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Update of P2Y receptor pharmacology: IUPHAR Review 27

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Eight G protein-coupled P2Y receptor subtypes respond to extracellular adenine and uracil mononucleotides and dinucleotides. P2Y receptors belong to the δ group of rhodopsin-like GPCRs and contain two structurally distinct subfamilies: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ (principally G_q protein-coupled $P2Y_1$-like) and $P2Y_{12-14}$ (principally G_i protein-coupled $P2Y_{12}$-like) receptors. Brain P2Y receptors occur in neurons, glial cells, and vasculature. Endothelial $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors induce vasodilation, while smooth muscle $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptor activation leads to vasoconstriction. Pancreatic $P2Y_1$ and $P2Y_6$ receptors stimulate while $P2Y_{13}$ receptors inhibits insulin secretion. Antagonists of $P2Y_{12}$ receptors, and potentially $P2Y_1$ receptors, are anti-thrombotic agents, and a $P2Y_2$/$P2Y_4$ receptor agonist treats dry eye syndrome in Asia. P2Y receptor agonists are generally pro-inflammatory, and antagonists may eventually treat inflammatory conditions. This article reviews recent developments in P2Y receptor pharmacology (using synthetic agonists and antagonists), structure and biophysical properties (using X-ray crystallography, mutagenesis and modelling), physiological and pathophysiological roles, and present and potentially future therapeutic targeting.

This article, contributed by members of the Nomenclature and Standards Committee of the International Union of Basic and Clinical Pharmacology (NC-IUPHAR) subcommittee for the P2Y receptors, confirms the existing nomenclature for these receptors, and reviews our current understanding of their structure, pharmacology and functions, and likely physiological roles in health and disease. More information on these receptors can be found in the Concise Guide to PHARMACOLOGY (http://onlinelibrary.wiley.com/doi/10.1111/bph.14748), and in the corresponding open access IUPHAR/BPS Guide to PHARMACOLOGY database (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=52).

1 | INTRODUCTION AND HISTORICAL OVERVIEW

1.1 | Initial characterization of P2 receptor subtypes

It is now >90 years since ATP and related adenine nucleotides and nucleosides were first isolated and shown to be pharmacologically active (Drury & Szent-Györgyi, 1929). It took, however, nearly another 50 years before the receptors through which they act were defined on the basis of pharmacological criteria. Burnstock (1978) proposed that P1 purinoceptors, that is, adenosine receptors, had an agonist potency order of adenosine > AMP > ADP > ATP, and methylxanthines, such as theophylline and caffeine, were selective antagonists. In contrast, at P2 purinoceptors, the agonist potency order was ATP > ADP > AMP > adenosine, and methylxanthines were inactive. This division received much support and was widely accepted.

Progress, thereafter, was much faster. First, Burnstock and Kennedy (1985) suggested the subdividing of P2 purinoceptors into P2X and P2Y subtypes, based on the relative agonist potency of ATP and several structural analogues in smooth muscle tissues. Shortly afterwards, Gordon (1986) identified P_{2T} purinoceptors on platelets that mediated aggregation in response to ADP, and P_{2Z} purinoceptors, which mediated ATP-induced degranulation of mast cells. Uracil nucleotides were also known to regulate cellular function and the P_{2U} receptor, activated by UTP and/or UDP, and in some cases, by ATP, was proposed (Abbracchio et al., 2006). P_{2D} purinoceptors sensitive to adenine dinucleotides were also identified (Abbracchio et al., 2006).

1.2 | Cloning of P2 receptors

Thus, numerous P2 purinoceptor subtypes had been named by the early 1990s, but their properties, distribution, and physiological roles were largely unclear, as there were no selective antagonists and the endogenous agonists had complex pharmacological profiles. These uncertainties were clarified in the next few years by the cloning of multiple adenine and/or uracil nucleotide-sensitive receptors. Seven ligand-gated cation channels, including the P_{2Z} purinoceptor, were all named P2X receptors, and eight GPCRs, including P_{2U} and P_{2T} purinoceptors, were called P2Y receptors (Abbracchio et al., 2006, 2019; Jacobson & Müller, 2016). The aim of this article is to review recent developments in our understanding of the pharmacological, biochemical, and biophysical properties of P2Y receptors, their physiological and pathophysiological roles, and how they are targeted clinically, both at present and potentially in the future. Key advances in this field since the last IUPHAR review (Abbracchio et al., 2006) include X-ray crystallographic structures of two representative subtypes and many new ligand tools.

The gaps in the numbering of P2Y receptors reflects the historical process in which newly cloned receptors were mis-assigned as purinergic receptors (e.g., P2Y10). Furthermore, several related orphan receptors, GPR171 and GPR17, appear in the literature as "P2Y-like" receptors. Several but not all reports support GPR17 activation by purinergic agonists and inhibition by antagonists (Burnstock, 2017; Fumagalli, Lecca, & Abbracchio, 2016).

