Purinergic Signalling (2009) 5:75–89
DOI 10.1007/s11302-008-9106-2

REVIEW

Development of selective agonists and antagonists of P2Y receptors

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Received: 19 March 2008 / Accepted: 10 April 2008 / Published online: 4 July 2008

Abstract Although elucidation of the medicinal chemistry of agonists and antagonists of the P2Y receptors has lagged behind that of many other members of group A G protein-coupled receptors, detailed qualitative and quantitative structure–activity relationships (SARs) were recently constructed for several of the subtypes. Agonists selective for $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors and nucleotide antagonists selective for $P2Y_1$ and $P2Y_{12}$ receptors are now known. Selective nonnucleotide antagonists were reported for $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors. At the $P2Y_1$ and $P2Y_{12}$ receptors, nucleotide agonists (5′-diphosphate derivatives) were converted into antagonists of nanomolar affinity by altering the phosphate moieties, with a focus particularly on the ribose conformation and substitution pattern. Nucleotide analogues with conformationally constrained ribose-like rings were introduced as selective receptor probes for $P2Y_1$ and $P2Y_6$ receptors. Screening chemically diverse compound libraries has begun to yield new lead compounds for the development of P2Y receptor antagonists, such as competitive $P2Y_{12}$ receptor antagonists with antithrombotic activity. Selective agonists for the $P2Y_4$, $P2Y_{11}$, and $P2Y_{13}$ receptors and selective antagonists for $P2Y_4$ and $P2Y_{14}$ receptors have not yet been identified. The $P2Y_{14}$ receptor appears to be the most restrictive of the class with respect to modification of the nucleobase, ribose, and phosphate moieties. The continuing process of ligand design for the P2Y receptors will aid in the identification of new clinical targets.

Keywords Nucleotide · Purine · Pyrimidine · G protein-coupled receptor · Structure activity relationship

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Introduction

The P2Y receptors are 7TM (containing seven transmembrane domains) receptors that couple to G protein-dependent and -independent signaling pathways, including ion channels [1]. Eight human subtypes of the P2Y receptor family have been defined. According to a dendrogram relating sequence homology, the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors form a cluster of preferentially G_q-coupled receptors, and the $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors form a cluster of preferentially G_i-coupled receptors [2, 3].

Elucidation of the medicinal chemistry of agonists and antagonists of the P2Y receptors has lagged behind characterization of many other members of the group A G protein-coupled receptors (GPCRs) [4–8]. Many of the early ligands suffered from promiscuity, affecting not only multiple P2Y metabotropic receptors but also P2X nucleotide-gated ion channels, various enzymes involved in controlling levels of extracellular nucleotides and nucleosides, and intracellular signaling pathways. However, the armamentarium of P2Y receptor ligands available has recently expanded greatly in both quantity and quality. Qualitative and quantitative structure–activity relationships (SARs) were recently constructed in detail for nucleotides acting at $P2Y_1$ and $P2Y_{12}$ receptors and with less detail for the $P2Y_2$ and $P2Y_6$ receptors. Molecular modeling of these receptors and docked ligands with a rhodopsin-based

template has aided in ligand design [2, 3]. Studies of radioligand binding have been successful at only a few of the P2Y receptors. In this review, we identify the most selective molecular probes reported for the P2Y receptors, which have enabled pharmacological studies in the nucleotide field. The availability of pharmacological tools for the various subtypes (along with genetically modified mouse strains) is also enabling biological studies.

The five major native ligands of the P2Y receptors (Fig. 1) are adenosine 5′-triphosphate (ATP) **1**, adenosine 5′-diphosphate (ADP) **2**, uridine 5′-triphosphate (UTP) **3**, uridine 5′-diphosphate (UDP) **4**, and UDP-glucose **5** (or other UDP sugars). Dinucleotides such as Ap_4U **6** are also naturally occurring P2Y agonists [9]. Adenosine monophosphate (AMP) and uridine monophosphate (UMP) are inactive at P2Y receptors. Figure 2 shows the correspondence of these nucleotides and the subtypes that they activate or antagonize, along with the diversity of representative biological effects induced by P2Y receptor activation. Some single nucleotides activate multiple subtypes (e.g., ATP and UTP) or activate one subtype and antagonize another (e.g., ATP and UDP). UDP acts as a competitive antagonist at the human but not rat $P2Y_{14}$ receptor [10].

Pharmacological studies of P2Y receptors are complicated by the presence of ectonucleotidases that degrade the native agonist and antagonist nucleotides. Products of the enzymatic hydrolysis might either be inactive or have activity at other P2Y receptor subtypes (e.g., 5′-triphosphate derivatives are converted to diphosphates) or at adenosine receptors (when adenosine is produced by the action of CD73/5′-nucleotidase on AMP [11]). Alternately, the 5′-diphosphates might be phosphorylated to biologically active 5′-triphosphates by the action of nucleoside diphosphokinase [12]. Thus, the state of activation of multiple P2Y receptors in a given tissue is a complex function of the kinetics of release, interconversion, and inactivation of the family of receptor-interactive nucleotides.

Various phosphate modifications, such as a terminal thiophosphate substitution, increase stability toward nucleotidases. Thus, ATPγS **7**, ADPβS **8**, UTPγS **9**, and UDPβS **10** have been used to activate P2Y receptors, as noted in Table 1. The terminal thiophosphates are generally well tolerated in the nucleotide-binding sites of the P2Y receptors. However, these compounds are susceptible to oxidation and are difficult to synthesize on a large scale. Thiophosphate substitutions at nonterminal phosphate groups are better tolerated at the $P2Y_1$ than at the $P2Y_2$ and $P2Y_4$ subtypes [15, 16]. Another type of modification that increases the stability of such nucleotide derivatives is replacement of a bridging oxygen of the phosphate chain with methylene or dihalomethylene; however, this modification tends to reduce potency at the P2Y receptors [17].

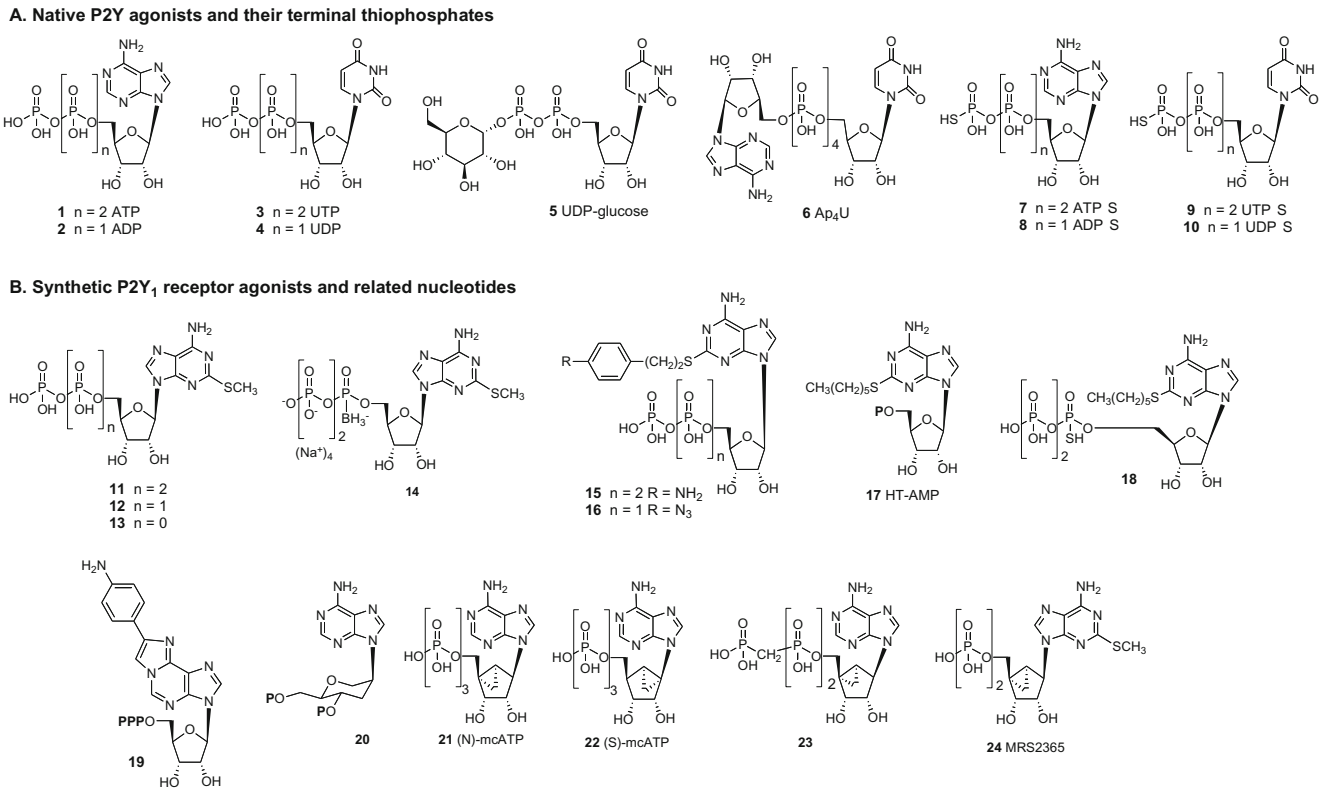

Fig. 1 **a** Naturally occurring P2Y receptor agonists and terminally thiophosphate-substituted analogues. **b** Structures of adenine-derived nucleotide agonists of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors

