughly $8 billion in 2008. Prasugrel (Effient) was recently approved by the FDA and the EMA for the prevention of clots in patients undergoing percutaneous coronary intervention. It has the advantage that its transformation into an active metabolite is more rapid and less variable than that of clopidogrel [57,58]. Other $P2Y_{12}$ antagonists acting in a reversible way are under development. Ticagrelor (Brilinta), an orally active reversible antagonist of $P2Y_{12}$, was compared to clopidogrel in the PLATO study and showed a greater efficacy in reducing cardiovascular death [52]: AstraZeneca submitted a New Drug Application to the FDA in November 2009. Cangrelor is a reversible $P2Y_{12}$ antagonist that must be administered by i.v. perfusion because it is a charged molecule with a very short half-life. Two recent phase III studies in percutaneous coronary intervention failed to demonstrate a benefit, and this program was discontinued [49,59]. A phase II study in coronary artery bypass graft surgery has been initiated. Elinogrel **48** (PRT128), a tricyclic benzothiazolo[2,3-c]thiadiazine, is another reversible $P2Y_{12}$ antagonist, which can be used orally or intravenously [60]. It is currently in phase II, and as part of an agreement with Portola Pharmaceuticals, Novartis will be responsible for its phase III development.

$P2Y_2$ receptor agonists are in phase III clinical trials for the treatment of cystic fibrosis and dry eye disease. This strategy developed by Inspire Pharmaceuticals is based on the observation that activation of $P2Y_2$ receptors on epithelial cells, in the airways and the eye (conjunctiva and cornea), stimulates the secretion of chloride via outwardly rectifying chloride channels, leading to mucus hydration and surface lubrication [61,62]. This action is independent from cystic fibrosis transmembrane regulator and can thus bypass the cystic fibrosis transmembrane regulator defect in cystic fibrosis. Furthermore, $P2Y_2$ activation inhibits sodium absorption and stimulates the other components of the mucociliary escalator – ciliary beating and mucus secretion [61]. A New Drug Application for diquafosol (Prolacria) eye drops in the treatment of dry eye disease has been submitted to the FDA, which requested a new phase III trial, which was initiated in 2009. In this placebo-controlled trial including 450 patients, the primary efficacy endpoint was the clearing of the fluorescein staining of the central region of the cornea after a six-week treatment. Inspire recently announced on its website that this endpoint was not met. However diquafosol was recently approved in Japan for the same indication. A first phase III trial of denufosol in cystic fibrosis (TIGER-1) has demonstrated a statistically significant increase in forced expiratory volume (1 s) in patients inhaling denufosol for 24 weeks. A second phase III trial (TIGER-2), comparing denufosol to placebo in 450 patients over 48 weeks, was initiated recently.

Other P2Y subtypes might become therapeutic targets, as suggested *inter alia* by the phenotype of knockout mice, which are now available for all P2Y subtypes (except $P2Y_{11}$, which is not present in the murine genome). Some findings support further developments in the two current areas of drug development related to P2Y receptors – platelet inhibition and topical stimulation of fluid secretion – but others suggest potential applications in entirely different therapeutic areas.

Platelet aggregation by ADP actually requires cooperation between two P2Y receptors: $P2Y_{12}$ and $P2Y_1$ [63]. $P2Y_1$ is involved in the initial platelet shape change and transient aggregation, whereas $P2Y_{12}$ is responsible for sustained aggregation and potentiation of secretion. $P2Y_1{}^{-/-}$ mice show defective platelet aggregation *ex vivo*, increased bleeding time and resistance to thrombosis [64,65]. Therefore, $P2Y_1$ antagonists might constitute a new class of antithrombotic agents [66].

The stimulatory effect of nucleotides on chloride and water secretion by epithelial cells is not restricted to the airways or the

eye. It also occurs in the gut, where it involves the $P2Y_4$ receptor. Indeed, both in jejunum and in colon, the UTP-induced Cl^- current was abolished in $P2Y_4$-deficient mice [67,68]. $P2Y_4$ agonists might thus be used to treat chronic constipation in a similar way to lubiprostone (Amitiza), which activates the CIC-2 chloride channel on the apical membrane of intestinal epithelial cells and thereby enhances intestinal fluid secretion and accelerates gastrointestinal transit [69].

Other studies suggest additional potentials of P2Y receptors as therapeutic targets, especially in cardiovascular diseases, inflammatory diseases (such as asthma) and neurodegeneration.

Multiple P2Y receptors might play a part in the development of atherosclerotic lesions, independently of their role in platelet activation. Aortic lesions were smaller in double ApoE/$P2Y_1$ knockout mice than in $ApoE^{-/-}$ mice [70]. This difference was unrelated to the role of $P2Y_1$ in platelet activation because it was unaffected by bone marrow transplantation from $P2Y_1$ wild-type mice, indicating the role of $P2Y_1$ in non-hematopoietic-derived cells, most probably endothelial cells. The $P2Y_6$ receptor might also be a target because it is functionally expressed in the three cell types that have a major role in the development of atherosclerotic lesions (i.e. endothelial cells, smooth muscle cells and macrophages) [71] and $P2Y_6$ mRNA is elevated in atherosclerotic plaques [72]. Acting on the $P2Y_6$ receptor, UDP might induce the expression of vascular cell adhesion molecule-1 (VCAM-1) on arterial endothelial cells (a key step in the infiltration of circulating monocytes), stimulate the growth of smooth muscle cells and amplify the release of cytokines by macrophages. Finally, the $P2Y_{13}$ receptor plays a part in reverse cholesterol transport, at the level of hepatocytes. It has, indeed, been shown that high-density lipoprotein Apo A-I activates an ecto-ATPase that generates ADP from ATP on the surface of hepatocytes [73]. ADP then stimulates the endocytosis of high-density lipoprotein particles via the activation of $P2Y_{13}$ receptors, as demonstrated by the use of siRNA [74].