2 | SELECTIVE LIGAND TOOLS TO STUDY P2Y RECEPTORS

P2Y receptors are activated by mononucleotides such as ADP (1), ATP (2), UDP (3), or UTP (4), by dinucleotides like diadenosine tetraphosphate (Ap$_4$A, 5) or diuridine tetraphosphate (Up$_4$U, 6), and/or by nucleotide sugars such as uridine 5′-diphosphoglucose (UDP-glucose, 7; see Figure 1). Selective agonists and antagonists are important for studying the roles of P2Y receptor subtypes in physiology and pathophysiology (Figures 1 and 2, Table 1, Abbracchio et al., 2019; Conroy, Kindon, Kellam, & Stocks, 2016; Jacobson & Müller, 2016; Rafehi & Müller, 2018). Their structure–activity relationship at each P2Y receptor subtype has been developed in detail through chemical efforts. In most studies, recombinant receptor proteins have been used for the analysis. Differences in native tissues may be due to a mixture of subtypes on cells, receptor oligomerization, differences in receptor density, and the metabolism of ligands by ecto-nucleotidases, such as CD39 (NTPDase-1), CD39L1 (NTPDase-2), and CD73 (NT5E).

2.1 | P2Y$_1$ receptor ligands

The G$_q$-coupled P2Y$_1$ receptor is activated by ADP (1) and analogues including the potent agonist 2-methylthio-ADP (8), which is also available as a radioligand [³H]2MeSADP (Abbracchio et al., 2006, 2019). ATP is an antagonist or partial agonist at the P2Y$_1$ receptor (Waldo & Harden, 2004). The Northern (N)-methanocarba analogue of 2-methylthio-ADP (MRS2365, 9) is more potent and, in addition, selective for the P2Y$_1$ receptor (Chhatriwala et al., 2004). Selective P2Y$_1$ receptor antagonists have been developed. The bisphosphonate derivative MRS2500 (2-iodo-N^6-methyl-(N)-methanocarba-2′-deoxyadenosine 3′,5′-bisphosphate, 10) is a potent and selective competitive antagonist (Hechler et al., 2006). The non-nucleotide compound MRS2950 (11) was identified by virtual screening of the modelled receptor orthosteric site (Costanzi, Kumar, Balasubramanian, Harden, & Jacobson, 2012). Another non-nucleotide compound, BPTU (N-[2-[2-(1,1-dimethylethyl)phenoxy]-3-pyridinyl]-N′-[4-(trifluoromethoxy)phenyl]urea, 12) and its analogues act as an allosteric inhibitors (Gao & Jacobson, 2017; Peng et al., 2018; Wang, Qiao, et al., 2013; Yuan et al., 2016; Zhang, Gao, et al., 2015).

The [³²P]-labelled and [¹²⁵I]-labelled analogues of MRS2500 are radioligands binding to P2Y$_1$ receptors (Ohlmann et al., 2010). An analogue of BPTU has been reported as a novel [¹⁸F]-tracer for imaging of tissue P2Y$_1$ receptor expression using PET (Moldovan et al., 2019). Tetrahydroquinolineamine derivative (13) was reported as a
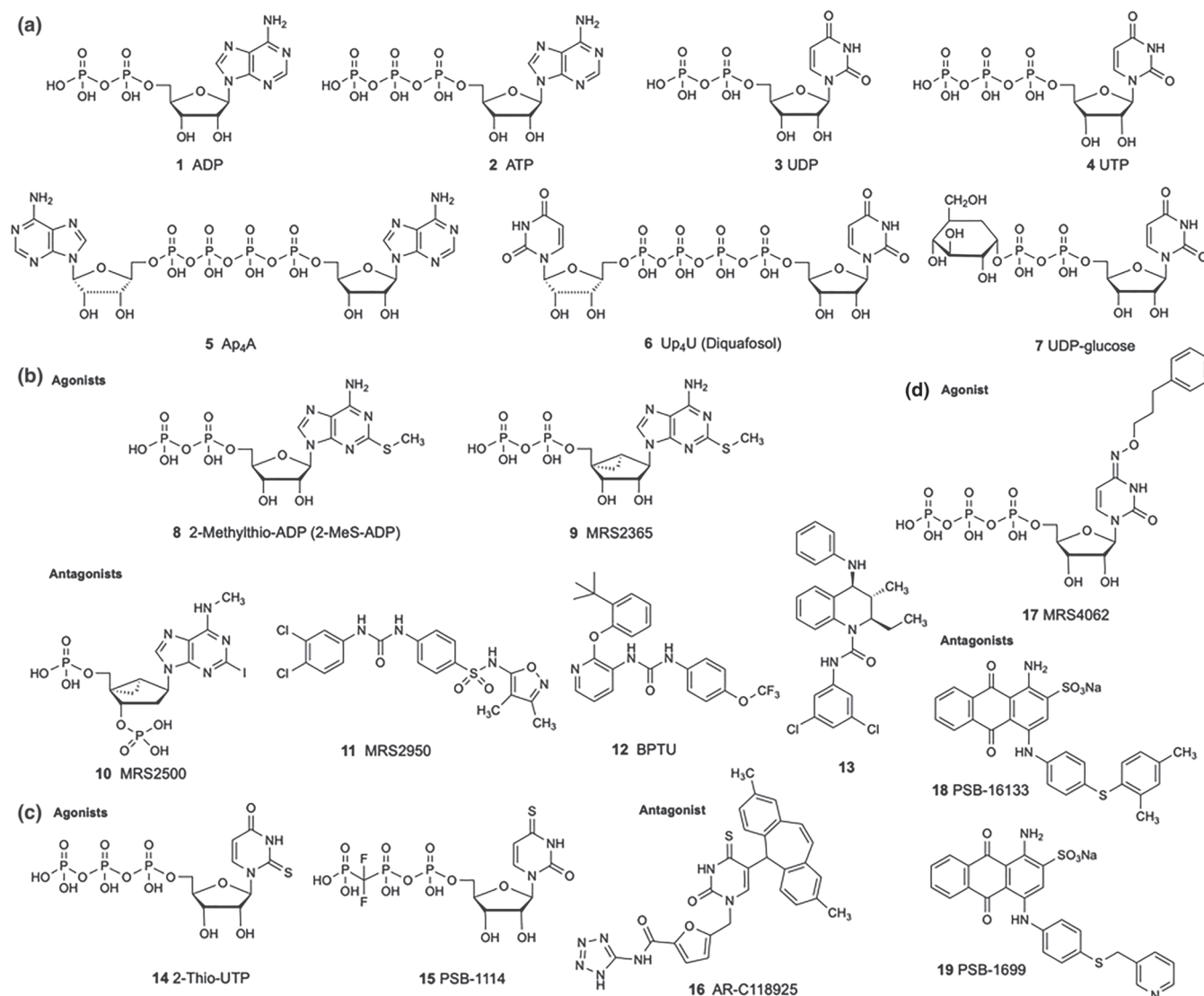
FIGURE 1 P2Y receptor ligands. (a) Natural agonists of P2Y receptors. (b) P2Y$_1$ receptor agonists and antagonists. (c) P2Y$_2$ receptor agonists and antagonist. (d) P2Y$_4$ receptor agonists and antagonists