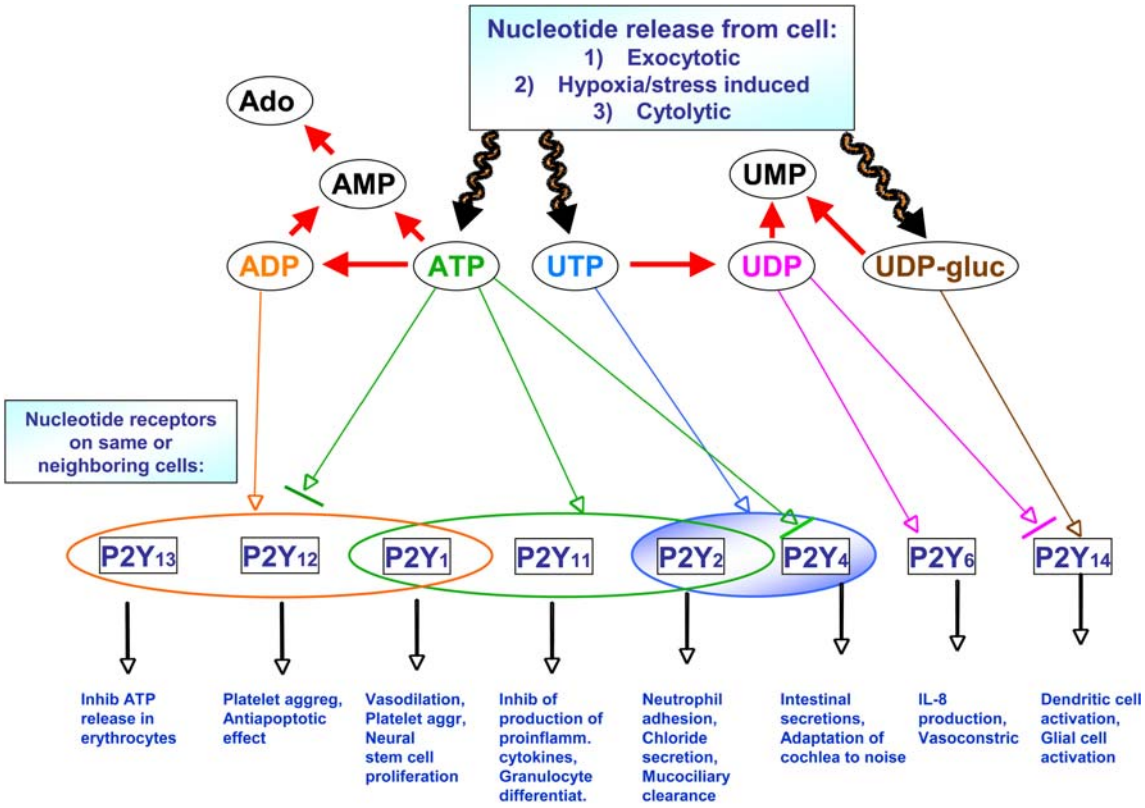

Fig. 2 Correspondence of the five principal native ligands of the human P2Y receptors and the subtypes they activate. Note that various dinucleotides also activate P2Y receptors. For example, dinucleoside triphosphates activate the $P2Y_6$ receptor

The P2Y receptor agonists are nearly exclusively nucleotide derivatives, which presents barriers to drug development because of their instability, low bioavailability, nonspecific binding to biological membranes, and tedious synthesis, purification, and structural verification. Screening of small-molecule ligands of diverse structure has not yet been carried out extensively for the P2Y family. Radioligand binding is effective for ligand screening in many other GPCRs, but biological assays at the P2Y receptors usually exploit functional endpoints, e.g., typically G_q-stimulated phospholipase C-β for the $P2Y_1$-like subfamily (Table 1). Suitable radioligand-binding methods are only available for the $P2Y_1$ and $P2Y_{12}$ receptors [18, 19]; those methods were developed after many attempts to use radiolabeled nucleotides were reported in the literature and later proved unsatisfactory.

Adenine nucleotide-responsive P2Y receptors

SAR of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors for 5′-diphosphates

The most prominent regions of distribution of these ADP-responsive receptors are $P2Y_1$ (platelets, endothelial cells, brain), $P2Y_{12}$ (platelets, brain), and $P2Y_{13}$ (immune system, dendritic cells). There is no striking sequence homology between the $P2Y_1$ receptor and $P2Y_{12}$ or $P2Y_{13}$ receptors. The sequence identity of the TM domains of the human $P2Y_1$ is 26.9% and 28.0% for the $P2Y_{12}$ and $P2Y_{13}$ receptors, respectively. In contrast, the sequence identity within the TM domains of the $P2Y_{12}$ and $P2Y_{13}$ receptors is 57.0%.

Modification of the phosphate moiety ADP **2** is the principal endogenous agonist at the $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors. ATP **1** interacts with less affinity and efficacy than ADP at the $P2Y_1$ and $P2Y_{12}$ receptors. At $P2Y_{12}$ receptors, the loss of efficacy is pronounced, such that ATP and other 5′-triphosphate derivatives act as antagonists. At $P2Y_{13}$ receptors, ADP and ATP both act as full agonists.

Modifications of the di- and triphosphate moieties of the nucleotide ligands have been probed for effects on P2Y receptor activity (Fig. 1). For example, when an ionizable oxygen of the α-phosphate of the triphosphate moiety of adenine nucleotide derivatives is substituted with a BH_2 moiety, it favors $P2Y_1$ receptor potency [20]. Thus, the $P2Y_1$ receptor can be activated by a 5′-(1-boranotriphosphate) derivative **14** of 2-methylthio-ATP **11**. Separation of two stable isomers of **14** demonstrated stereoselectivity in activation of the rat $P2Y_1$ receptor (EC_{50}=2.6 nM, for the more potent R-isomer of **14**).

Table 1 Potencies and selectivities of natural and synthetic ligands at the human P2Y receptors (references in brackets)