Multiple P2Y receptors are expressed in the heart. The $P2Y_2$ and $P2Y_6$ receptors are expressed on cardiomyocytes [75], whereas the $P2Y_4$ receptor is present on microvascular endothelial cells (Horckmans *et al.*, unpublished). Nucleotides are released from cardiomyocytes in response to mechanical stretch [76] or ischemia [75]. Pharmacological experiments suggest that the $P2Y_2$ receptor might have a role in protection of cardiomyocytes against ischemia [77], and the use of siRNA revealed that the $P2Y_6$ receptor has a role in cardiac fibrosis resulting from pressure overload [76].

P2Y receptors are involved at various steps in the inflammatory process. ATP released from neutrophils amplifies their attraction by chemotactic signals [78], and its release from apoptotic cells constitutes a 'find-me signal' for monocytes and macrophages [2]. These actions are abrogated in leukocytes from $P2Y_2^{-/-}$ mice. Nucleotides upregulate the expression on endothelial cells of VCAM-1, which has a crucial role in the tissue infiltration of eosinophils and monocytes. This action is $P2Y_2$-receptor-mediated in coronary arteries [79], but $P2Y_4$ and $P2Y_6$ receptors might also be involved in other vascular beds. Nucleotides also stimulate the release of various cytokines and chemokines. For instance, UTP stimulates the release of CCL20 from human nasal epithelial cells [80], and UDP amplifies the release of IL-8 from human monocytes via the autocrine activation of the $P2Y_6$ receptor [81]. P2Y receptors are also involved in adaptive immunity. In particular, ATP induces via the $P2Y_{11}$ receptor the semi-maturation of human monocyte-derived dendritic cells, characterized by an upregulation of co-stimulatory molecules and the inhibition of IL-12 secretion, resulting in an enhanced ability to induce Th2 differentiation of T lymphocytes [82,83]. These various mechanisms of action might play a part in asthma and, indeed, allergen challenge causes an acute accumulation of ATP in the airways of asthmatic patients and mice with experimental asthma [1]. Neutralizing this increase in ATP by the ATP-hydrolyzing enzyme apyrase reduced airway inflammation in sensitized mice. Furthermore, ATP derived from commensal bacteria stimulates the differentiation of Th17 cells in the intestinal lamina propria [84], probably via the $P2Y_{11}$ receptor and an increase of cAMP in dendritic cells [85]. These data suggest that antagonists of $P2Y_2$, $P2Y_6$ and/or $P2Y_{11}$ receptor might be beneficial in asthma and inflammatory bowel disease.

Microglia from $P2Y_{12}^{-/-}$ mice are unable to polarize, migrate or extend processes towards ADP, and *in vivo* they showed decreased directional branch extension towards sites of laser-induced cortical damage [86]. Independent of this chemotactic action of ADP, UDP stimulates the uptake of microspheres by rat microglia, and this action was blocked by an antisense oligonucleotide targeting the $P2Y_6$ receptor [87]. These complementary actions of ADP, a find-me signal, and UDP, an eat-me signal, involving a cooperation between $P2Y_{12}$ and $P2Y_6$, might be beneficial in neurodegenerative conditions such as Alzheimer's disease, via an increased clearance of amyloid-β deposits. Microglia can be a double-edged sword, however, and they play a key part in neuropathic pain resulting from nerve injury. Tactile allodynia after nerve injury, a hallmark of neuropathic pain, was decreased in $P2Y_{12}^{-/-}$ mice [88,89]. These data suggest that a $P2Y_{12}$ antagonist might be effective in neuropathic pain, and a $P2Y_6$ agonist could be beneficial in Alzheimer's disease.

Concluding remarks

Probing the SAR of the P2Y receptor family by medicinal chemical approaches is an ongoing process. Selective agonists for $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors have been reported, but there are not yet any selective agonists for the $P2Y_4$, $P2Y_{11}$ and $P2Y_{13}$ receptors. Among the methods used recently to achieve selectivity of nucleotides for P2Y receptor subtypes is the conformational locking of the ribose moiety. Selective antagonists for $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$ receptors are also known, but antagonists that are truly selective for the $P2Y_4$ and $P2Y_{14}$ receptors are still needed. For antagonist development, screening of chemically diverse compound libraries has begun to yield new lead compounds for the development of $P2Y_1$ receptor antagonists and directly acting $P2Y_{12}$ receptor antagonists, both of which are sought for antithrombotic activity. The discovery of new, selective P2Y receptor agonists and antagonists holds promise of providing new opportunities for therapeutics.

Acknowledgements

We thank the Intramural Research Program of the NIH, National Institute of Diabetes and Digestive and Kidney Diseases, as well as

the Fonds de la Recherche Scientifique Médicale (FRSM), the Communauté Française de Belgique (Action de Recherche Concertée) and the Walloon Region (Programme d'Excellence CIBLES).

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