structurally novel P2Y$_1$ receptor antagonist with binding K_i 0.5 μM (Morales-Ramos et al., 2008).

2.2 | P2Y$_2$ receptor ligands

The G$_q$-coupled **P2Y$_2$ receptor** is activated by both ATP and UTP (Abbracchio et al., 2006, 2019) as well as by dinucleoside polyphosphates including Ap$_4$A and Up$_4$U (**diquafosol**, INS365; Xu et al., 2018). Diquafosol is approved in Japan and South Korea for the treatment of dry eye syndrome (Keating, 2015; Yamane et al., 2015). **2-Thio-UTP** (**14**) and related analogues are selective P2Y$_2$ receptor agonists (El-Tayeb, Qi, Nicholas, & Müller, 2011; Xu et al., 2018). 4-Thiouridine-5′-O-(β,γ-difluoromethylene)triphosphate (**PSB-1114**, **15**) is more than 60-fold selective for the P2Y$_2$ receptors over P2Y$_4$ and P2Y$_6$ receptors (El-Tayeb et al., 2011).

AR-C118925 (5-[[5-(2,8-dimethyl-5H-dibenzo[a,d]cyclohepten-5-yl)-3,4-dihydro-2-oxo-4-thioxo-1(2H)-pyrimidinyl]methyl]-N-2H-tetrazol-5-yl-2-furancarboxamide, **16**) is a potent and selective antagonist for P2Y$_2$ receptors (Kindon et al., 2017; Rafehi, Burbiel, Attah, Abdelrahman, & Müller, 2017; Rafehi, Neumann, et al., 2017).