Subfamily and Pharmacologic Characteristics	Receptor Subtype	Ligands (potency and selectivity)							
		Native agonist	pEC_{50} (ref)	Synthetic agonist	pEC_{50} (ref)	Cross reactivity	Synthetic (or native) antagonist	pEC_{50} (ref)	Cross reactivity
$P2Y_1$-like, Gq-coupled	$P2Y_1$	ADP **2**	5.09 [6]	MeSADP **12**	8.22 [6]	$P2Y_{12,13}$	A3P5P **25**	6.08 [35]	none
				ADP-β-S[a] **8**	7.02 [6]	$P2Y_{12,13}$	MRS2179 **26**	6.48 [36]	none
				MRS2365 **24**	9.40 [34]	none	MRS2279 **29**	7.28 [6]	none
							MRS2500 **30**	9.02 [39]	none
	$P2Y_2$	UTP **3**	8.10 [4]	UTP-γ-S[a] **9**	6.62 [4]	none	Suramin **48a**	4.32 [6]	$P2Y_{11}$
		ATP **1**	7.07 [4]	NS365 **68c**	7.00 [4]	$P2Y_4$	AR-C126313 **52**	6 [4,74]	
				INS37217 **69**	6.66 [4]	$P2Y_4$	MRS2576[b] **57**	4.04 [81]	$P2Y_{1,4,6}$
				MRS2698**63b**	8.10 [64]	none			
	$P2Y_4$	UTP **4**	5.60 [6]	2'-azido-dUTP **64**	7.14 [12]	$P2Y_4$	PPADS **49**	< 5.00 [6]	$P2Y_2$
							ATP **1**	4.37 [6]	various (ago), $P2Y_{12}$
							MRS2577[b] **56**	4.01 [81]	$P2Y_6$
	$P2Y_6$	UDP **4**	6.52 [6]	UDP-β-S **10**	7.33 [4]	none	MRS2578[b] **55**	7.43 [4]	none
				INS48823 **71**	6.90 [4]	none			
				MRS2633 **67**	6.64 [68]	none			
				MRS2693 **66**	7.83 [68]	None			
	$P2Y_{11}$	ATP **1**	4.77 [6]	AR-C67085 **35**	5.05 [50]	$P2Y_{12,13}$ (ant)	Suramin **48a**	4.79 [6]	$P2Y_2$
				ATP-γ-S[a] **7**	5.52 [6]	$P2Y_{1,2,12}$	AMP-α-S[a] **18**	partial agonist [50]	none
$P2Y_1$-like, Gi-coupled	$P2Y_{12}$	ADP **2**	7.22 [1]	MeSADP **12**	7.85 [1]	$P2Y_{1,13}$	MeSAMP **13**	4.00 [82]	none
				ADP-β-S[a] **8**	6.72 [1]	$P2Y_{1,13}$	ATP **1**	3.60 [40]	various (ago), $P2Y_4$
							AR-C67085 **36**	4.52 [45]	$P2Y_{11}$ (ago), $P2Y_{13}$
							AR-C69931MX **37**	9.40 [40]	$P2Y_{11}$ (ago), $P2Y_{13}$
							AZD6140 **38**	7.90 [45]	none
							INS50589 **40**	7.80 [41]	none
							Clopidogrel[c] **41**	5.74 [42]	none
	$P2Y_{13}$	ADP **2**	7.94 [84]	MeSADP **12**	7.85 [84]	$P2Y_{1,12}$	MRS2211 **50**	5.97 [83]	none
							AR-C67085 **36**	6.67 [50]	$P2Y_{11}$ (ago), $P2Y_{12}$
							AR-C69931MX **37**	8.40 [83]	$P2Y_{11}$ (ago), $P2Y_{13}$
	$P2Y_{14}$	UDP-glucose **75**	6.45 [71]	MRS2690 **78**	7.31 [71]	none	UDP **4**	7.28 [10]	$P2Y_6$ (ago)
		UDP-galactose **76**	6.17 [71]						
		UDP-glucosamine **77**	5.36 [71]						

a, unstable to oxidation.
b, an insurmountable antagonist, which is hydrophobic and reactive toward nucleophiles and aqueous medium.
c, active only *in vivo,* through a thiol-reactive metabolite.
ago, agonist; ant, antagonist

Modification of the adenine moiety The SAR around the adenine moiety of the nucleotides has been extensively explored at the $P2Y_1$ and $P2Y_{12}$ receptors. Broad freedom of substitution has been observed at the C2 position, and sterically bulky groups and extended chains at this position are often tolerated in receptor binding.

A small hydrophobic pocket in the receptor-binding site surrounds the N^6-position of adenine nucleotides acting as $P2Y_1$ agonists and antagonists [21], and the $P2Y_{12}$ receptor has a considerably larger pocket [22]. Amino, ether, and thioether derivatives at the 2-position of the adenine ring of ATP were compared in $P2Y_1$ potency; thioethers, in general, are the most potent [23].

2-Alkylthio and 2-(arylalkylthio) ethers of ADP are highly potent agonists at these subtypes (Fig. 1b) [24, 25]. 2-Methylthio-ADP **12** is a potent agonist (EC_{50} in nM) at human $P2Y_1$ (3), rat $P2Y_{12}$ (1), and human $P2Y_{13}$ (1) receptors [2, 7, 26]. 2-Methylthio (2-MeS)-ATP **11** is less potent (EC_{50}=8 nM) than **12** at the $P2Y_1$ receptor and also less selective because certain P2X receptors are activated [26]. A 2-[2-(4-aminophenylethyl)] 5′-triphosphate derivative **15** exhibits an EC_{50} of 1 nM at the $P2Y_1$ receptor, and a related azido 5′-diphosphate derivative **16** serves as a photoaffinity label of P2Y receptors in platelets [27]. 2-Alkynyl substituents on adenine nucleotide analogues provide high potency as either $P2Y_{1/12}$ receptor agonists or antagonists [28].

Although AMP is inactive at the $P2Y_1$ receptor, adding a 2-thioether substituent as a receptor "anchor" allows adenosine 5′-monophosphate analogues to bind to and activate the $P2Y_1$ receptor. Among these derivatives, 2-(hexylthio) (HT)-AMP **17** is especially potent, with an EC_{50} of 59 nM at the turkey $P2Y_1$ receptor [29]. 2-MeS-AMP **13** weakly activates the $P2Y_1$ receptor but also acts as a weak antagonist of the $P2Y_{12}$ receptor [30]. α-Thio ATP derivatives, such as compound **18**, are potent $P2Y_1$ receptor agonists (EC_{50}= 17 nM). The corresponding α-thio AMP derivatives are weak as P2Y receptor agonists, but the simultaneous inhibition of ATP diphosphohydrolase [ecto-apyrase nucleoside triphosphate diphosphohydrolase-1 (NTPDase1) or CD39, present in blood, neuronal, endothelial, pancreatic, and smooth muscle cells [31]] complicates their use as pharmacological probes [15].

Limited alteration of the heterocyclic ring of P2Y-active nucleotides is possible. 8-Aza and 1-deaza modifications are generally tolerated at $P2Y_1$ and $P2Y_{12}$ receptors [22, 34, 87], but other modifications, such as N1, N6-etheno, result in inactivity at P2Y receptors [32]. A fluorescent derivative of ATP **19** containing a tricyclic nucleobase is strikingly potent in activation of the $P2Y_1$ receptor [33].

Modification of the ribose moiety Replacement of the ribose 2′ and/or 3′ hydroxyl groups with H in adenine nucleotide agonists greatly reduces the potency at $P2Y_1$ receptors. A 1,5-anhydrohexitol bisphosphate derivative **20** activates the $P2Y_1$ receptor (EC_{50}=6.29 μM at turkey $P2Y_1$ receptor) [34] (Fig. 1b).

Simple carbocyclic (cyclopentyl) analogues of ATP enhance antagonist affinity at the $P2Y_{12}$ receptor [22]. Among the more successful examples of the use of carbocyclic or sterically constrained carbocyclic substitution of the ribose moiety for P2Y receptor interactions are the bicyclic "methanocarba" analogues [35, 36]. Two isomeric forms of the methanocarba (bicyclo[3.1.0]hexane) ring system have been used as ribose replacements in ATP, representing a North (N), 2′-*exo* envelope **21** or South (S), 2′-*endo* envelope **22** conformation. The addition of a 2-MeS group to **21** to form **24** provides a highly potent and selective $P2Y_1$ agonist, MRS2365 (EC_{50}=0.40 nM) [37]. Unlike 2MeS-ADP, this compound does not activate $P2Y_{12}$ or $P2Y_{13}$ receptors [38]. (N)-methanocarba derivative **23** is a full agonist at the $P2Y_1$ receptor (EC_{50}=158 nM); the corresponding 9-riboside, β,γ-methylene-ATP, is a partial weak agonist at that subtype.

A successful approach to designing potent and selective $P2Y_1$ receptor antagonists became possible with the observation by Boyer et al. that naturally occurring adenosine bisphosphate derivatives such as A3P5P **25** (Fig. 3a) act as partial agonists or antagonists of the receptor (EC_{50}= 0.83 μM) [39]. This has led to improved 2′-deoxyribose 3′,5′-bisphosphate derivatives MRS2179 **26** (EC_{50}=0.33 μM) and MRS2216 **27** (EC_{50}=0.21 μM), which are potent and selective $P2Y_1$ receptor antagonists [34]. A C-nucleotide-based antagonist **28** of the $P2Y_1$ receptor is patterned after MRS2179 [40]. (N)-methanocarba substitution within the family of bisphosphate antagonists yields MRS2279 **29** and MRS2500 **30**, which display nanomolar potency at the $P2Y_1$ receptor (EC_{50}=52 nM and 0.95 nM, respectively) [18]. Moderate antagonist activity at the $P2Y_1$ receptor is retained after removal of the 5′-phosphate group of MRS2500 in **31** (EC_{50}=1560 nM) [41].