2.3 | P2Y$_4$ receptor ligands

The human G$_q$-coupled **P2Y$_4$** receptor is activated by UTP, but not by ATP or nucleoside diphosphates (Abbracchio et al., 2006, 2019). **MRS4062** (N^4-(phenylpropoxy)-CTP, **17**), **MRS2927** (a 3′-deoxy-3′-fluoroglucose analogue), and **N^4-(phenylethoxy)-CTP** are potent P2Y$_4$ receptor agonists (Maruoka et al., 2011). PSB-16133 (**18**) and PSB-1699 (**19**) are novel P2Y$_4$ receptor antagonists acting at submicromolar concentrations (Rafehi, Malik, et al., 2017). PSB-16133 is somewhat more potent, but PSB-1699 is more selective for the P2Y$_4$ receptor.
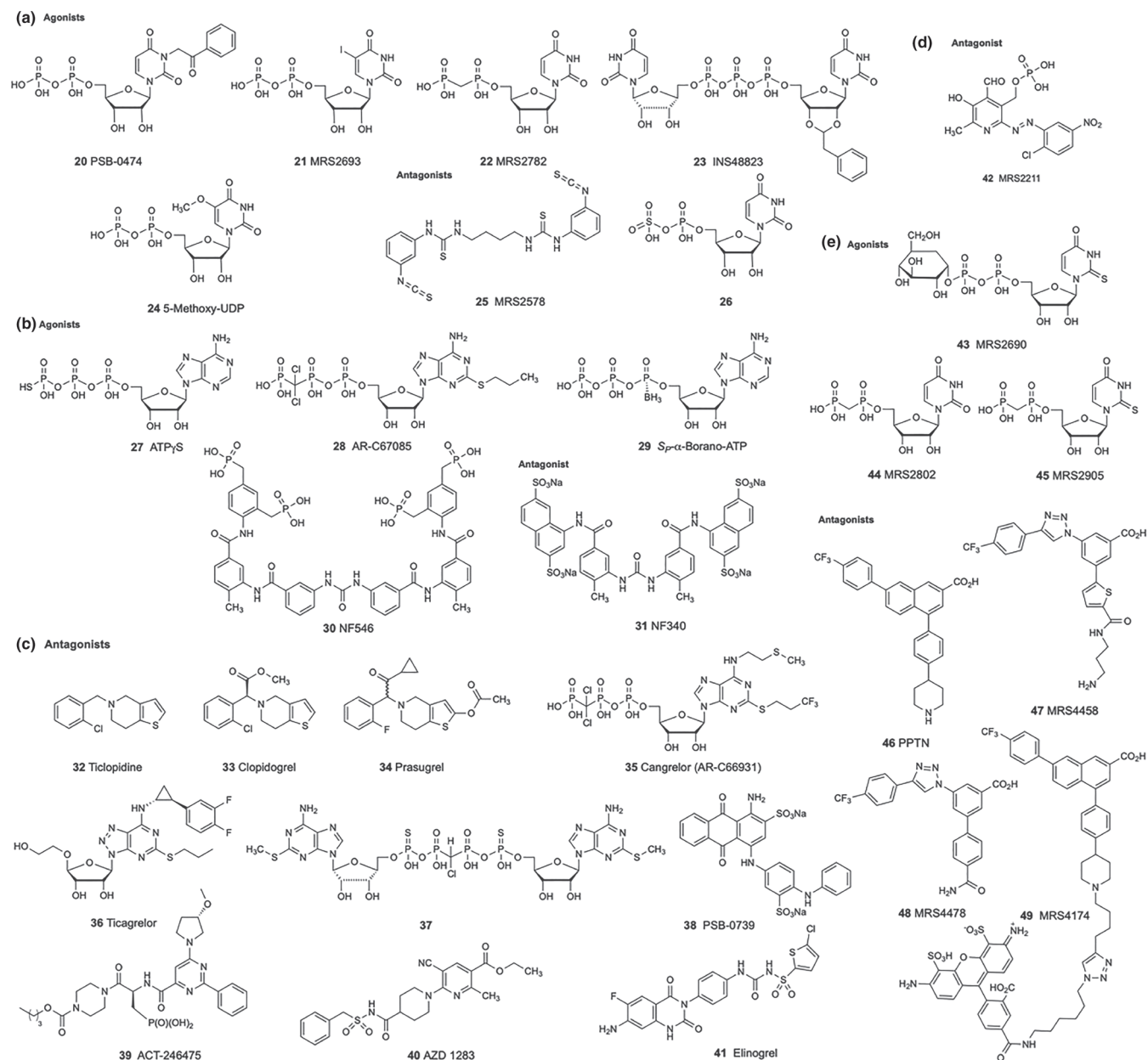
FIGURE 2 P2Y receptor ligands. (a) $P2Y_6$ receptor agonists and antagonists. (b) $P2Y_{11}$ receptor agonists and antagonists. (c) $P2Y_{12}$ receptor agonists and antagonists. (d) $P2Y_{13}$ receptor antagonist. (e) $P2Y_{14}$ receptor agonists and antagonists

2.4 | $P2Y_6$ receptor ligands

The G_q-coupled **$P2Y_6$ receptor** prefers UDP as an agonist (Abbracchio et al., 2006, 2019). Analogues including **3-phenacyl-UDP** (PSB-0474, **20**; El-Tayeb et al., 2011), 5-iodo-UDP (**MRS2693**, **21**; Ko et al., 2008), α,β-methylene-UDP (**MRS2782**, **22**; Ko et al., 2008), **INS48823** (**23**, Korcok, Raimundo, Du, Sims, & Dixon, 2005; Rafehi & Müller, 2018), and 5-methoxy-UDP (**24**, Ginsburg-Shmuel et al., 2012) are much more potent agonists.

The $P2Y_6$ receptor is blocked by **MRS2578** (N,N″-1,4-butan ediylbis[N′-(3-isothiocyanatophenyl)thiourea, **25**; Mamedova, Joshi, Gao, von Kügelgen, & Jacobson, 2004) and uridylyl phosphosulfate (**26**, Meltzer et al., 2015).