Acyclic bisphosphate antagonists of the $P2Y_1$ receptor and related derivatives **32**–**35** have been characterized [42, 43]. Short alkyl chains bearing two phosphate groups attached at the adenine 9-position are favored over long chains. The bisphosphate derivative MRS2298 **32** is a potent antagonist of the $P2Y_1$ receptor (binding K_i= 29.6 nM, human). MRS2496 **34** is a bisphosphonate derivative, which remains tolerated in the $P2Y_1$ receptor binding site (binding K_i=76 nM, human). Compound **35**, which contains a cycloproyl ring within the 9-alkyl subsituent, is a $P2Y_1$ antagonist with micromolar affinity. Although various phosphate derivatives of the adenine 9-ribosides (cyclic) may be either agonists or antagonists of the $P2Y_1$ receptor, only antagonism has been achieved in the acyclic series.

The observation that ATP analogues inhibit platelet aggregation by antagonism of the $P2Y_{12}$ receptor enabled development of the 5′-triphosphate derivatives AR-C67085 (EC_{50}=30 μM) **36** and AR-C69931MX **37** (Cangrelor, EC_{50}=0.4 nM) as antithrombotic agents, which were in clinical testing [44] (Fig. 3b). Other nucleoside–nucleotide derivatives were investigated for $P2Y_{12}$ receptor antagonism [42, 45]. For example, AZD6140 **38a** is an uncharged nucleoside-based antagonist of the $P2Y_{12}$ receptor of high potency (pIC_{50}=7.9) that has been in clinical trials [22, 89]. A similar carbocyclic derivative **38b** containing a 1H-tetrazol-5-yl group was recently reported to bind to the $P2Y_{12}$ receptor with an IC_{50} value of 2 nM [87]. The (1*R*,2*S*)-2-phenylcyclopropyl derivative **38b** was found to be 30-fold more potent than the corresponding (1*S*,2*R*) isomer. The uncharged nucleoside derivative MRS2395 **33** [2,2-dimethyl-propionic acid 3-(2-chloro-6-methylamino-purin-9-yl)-2-(2,2-dimethyl-propionyloxymethyl)-propyl ester] is a weak antagonist of the $P2Y_{12}$ receptor [5]. The ADP derivative INS49266 **39** (EC_{50}=0.052 μM) and the AMP derivative INS50589 **40** (EC_{50}=0.011 μM) act as potent competitive antagonists of the $P2Y_{12}$ receptor [46].

Nonnucleotide antagonists of P2 receptors have also been identified and modified to achieve $P2Y_{12}$ receptor selectivity (Fig. 4). The clinical success of the thienopyridine clopidogel **41** as an antithrombotic agent has stimulated the development of other $P2Y_{12}$ antagonists. The thienopyridines act as liver-activated prodrugs that are irreversible

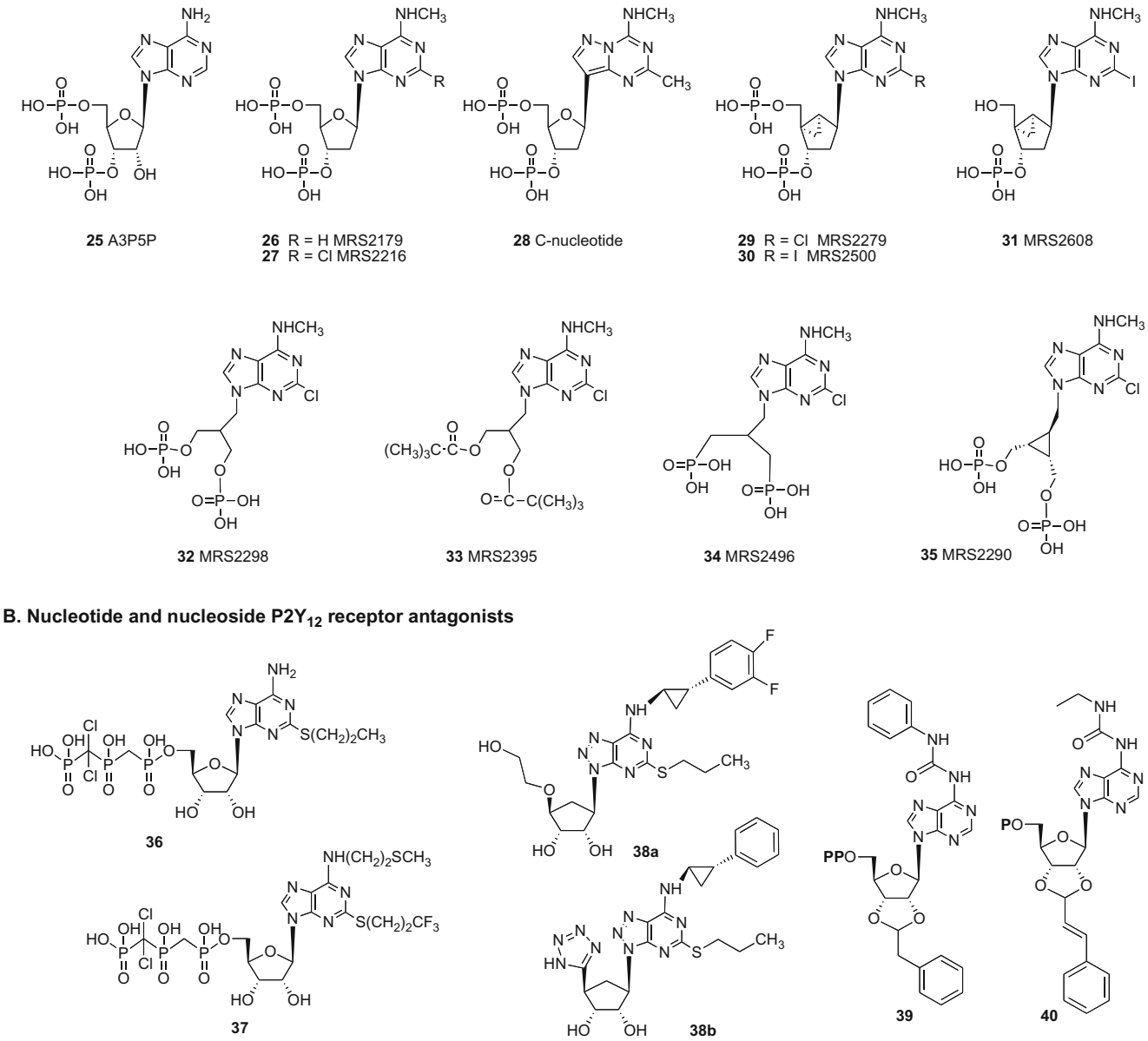

Fig. 3 Structures of nucleotide-based antagonists of $P2Y_1$ and $P2Y_{12}$ receptors

inhibitors of the $P2Y_{12}$ receptor [47]. Thiol **41b** is reported to be the active metabolite of clopidogrel. Prasugrel **42** is a $P2Y_{12}$ antagonist with similar action, which shows clinical promise [48].

Competitive antagonists of $P2Y_{12}$ receptors that do not require chemical conversion to an active species in vivo and that are nonnucleotides, i.e., not highly charged phosphate derivatives, are also sought as antithrombotic agents [45]. Screening of compound libraries has aided hit-and-lead identification in this effort. Compound **43** is a tricyclic benzothiazolo[2,3-c]thiadiazine antagonist of the $P2Y_{12}$ receptor (EC_{50}=0.18 μM) [19]. Compound **44** is a competitive $P2Y_{12}$ receptor antagonist [49]. The directly acting (i.e., not requiring chemical transformation in vivo prior to interacting with the receptor) $P2Y_{12}$ antagonist BX677 **45** exhibits a wider therapeutic index than does clopidogrel in experimental models of thrombosis [50]. Other nonnucleotide heterocyclic derivatives have been discovered that act as selective $P2Y_1$ receptor antagonists [51]. Recently, PRT128 (structure not disclosed) was introduced as an orally active direct-acting and reversible $P2Y_{12}$ receptor antagonist [88].