2.5 | $P2Y_{11}$ receptor ligands

The **$P2Y_{11}$ receptor** couples to both G_q and G_s proteins. ATP activates human $P2Y_{11}$ receptors, whereas ADP is a canine $P2Y_{11}$ receptor agonist (Abbracchio et al., 2006, 2019). Human $P2Y_{11}$ receptors are also activated by **ATPγS** (**27**), the $P2Y_{12}$ receptor antagonist 2-propylthio-β,γ-dichloromethylene-ATP (**AR-C67085**, **28**) and adenosine-5′-O-(α-boranotriphosphate) with a preference for the diastereomer that has an S-configuration at the α-phosphorus atom (compound **29**; Ecke, Tulapurkar, Nahum, Fischer, & Reiser, 2006; Meis et al., 2010). Moreover, the **suramin** analogue **NF546** (4,4′-(carbonylbis (imino-3,1-phenylene-carbonylimino-3,1-(4-methyl-phenylene)carbonylimino))-bis(1,3-xylene-α,α′-diphosphonic acid, **30**) has

TABLE 1 Properties of human P2Y receptors and key agonist/antagonist ligands (Abbracchio et al., 2019; Jacobson et al., 2015; Rafehi & Müller, 2018)

Group	Subtype, gene symbol, chromosome	Principal G protein coupling; distribution	Ligand agonist/antagonist	pEC_{50}, pIC_{50}, or pK_i	Other interactions, Subtype
P2Y$_1$-like	P2Y$_1$, P2RY1, 3q25.2	G$_q$; platelets, heart, skeletal muscle, brain, intestine	ADP **1**	5.09	P2Y$_{12,13}$
			2-MeSADP **8**	5.60	P2Y$_{12,13}$
			MRS2365 **9**	9.40	Selective
			ADPβS	5.62	P2Y$_{12}$
			MRS2500 **10**[d]	9.02	Selective
			MRS2279[d]	8.10	Selective
			MRS2179[d]	6.48	Selective
			MRS2298	7.20	Selective
			MRS2496	5.82	Selective
	P2Y$_2$, P2RY2, 11q13.4	G$_q$, G$_i$; endocrine, reproductive and immune systems, skeletal and cardiac muscle, lung, intestine	UTP **4**	7.22	P2Y$_4$
			MRS2698	8.10	Selective
			ATP **2**	7.07	P2Y$_{4,11}$ + others
			Ap$_4$A **5**	6.1	P2Y$_{12}$ and others
			Up$_4$U **6**	6.68	P2Y$_4$ (6.89), P2Y$_6$ (5.94)
			PSB-1114 **15**	6.87	Selective
			MRS2768	5.72	Selective
			Denufosol	6.66	P2Y$_4$ (6.10)
			AR-C118925 **16**	7.24	selective
	P2Y$_4$, P2RY4, Xq13.1	G$_q$, G$_i$; placenta, brain, intestine, lung, heart, prostate, fat	UTP **4**	6.26	P2Y$_2$
			GTP	5.18	Nonselective
			MRS4062 **17**	7.64	selective
			ATP **2**[a]	6.15	P2Y$_{2,11}$ + others
			PSB-16133 **18**	6.63	Selective
			PSB-1699 **19**	6.39	Selective
	P2Y$_6$, P2RY6, 11q13.4	G$_q$; lung, heart, spleen, placenta, kidney	UDP **3**	6.28	P2Y$_{14}$ (6.80)
			Up$_3$U	6.57	P2Y$_2$ (5.88), P2Y$_4$ (6.06)
			MRS2957	7.92	P2Y$_2$ (6.77), P2Y$_4$ (6.10)
			PSB-0474 **20**	7.15	Selective
			MRS2693 **21**	7.83	Selective
			MRS2782 **22**	6.47	P2Y$_{14}$ (7.94)
			INS48823 **23**	6.90	Selective
			5-MeO-UDP **24**	7.10	Selective
			MRS2795	7.38	Selective
			MRS2578 **25**[f]	7.43	Selective
			U-phosphosulfate **26**	3.95	Selective
	P2Y$_{11}$, P2RY11, 19p13.2	G$_q$, G$_s$; spleen, intestine, immunocytes	ATP **2**	4.77	P2Y$_{2,4}$ + others
			ATPγS **27**	4.62	P2Y$_{2,4}$ + others
			AR-C67085 **28**	8.5	P2Y$_{12}$
			Sp-α-borano-ATP **29**	6.47	P2Y$_1$ (5.92)
			NF546 **30**[c]	6.27	Selective
			NF157	7.35	Also blocks P2X1,2,3Rs
			NF340 **31**	7.77	Selective

(Continues)

TABLE 1 (Continued)

Group	Subtype, gene symbol, chromosome	Principal G protein coupling; distribution	Ligand agonist/antagonist	pEC_{50}, pIC_{50}, or pK_i	Other interactions, Subtype
P2Y12-like	P2Y12,[b] P2RY12, q25.1	Gi; platelets, brain, immunocytes	ADP **1**[d]	7.22	P2Y1,13
			2-MeSADP **8**	8.3	P2Y1,13
			Ap4A **5**	6.0	P2Y4 (5.9), P2Y13 (6.7)
			PSB-0739 **38**	9.8	Selective
			AZ11931285[d]	~9	Selective
			AR-C67085 **28**[d]	8.2	P2Y11
			AR-C69931MX **35**	9.40	P2Y13 (8.3)
			ticagelor **36**	7.90	Selective
			Ap4A analogue **37**	6.66	P2Y1 (5.67)
			ACT246475 **39**	9.00	Selective
			AZD1283 **40**	7.50	Selective
			elinogrel **41**	7.64	Selective
	P2Y13,[b] P2RY13, 3q25.1	Gi; spleen, brain, myeloid cells	ADP **1**	7.94	P2Y1,12
			2-MeSADP **8**	7.72	P2Y1,12
			MRS2211 **42**	5.97	Selective
	P2Y14, P2RY14, 3q25.1	Gi; brain, endocrine and immune systems, muscle, lung, pancreas, intestine, kidney	UDP **3**[d]	6.80	P2Y6
			UDP-glucose **7**[d]	6.45	P2Y2
			MRS2690 **43**	7.31	Selective
			MRS2802 **44**	7.20	Selective
			MRS2905 **45**	8.70	Selective
			PPTN **46**	9.36, 8.22[e]	Selective
			MRS4458 **47**	6.77[e]	Selective
			MRS4478 **48**	6.57[e]	Selective
			MRS4147 **49**	10.10	Selective