Other classes of nonnucleotide antagonists of P2 receptors have also been identified and modified to achieve P2Y receptor selectivity (Fig. 4). Derivatives of pyridoxal phosphate, e.g., pyridoxal-phosphate-6-azophenyl-2′,4′-

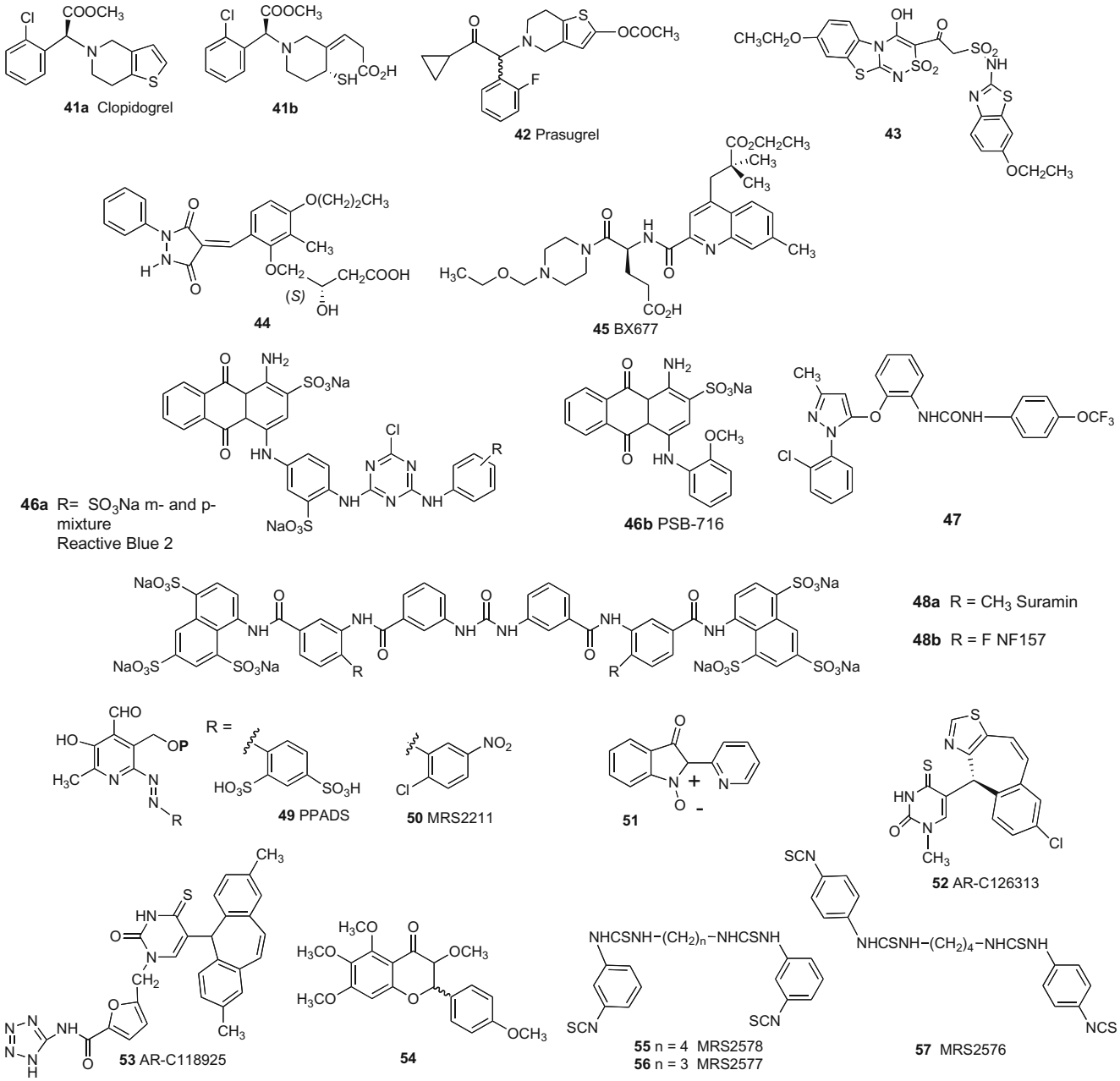

Fig. 4 Structures of nonnucleotide antagonists of P2Y receptors

disulfonate (PPADS) **49**, are generally relatively nonselective antagonists of the $P2Y_1$ receptor and other subtypes [4]. The SAR of P2 receptor antagonists derived from pyridoxal phosphate was reviewed by Lambrecht and coworkers [52]. Pyridyl isatogen **51** antagonizes the $P2Y_1$ receptor.

Recently, a nonnucleotide antagonist of the $P2Y_1$ receptor **47** has been reported through chemical optimization of a library hit [86]. This compound has a Ki value of 90 nM at the human $P2Y_1$ receptor and is proposed for use as an antithrombotic agent. It is orally bioavailable with a half life in rats of 2.8 h.

A moderately selective antagonist of the $P2Y_{13}$ receptor, MRS2211 **50**, has been identified [53]. Nucleotide derivatives AR-C67085 **36** (EC_{50}=4 nM) and AR-C69931MX **37** (EC_{50}=213 nM) potently antagonize the $P2Y_{13}$ receptor.

SAR of $P2Y_{11}$ and $P2Y_2$ receptors for 5′-triphosphates

$P2Y_{11}$ receptor agonists

Ten years ago, the human $P2Y_{11}$ receptor was cloned from human placenta [54]. This P2Y receptor subtype is also found on lymphocytes, kidney, and pancreatic-duct epithe-

lial cells [55, 56]. The $P2Y_{11}$ receptor exhibits moderate amino acid identity with other P2Y receptors. The $P2Y_1$ receptor is the closest homologue of the $P2Y_{11}$ receptor subtype, with 37.8% identity in the TM domains. The amino acid identity of the TM domains of the $P2Y_{11}$ receptor with the $P2Y_2$ receptor is 35.2%. The $P2Y_{11}$ receptor demonstrates the least homology (20%–22%) with the $P2Y_{12}$-like receptor subfamily.

As with the $P2Y_1$ and $P2Y_2$ receptors, the $P2Y_{11}$ receptor can be activated by adenosine 5′-triphosphate (ATP) **1**, which is its native agonist. ATP-induced activation of the $P2Y_{11}$ receptor stably expressed in CHO-K1 cells and in 1321N1 astrocytoma cells results in accumulation of both cyclic AMP and IP_3. These findings indicate that the $P2Y_{11}$ receptor is coupled to both the adenylyl cyclase (via G_s) and the phosphoinositide (via G_q) pathways [54, 57].

It was suggested that nonadenine triphosphates, including UTP **3**, guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP), thymidine 5′-triphosphate (TTP), inosine 5′-triphosphate (ITP), and UDP **4** are inactive at the $P2Y_{11}$ receptor [54, 57]. Another report characterized UTP as a Ca^{2+}-mobilizing agonist in $P2Y_{11}$ receptor-expressing 1321N1 astrocytoma cells, whose potency and maximal response are similar to ATP. However, the production of IP_3 does not increase during activation of the $P2Y_{11}$ receptor by UTP [58].

Dinucleotide polyphosphates activate various subtypes of P2Y receptors [59] (Fig. 5b). At the $P2Y_1$ receptor, diadenosine pentaphosphate (Ap_5A **70c**, EC_{50}=0.32 μM) is more potent than ATP (EC_{50}=0.65 μM), and Ap_3A **70a** (EC_{50}=0.011 μM) is equipotent to ADP (EC_{50}=0.014 μM) [60]. However, none of the diadenosine polyphosphates activate the $P2Y_{11}$ receptor, with the exception of Ap_4A **70b**, which increases the level of intracellular [Ca^{2+}] at a concentration of 1 mM [54, 57, 61].

Experiments performed on human $P2Y_{11}$ receptor-infected 1321N1 astrocytoma cells indicated increased intracellular production of IP_3 and cyclic AMP followed by elevation of Ca^{2+} induced by nicotinamide adenine dinucleotide **72** (β-NAD^+) in a concentration range of 1–100 μM [62]. Thus, extracellular β-NAD^+ appears to be an agonist of the $P2Y_{11}$ receptor [12]. Moreover, nicotinic acid adenine dinucleotide phosphate **73** ($NAADP^+$) also activates the $P2Y_{11}$ receptor [63].

Various analogues of ATP **1** have been examined as potential agonists of the $P2Y_{11}$ receptor (Fig. 1). The rank order of agonist potency is ATP > ADP >> AMP = adenosine in erythroleukemic K562 cells and in acute monocytic leukemia U937 cells stably transfected with the $P2Y_{11}$ receptor (K11 and U11 cells, respectively) [64]. NB4 promyelocytic cells behave similarly [16]. ATP-γ-S **7** is a more potent $P2Y_{11}$ receptor agonist than ATP in 1321N1, CHO-K1, U11, K11, and NB4 cells. Also in U11 and K11 cells, ATP-α-S and ADP-β-S **8** are more potent than ATP. Interestingly, ADP-β-S produces no agonist effect at the $P2Y_{11}$ receptor in NB4 cells [65]. Adenosine 5′-thiomonophosphate (α-thio-AMP) is almost inactive at the $P2Y_{11}$ receptor [64, 65].

BzATP (2′ or 3′-O-(4-benzoyl)benzoyl-ATP) is equipotent to ATP-γ-S in 1321N1, CHO-K1, U11, and NB4 cell lines. In contrast, in K11 cells, BzATP (EC_{50}=63 μM) is one of the weakest $P2Y_{11}$ receptor agonists. Interestingly, 2′-dATP is a more potent agonist of this receptor than ATP in all these cell lines [54, 57, 64].

Replacement of the α,β- or β,γ-oxygen atom by a methylene group decreases the potency of the ligand in comparison with ATP [64, 65]. Also, 2-MeS-ATP **11** is weaker than ATP at the $P2Y_{11}$ receptor subtype expressed in 1321N1, CHO-K1, and U11 cells but not in K11 cells. In addition, 2-Cl-ATP is a weak $P2Y_{11}$ receptor agonist [66]. However, 2-propylthio-β,γ-dichloromethylene-ATP (AR-C67085, **36**, an antagonist of the $P2Y_{12}$ receptor) is the most potent reported agonist of the $P2Y_{11}$ receptor (EC_{50}=8.9 μM) [57, 67].

The effects of replacement of the ATP ribose moiety by a (N)- or a (S)-methanocarba-constrained carbocyclic ring were studied at the human $P2Y_4$ receptor [36]. The potency of (N)-methanocarba-ATP **21** (EC_{50}=34.5 μM) is equivalent to unmodified ATP (EC_{50}=17 μM). In contrast, (S)-methanocarba-ATP **22** produces no effect at the $P2Y_{11}$ receptor at a concentration of 100 μM. It is possible that not only (N)-methanocarba analogues but also the (N)-conformation of the ribose ring of ATP analogues are preferred at the $P2Y_{11}$ receptor. In addition, these data demonstrate that the oxygen atom of the ATP ribose ring is not essential for agonist potency.

The preferences of green fluorescent protein (GFP)-tagged $P2Y_{11}$ receptor stably expressed in 1321N1 astrocytoma cells for ATP-α-S and ATP-α-S diastereoisomers were the focus of recent studies [66]. Rp-ATP-α-S (EC_{50}=0.27 μM) and Sp-ATP-α-B (EC_{50}=0.34 μM) isomers are more potent than their respective Sp (EC_{50}=1.71 μM) and Rp (EC_{50}=2.38 μM) isomers. Moreover, these compounds are more potent than ATP (EC_{50}=2.83 μM). In addition, both Rp- and Sp-2-MeS-ATP-α-S (EC_{50}=0.64 and 2.64 μM) are more potent at the $P2Y_{11}$ receptor than is 2-MeS-ATP (EC_{50}=13.8 μM) [66]. Interestingly, for the $P2Y_1$ receptor, these ligands display the opposite stereoselectivity, but for the $P2Y_2$ and $P2Y_4$ receptors, Rp-UTP-α-S (EC_{50} $P2Y_2$=5.4 μM, EC_{50} $P2Y_4$=27 μM) is more potent than the Sp-UTP-α-S-analogue (EC_{50} $P2Y_2$=14 μM, EC_{50} $P2Y_4$=81 μM) [16].

Various nonnucleotide derivatives have been shown to inhibit $P2Y_{11}$ receptors (Fig. 4). Suramin **48a**, a drug used to treat trypanosomiasis and onchocerciasis, weakly antagonizes various P2Y and P2X receptors, and its SAR is known in detail [52]. Suramin also inhibits G protein signaling intracellularly. $P2Y_{11}$ receptor antagonists derived

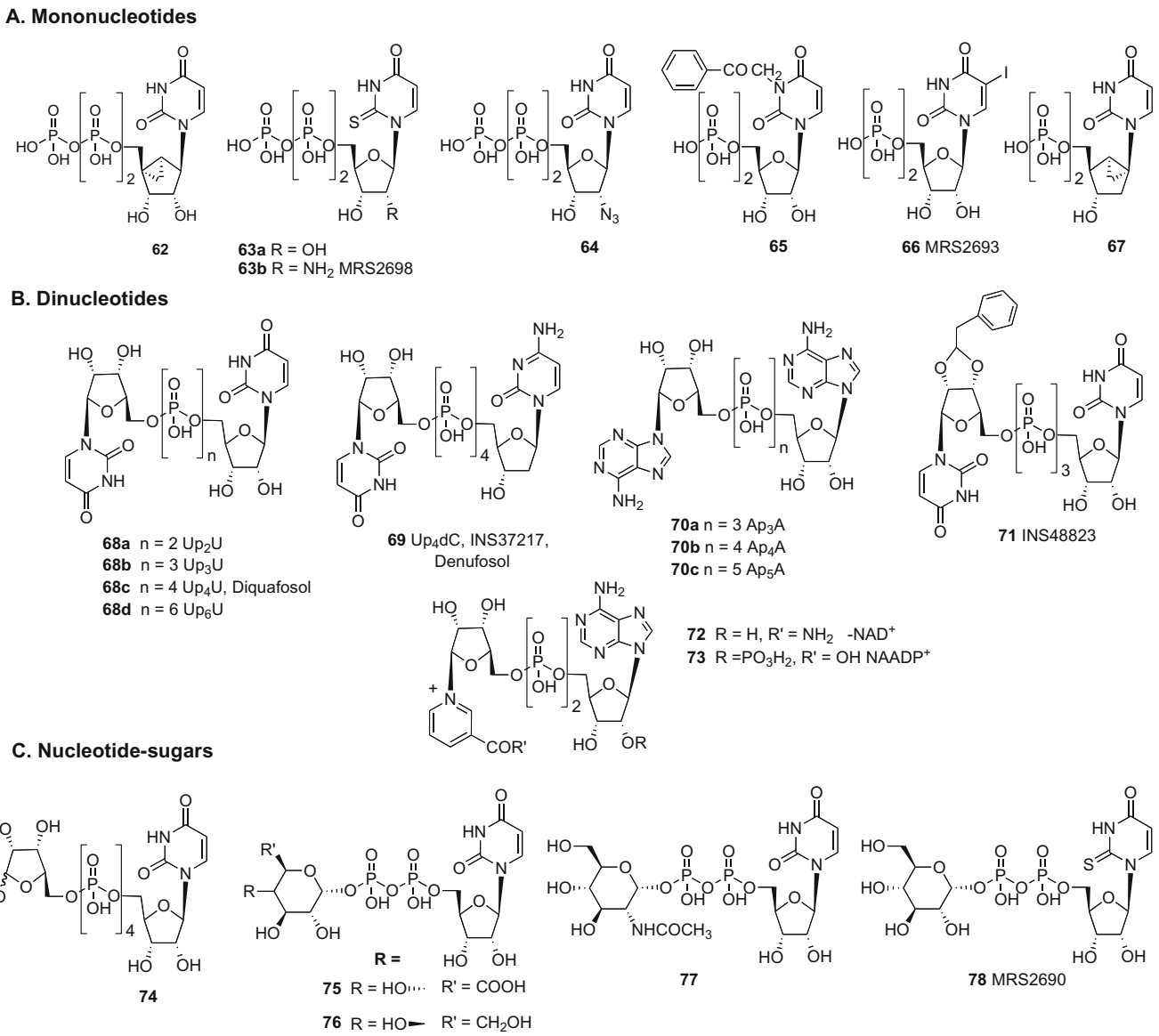

Fig. 5 Structures of additional uracil-derived nucleotide agonists of $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{14}$ receptors

from suramin exhibit nanomolar potency [68]. NF157 **48b** has a pK_i value of 7.35 at the $P2Y_{11}$ receptor and displays the following selectivity ratios (x-fold) over $P2Y_1$ (>650), $P2Y_2$ (>650), $P2X_2$ (1), $P2X_2$ (3), $P2X_3$ (8), $P2X_4$ (>22), and $P2X_7$ (>67) receptors.