Note. If no compound number is given, the structure is not shown. The affinity and selectivity in other species is not shown.

[a]ATP acts as antagonist at the human P2Y4 receptor, but an agonist at the rat or mouse P2Y4 receptor.

[b]Selective agonists not yet available.

[c]NF546 activates the P2Y11 receptor, although it belongs to a structural class of antagonists.

[d]Used as a radioligand, when labelled with [^{3}H], [^{32}P], [^{33}P], or [^{125}I], as appropriate.

[e]Fluorescent antagonist **49** binding assay, underestimates affinity in functional assays.

[f]Non-competitive antagonist.

been reported to act as an agonist (Meis et al., 2010). Another suramin analogue, **NF340** (4,4′-(carbonylbis (imino-3,1-(4-methyl-phenylene) carbonylimino))bis (naphthalene-2,6-disulfonic acid, **31**), is a potent antagonist with a pA_2 value of 8.0 (Meis et al., 2010).

2.6 | P2Y12 receptor ligands

The Gi-coupled **P2Y12 receptor** is activated by ADP and its potent analogue 2-methylthio-ADP (**8**, Abbracchio et al., 2006, 2019; Hollopeter et al., 2001). Many P2Y12 receptor antagonists have been developed (Baqi & Müller, 2019). Some of them are used to reduce platelet aggregation for the prevention or treatment of cardiovascular events such as myocardial infarction or stroke. The thienopyridine compounds **ticlopidine** (**32**), **clopidogrel** (**33**, Savi et al., 2006), and **prasugrel** (**34**, Sugidachi, Asai, Ogawa, Inoue, & Koike, 2000;

Sugidachi, Mizuno, Ohno, Jakubowski, & Tomizawa, 2016) are liver-activated prodrugs. Their active metabolites, such as **R-138727** (structure not shown, Dansette, Levent, Hessani, & Mansuy, 2015), have been shown to interact in an irreversible manner with the human P2Y12 receptor protein (Algaier, Jakubowski, Asai, & von Kugelgen, 2008; Ding, Bynagari, Mada, Jakubowski, & Kunapuli, 2009; Savi et al., 2006; Zhang, Zhang, Gao, Zhang, et al., 2014). Competitive P2Y12 receptor antagonists include the nucleotide derivatives **cangrelor** (**35**, AR-C69931MX, N^6-(2-methylthioethyl)-2-(3,-3,3-trifluoropropylthio)-β,γ-dichloromethylene-ATP) and AR-C67085 (2-propylthio-β,γ-dichloromethylene-D-ATP, **28**; Ingall et al., 1999) as well as nucleoside derivatives such as the orally active **ticagrelor** (**36**, AZD6140, Springthorpe et al., 2007; Hoffmann et al., 2009; Hoffmann et al., 2014). Ticagrelor has been reported to act as an inverse agonist under some experimental conditions (Aungraheeta

et al., 2016; Garcia et al., 2019). A series of novel analogues of Ap$_4$A blocks P2Y$_{12}$ receptors at nanomolar concentrations; one of the most potent derivatives was compound **37** (Yanachkov et al., 2016).

PSB-0739 (1-amino-9,10-dihydro-9,10-dioxo-4-[[4-(phenylamino)-3-sulfophenyl]amino]-2-anthracenesulfonic acid, **38**) is a potent and competitive non-nucleotide P2Y$_{12}$ receptor antagonist with a pA$_2$ value of 9.8 (Baqi & Müller, 2019; Hoffmann et al., 2014). Further novel P2Y$_{12}$ receptor antagonists include piperazinyl glutamates and derived phosphonates such as ACT-246475 (**39**, Caroff et al., 2015), ethyl 6-aminonicotinate acyl sulfonamides, for example, **AZD1283** (**40**, Bach et al., 2013; Zhang, Zhang, Gao, Zhang, et al., 2014), and related sulfonamide derivatives such as elinogrel (PRT060128, **41**). The reversible and very potent antagonist ACT-246475 (Caroff et al., 2015) is being tested in clinical trials for use in the prevention or treatment of cardiovascular events (Baldoni et al., 2014). In addition, a variety of moderately potent P2Y$_{12}$ receptor antagonists have been described, among them 4-benzhydrylmorpholines (Ahn et al., 2016), flavonolignans (Bijak, Szelenberger, Dziedzic, & Saluk-Bijak, 2018), and salvianolic acids (Liu et al., 2018). [^{3}H] **PSB-0413** is an antagonist radioligand, the tritiated form of AR-C67085 (**28**), which binds to P2Y$_{12}$ receptors with a K_D value of 4.6 nM (Ohlmann et al., 2013).