ATP derivatives as $P2Y_2$ receptor agonists

The $P2Y_2$ receptor is activated by both uracil and adenine 5′-triphosphates (Fig. 1). The SAR of UTP derivatives at the $P2Y_2$ receptor are treated separately below.

In contrast to UTP **3**, which can activate only $P2Y_2$ and $P2Y_4$ receptors, ATP **1** is an agonist for three P2Y receptor subtypes $P2Y_2$ (EC_{50}=0.085 μM), $P2Y_1$ (EC_{50}=1.5 μM), and $P2Y_{11}$ (EC_{50}=17.3 μM) [36]. For this reason the design of ATP analogues with $P2Y_2$ receptor selectivity is a difficult problem.

BzATP and ATP-γ-S are weaker than ATP, and ATP-α-S is inactive at the mouse $P2Y_2$ receptor [69, 70]. Substitution of the α,β-oxygen atom of ATP by a methylene group (α,β-MeATP) results in a ligand that is virtually ineffective at the $P2Y_2$ receptor [71]. In addition, replacement of the β,γ oxygen atom in the 5′-triphosphate chain of ATP by a β,γ-methylene or β,γ-imido group leads to ligands with no effect at the $P2Y_2$ receptor at concentration of 1 mM [69, 70]. Although the $P2Y_1$ receptor can be potently activated by 2-MeS-ATP **11**, this ligand is inactive at the $P2Y_2$ subtype. However, 2-Cl-ATP is a weak agonist of the $P2Y_2$ receptor (EC_{50}=2.30 μM). The potency of 8-Br-ATP is reported to be EC_{50}=23.0 μM [72].

Studies of the preferred conformation of the ribose ring indicate that at the $P2Y_2$ receptor (N)-methanocarba-ATP (**21**, EC_{50}=0.091 μM) and (N)-methanocarba-UTP (**62**, EC_{50}= 0.0159 μM) are equipotent to ATP and UTP, respectively. In contrast, (S)-methanocarba-ATP (**22**, EC_{50}=3.7 μM) is significantly weaker than ATP.

Uracil nucleotide-responsive P2Y receptors

SAR of $P2Y_2$ and $P2Y_4$ receptors for 5′-triphosphates

The most prominent tissues of distribution of these UTP-responsive receptors are $P2Y_2$ (vascular cells, epithelial cells) and $P2Y_4$ (gastrointestinal cells, placenta, cochlear cells). These two receptors display the highest identity in the sequences of their TM domains (66.8%) of all P2Y receptor subtypes.

Phosphate modifications and dinucleotides The $P2Y_2$ receptor is activated nearly equipotently by UTP **3** (EC_{50}= 0.049 μM) and ATP **3** (EC_{50}=0.085 μM) (Fig. 1). However, it is not activated by the 5′-diphosphates UDP **4** and ADP **2**. An early publication showed UDP to be active, but this was later attributed to the presence of UTP in commercial preparations of UDP [73]. Uridine γ-thiodiphosphate (UTP-γ-S) **9** is a selective agonist for $P2Y_2$ (EC_{50}= 0.24 μM) and $P2Y_4$ (EC_{50}=1.6 μM) receptors [43].

The $P2Y_2$ receptor is activated by dinucleotide molecules [72] as well as uracil or adenine triphosphates. The dinucleotides Up_4U (INS365, Diquafosol) **68c** and Up_4dC (INS37217, Denufosol) **69** (Fig. 5) have the optimal phosphate chain length for activation of the $P2Y_2$ receptor (EC_{50}=0.1 and 0.22 μM) and tend to be more resistant to degradation by nucleotidases than nucleoside triphosphates [59, 60]. Up_4-[5′]-ribose **74** activates the $P2Y_2$ receptor with an EC_{50} of 1.88 μM, i.e., ninefold less potent than Up_4U **68c**. Up_4-[5′]-ribose **74** still activates the $P2Y_2$ receptor and serves as a truncated form of Up_4U **68c** for purposes of modeling receptor docking [74]. On the other hand, Up_2U **68a**, containing only two phosphate groups, is nearly inactive as an agonist, and Up_6U **68d** is 98-fold weaker than Up_4U at the $P2Y_2$ receptor.

The model of receptor-docked Up_4U is consistent with a tetraphosphate chain having the optimal length for dinucleotide binding [74]. The phosphate side chain of diuridine diphosphates apparently is too short to allow uridineII to reach its binding pocket. However, in the case of Up_6U, its phosphate chains are too large to be fully accommodated inside the receptor. Also, as discussed by Brunschweiger and Müller [4], among various linear dinucleotides, only dinucleoside tetraphosphates have the same number of negatively charged oxygen atoms as UTP.

Ribose and uracil modifications Both 2′-deoxy-2′-amino-UTP and 2-thio-UTP **63a** preserve the agonist potency of UTP at the $P2Y_2$ receptor (Fig. 5a). Combination of these two modifications yields 2′-amino-2-thio-UTP, compound **63b**, synergizes to enhance both potency (8 nM EC_{50}) and selectivity (300-fold $P2Y_2$-selective versus $P2Y_4$). 2′-Amine acetylation reduces potency, and trifluoroacetylation produces intermediate potency.

Modification at position 5, such as 5-bromo-UTP (EC_{50}= 0.75 μM) and 5-iodo-UTP (EC_{50}=0.83 μM), suggests that introducing a small hydrophobic group might be beneficial at the $P2Y_2$ receptor. However, 5-methyl UTP (EC_{50}= 0.48 μM) does not enhance potency, and the 5-amino (EC_{50}=5.6 μM) and 5-azido (EC_{50}=1.8 μM) analogues are less potent at the $P2Y_2$.

2′-Deoxy-UTP (EC_{50}=1.08 μM) is 22-fold less potent than UTP (EC_{50}=0.049 μM); replacement of the 2′-hydroxyl group by a 2′-methoxy group (EC_{50}=14.3 μM) reduces the potency further (290-fold weaker than UTP). Thus, the hydroxyl group at the 2′-position appears to be important for agonist activities as a donor of H-bonding to the $P2Y_2$ receptor.

AR-C126313 **52** and its higher-molecular-weight analogue AR-C118925 **53** are selective antagonists of the $P2Y_2$ receptor [75] (Fig. 4). Recently, flavonoid derivatives, e.g., **54**, [76], and derivatives of Reactive Blue 2 **46a**, such as 1-amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone (PSB-716) **46b** [77], have been explored as $P2Y_2$ receptor antagonists. Flavonoid antagonists reduce the amplitude of the $P2Y_2$ receptor response to UTP but not the EC_{50} value, indicating allosteric antagonism [76].

New ligand tools are needed to distinguish $P2Y_2$ and $P2Y_4$ receptor subtypes pharmacologically and to extend the characterization of the few existing tools to a wider range of species. Selective antagonists of the $P2Y_4$ receptor have not yet been identified. Suramin **48a** is more potent as a competitive antagonist at the human $P2Y_2$ receptor than at the $P2Y_4$ receptor [4, 5]. The order of potency of agonists is useful in this regard [16]. 2′-Azido-2′-deoxyUTP **64** is fivefold more potent in activation of phospholipase C (PLC0 at the human $P2Y_4$ receptor than at the $P2Y_2$ receptor (EC_{50}=0.54 μM *vs.* 0.78 μM). 4-Thio-UTP (EC_{50}= 0.023 μM) is 15-fold more potent than 2-thio-UTP (EC_{50}= 0.35 μM) in activating the human $P2Y_4$ receptor.