2.7 | P2Y$_{13}$ receptor ligands

ADP and 2-methylthio-ADP (**8**) activate the G$_i$-coupled **P2Y$_{13}$ receptor** (Communi et al., 2001; Abbracchio et al., 2006, 2019). **MRS2211** (2-[(2-chloro-5-nitrophenyl)azo]-5-hydroxy-6-methyl-3-[(phosphonooxy)methyl]-4-pyridinecarboxaldehyde, **42**) is a competitive antagonist with a pA$_2$-value of 6.3 (Kim et al., 2005). Cangrelor, in contrast, blocks the human and the rat P2Y$_{13}$ receptors in a non-competitive mode of action (Fumagalli et al., 2004).

2.8 | P2Y$_{14}$ receptor ligands

UDP and sugar derivatives such as UDP-glucose and **UDP-galactose** are G$_i$-coupled P2Y$_{14}$ receptor agonists (Abbracchio et al., 2006, 2019; Carter et al., 2009). The analogues **MRS2690** (diphosphoric acid 1-α-D-glucopyranosyl ester 2-[(4′-methylthio)uridin-5″-yl] ester, **43**), **MRS2802** (α,β-difluoromethylene-UDP, **44**), and **MRS2905** (α,β-methylene-2-thio-UDP, **45**) are much more potent agonists (Abbas et al., 2018; Carter et al., 2009; Das et al., 2010).

PPTN (4-[4-(4-piperidinyl)phenyl]-7-[4-(trifluoromethyl)phenyl]-2-naphthalenecarboxylic acid, **46**) is a potent antagonist acting at the P2Y$_{14}$ receptor (Barrett et al., 2013). Novel antagonists include **MRS4458** (3-{5-[(3-aminopropyl)carbamoyl]thiophen-2-yl}-5-{4-[4-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-1-yl}benzoic acid, **47**) and **MRS4478** (4′-carbamoyl-5-{4-[4-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-1-yl]-[1,1′-biphenyl]-3-carboxylic acid, **48**; Yu et al., 2018). **MRS4174** (**49**) is a high-affinity fluorescent ligand with antagonistic properties.

3 | THE EVOLUTIONARY HISTORY OF P2Y RECEPTORS AND RELATED GPCRS

The availability of genomic sequences of human and other animal species allowed for comprehensive phylogenetic studies and meaningful analysis of the evolutionary history of the GPCR superfamily revealing several major classes of GPCRs—the rhodopsin-, adhesion/secretin-, frizzled-, and glutamate-like metabotropic receptor classes (Fredriksson & Schiöth, 2005). Based on amino acid sequence similarities, P2Y receptors belong to the rhodopsin-like receptor class, specifically to the δ group within this class. The δ group does not only include P2Y receptors but also receptors for lipids, hydroxycarboxylic acids, and peptides (Figure 3a).

The phylogenetic relation of P2Y receptors subdivides them into at least two groups (Figure 3a) that also correspond to their preferential G protein coupling. One group comprises P2Y$_1$, P2Y$_2$, P2Y$_4$, P2Y$_6$, and P2Y$_{11}$ receptors (G$_q$-coupled P2Y$_1$ receptor-like) while the second group contains P2Y$_{12-14}$ receptors (G$_i$-coupled P2Y$_{12}$ receptor-like). The diversity of P2Y receptor signalling pathways is discussed below in the context of neural cells. Interestingly, several other structurally related δ group GPCRs, such as the 2-oxoglutarate receptor (**OXGR1** and GPR99) and the succinate receptor (**SUCNR1** and GPR91), as well as the orphan receptors **GPR34**, **GPR82**, GPR171, and **GPR87** cluster into one of both groups (Figure 3a).

The phylogenetic clustering of P2Y receptors correlates, at least in part, with the genomic localization of P2Y receptors. Chromosomal clustering in the human genome is found for P2Y$_{12-14}$ receptor genes at chromosome 3 (Figure 3b). Here, all genes show the same orientation in a tandem-like fashion within an intron of the reverse-oriented Mediator complex subunit 12 like (Med12L) gene. Such condensed genomic localization of related genes most probably arose from multiple rounds of local gene duplications. Interestingly, SUCNR1 and P2Y$_1$ receptors are in close genomic proximity to this P2Y$_{12}$ receptor-like receptor gene cluster. However, both genes are in reverse orientation to the P2Y$_{12}$ receptor cluster (Figure 3b) indicating an independent genomic history. Chromosomal clustering is also found for P2Y$_2$ and P2Y$_6$ receptors at chromosome 11, the **hydroxycarboxylic acid (HCA$_{1-3}$) receptors** at chromosome 12, and seven δ group members at chromosome X (Figure 3b).