SAR of $P2Y_6$ receptor for 5′-diphosphates

The major tissue distribution of these UDP-responsive receptors includes vascular smooth muscle cells, microglial cells, and neutrophils. This receptor exhibits significant homology with the TM domains of other $P2Y_1$-like receptors, such as $P2Y_1$ (43.0%), $P2Y_2$ (47.7%), $P2Y_4$ (48.2%), and $P2Y_{11}$ (31.6%).

UDP **4** is a selective agonist at the $P2Y_6$ receptor. UDP-β-S **10** (EC_{50}=47 nM) is more potent than UDP (EC_{50}= 300 nM) in activation of the $P2Y_6$ receptor and is more stable toward ectonucleotidases. 5-Br-UDP (EC_{50}=800 nM) and the dinucleotide triphosphate (**71**, INS48823) are potent and/or stable agonists of the $P2Y_6$ receptor (EC_{50}=125 nM) [72, 78] (Figs. 1 and 5). The SAR and molecular modeling of the uracil nucleotide-activated $P2Y_6$ receptor were investigated recently [79, 80]. Novel UDP analogues were synthesized and assayed for activity at the human $P2Y_6$ receptor.

The $P2Y_6$ receptor is generally selective for 5′-diphosphate derivatives. Thus, uridine 5′-monophosphate, 2′-deoxyuridine 3′,5′-bisphosphate, a cyclic 3′,5′-diphosphate analogue, and a uridine 3′-diphosphate derivative are inactive as either agonists or antagonists.

Within a series of uracil dinucleoside 5′,5′-polyphosphates, the triphosphates (**68b** and **71)** are the most potent and selective (EC_{50}=0.2 and 0.125 μM). The simple Up_2U **68a** displays low potency at the $P2Y_6$ receptor.

Modification of ribose hydroxyl groups of UDP has not yielded more potent $P2Y_6$ receptor agonists. Substitution with a 2′-azido or 2′-amino group reduces potency at the $P2Y_6$ receptor. Removal of either the 2′- or 3′-hydroxyl group of UDP yields a >100-fold decrease in potency. The (S) conformation of the ribose moiety is preferred for ligand recognition by the $P2Y_6$ receptor [79]. A UDP derivative locked in the (N) conformation by an (N)-methanocarba ring is completely inactive, and the 2′-deoxy-(S)-methanocarba derivative **67** is moderately potent in activating the $P2Y_6$ receptor (Fig. 5A). This is a rare example of the use of molecular modeling to predict the conformational preference in a putative GPCR binding site for rational ligand design. The computational prediction led to the subsequent synthesis of a novel analogue containing a rigid carbocyclic ribose substitute that is more potent than the corresponding 9-(2′-deoxyriboside) as a $P2Y_6$ agonist.

The uracil ring was modified at the 2-, 3-, 4-, and 5-positions. Modification at the 3-position with N-CH_3 markedly decreases potency, but halogenation of the 5-position in the iodo analogue **66** (Fig. 5A) yields a molecule that is equal in potency (EC_{50}=0.15 μM) to UDP and is similar in potency to a 5-bromo analogue previously reported to be roughly equipotent to UDP [80]. A 5-iodo modification maintains potency at the $P2Y_6$ receptor, and 5-halo substitution of the uracil ring may therefore provide a basis for achieving selectivity at this subtype. In a series of uracil-modified compounds, 2-thio-UDP (EC_{50}=0.06 μM) and 4-thio-UDP (EC_{50}=0.08 μM) are five- to six-fold less potent than UDP. 4-Thioether derivatives and 4-carboxyalkylthio derivatives exhibit lower potency than the parent compound 4-thio-UDP. 3-Phenacyl-UDP **65** (EC_{50}=70 nM) is a potent and selective $P2Y_6$ receptor agonist [81].

Only one class of antagonists of the $P2Y_6$ receptor has been reported. The diisothiocyanate derivative MRS2578 **55** (EC_{50}=0.037 μM) is a potent, insurmountable antagonist of $P2Y_6$ nucleotide receptors [82] (Fig. 4). Related compounds **56** and **57** have varied selectivity for P2Y receptor subtypes. These isothiocyanate derivatives likely bind covalently to P2Y receptors; they are hydrophobic and have limited stability in aqueous solution.

SAR of $P2Y_{14}$ receptor for UDP-sugars

The most atypical P2Y receptor is the $P2Y_{14}$ receptor, which is distributed in the immune system, including in dendritic cells and the central nervous system. The identity of amino acid residues of TM domains of the $P2Y_{14}$ receptor with $P2Y_{12}$ and $P2Y_{13}$ subtypes is 57.0% and 52.8%, respectively. However, in contrast to other P2Y receptors, the $P2Y_{14}$ receptor is not activated by uridine 5′-di- or triphosphates or by adenine nucleotides [83]. The $P2Y_{14}$ receptor is the only subtype to respond principally to UDP-glucose **5** (EC_{50}= 0.35 μM) and other UDP-sugars, such as UDP-glucuronic acid **75** (EC_{50}=102 nM), and the endogenous nucleotides UDP-galactose **76** (EC_{50}=0.67 μM) and UDP-*N*-acetylglucosamine **77** (EC_{50}=4.38 μM) (Fig. 5C). The SAR of analogues of UDP-glucose at the $P2Y_{14}$ receptor was recently systematically explored [84]. Novel analogues modified on the nucleobase, ribose moiety, and glucose moieties were synthesized and characterized biologically at the recombinant human $P2Y_{14}$ receptor.

The $P2Y_{14}$ receptor appears to be the most restrictive of the P2Y family with respect to modification of the nucleobase, ribose, and phosphate moieties. Most of the novel analogues of **5** modified on the uracil or ribose moieties are inactive. All compounds modified at the 5-position of uracil moiety (i.e., with azido, amino, and iodo) or at the 2′, 3′ hydroxy groups of ribose ring are inactive. Analogues with other nucleosides, such as adenosine, guanosine, cytidine, thymidine, deoxyuridine and deoxycytidine, are inactive. The dinucleotides Up_2U **68a** and Up_4U **68c** are inactive at the $P2Y_{14}$ receptor.

Among the analogues of **5**, a 2-thiouracil analogue, MRS2690 **78**, is tenfold more potent (EC_{50}=49 nM). A 4-thiouracil (EC_{50}=0.29 μM) analogue is equipotent to **5**, but a 4-methyl-4-thiouracil moiety is not tolerated. The 2- and 4-thio modifications preserve potency at all uracil nucleotide-responsive subtypes but most effectively at the $P2Y_2$ and $P2Y_4$ receptors.

The high potency of analogues **75 (**UDP-glucuronic acid**)** and **76 (**UDP-galactose**)** suggests a flexibility of structural modification at the glucose C4 and C6 positions. The 12-fold-lower potency of **77** (UDP-N-acetylglucosamine) in comparison with **5** suggests a limited tolerance for steric bulk at the glucose C2 position. Modification of the glucose moiety

of **5** to yield, for example, UDP-fructose, UDP-mannose, and UDP-inositol, preserved agonist potency at the $P2Y_{14}$ receptor, as predicted in a modeling study [85].

Conclusion

The medicinal chemistry of several of the P2Y receptors has recently acquired detailed qualitative and quantitative SARs. Selective agonists (for $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors) and selective antagonists (for $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ receptors) have been identified. At the $P2Y_1$ and $P2Y_{12}$ receptors, 5′-diphosphate agonist derivatives have been transformed into potent and selective antagonists by alteration of the phosphate moieties and nucleobase and substitution pattern and introduction of carbocyclic substitution of the ribose moiety. Empirical and computational methods have identified conformations of the ribose moiety that enhance affinity at the $P2Y_1$ and $P2Y_6$ receptors have been identified through—and these conformations have been chemically locked to provide novel—selective ligands.

Screening of chemically diverse compound libraries has begun to yield new lead compounds for the development of P2Y receptor antagonists, e.g., directly acting $P2Y_{12}$ receptor antagonists with antithrombotic activity. Challenges remaining include identification of selective agonists for the $P2Y_4$, $P2Y_{11}$, and $P2Y_{13}$ receptors and of selective antagonists for the $P2Y_4$ and $P2Y_{14}$ receptors. The discovery of new, selective P2Y receptor ligands and optimization of existing structural leads hold promise for the identification of new clinical agents that exploit nucleotide receptor signalling.

Acknowledgment Support from the NIDDK Intramural Research Program is acknowledged.

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