Several P2Y receptors and other members of the δ group cluster have prominent genomic clusters at chromosome 3 and chromosome X. The P2Y$_{12}$-like receptors are found in an intron of the MED12L gene at chromosome 3. Similarly, GPR34 and GPR82 are found in an intron of the reverse-oriented CASK gene at Xp11.4. Phylogenetic analysis showed relation of cysteinyl-LT receptor and **lysophosphatidic acid (LPA) receptors** (see Figure 3a). Interestingly, **CysLT$_2$** and **LPA$_6$** receptors and **GPR183** are closely located at chromosome 13 (13q14.2, not shown), whereas **CysLT$_1$** genomically clusters with **LPA$_4$** and **P2Y10** receptors and **GPR174**, at the X chromosome.
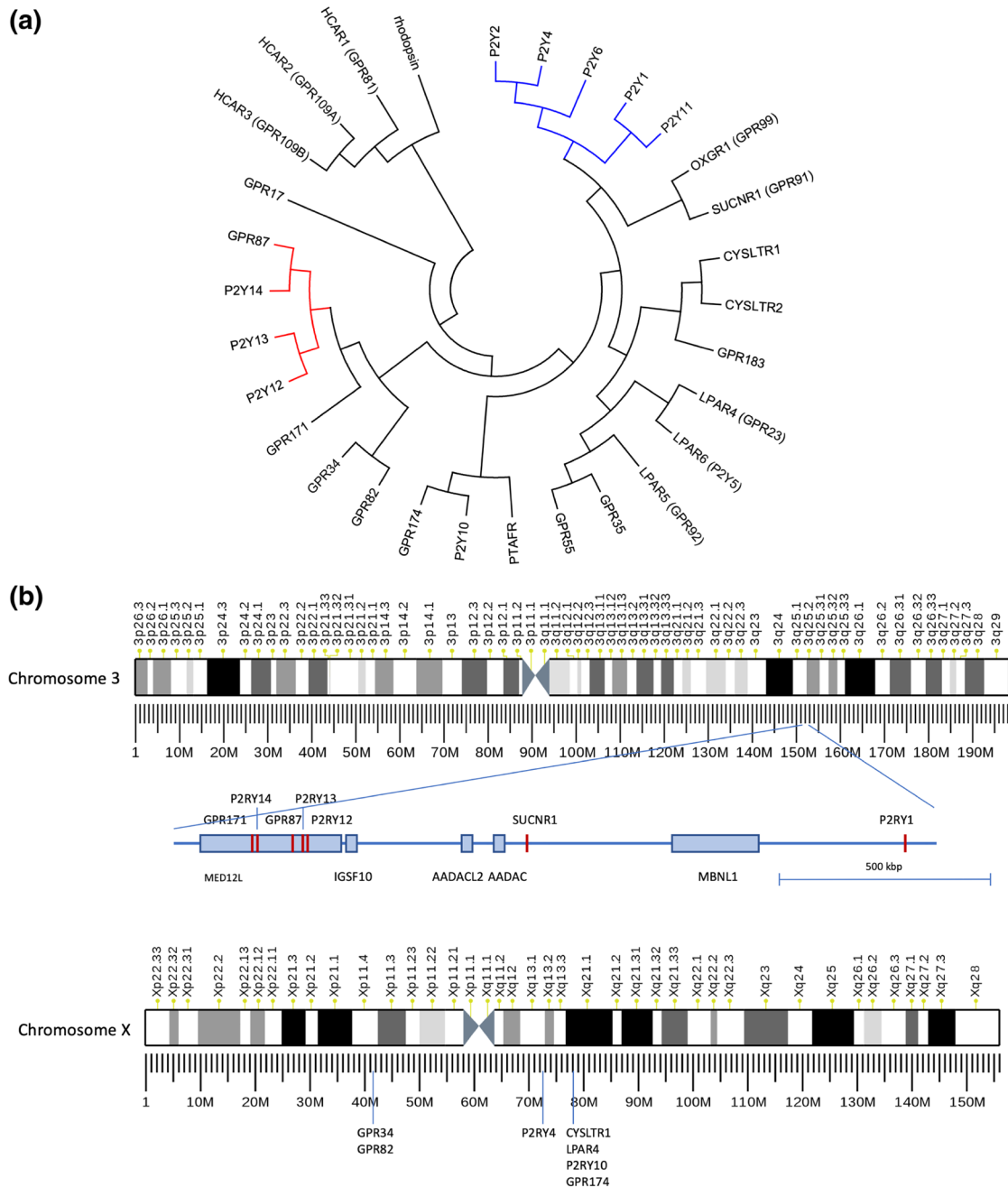
FIGURE 3 (a) Evolutionary relationships of human P2Y$_1$- and P2Y$_{12}$-like receptors of the δ group of rhodopsin-like GPCRs (Fredriksson & Schiöth, 2005). P2Y$_1$- and P2Y$_{12}$-like receptors form separated clusters of receptors indicating that they evolved independently. The evolutionary history was inferred using the neighbour-joining method by extending a previous analyses (Le Duc et al., 2017). The optimal tree with the sum of branch length = 14.64350117 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved the amino acid sequences of 30 human receptors which were aligned by using the PAM matrix and default parameters. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 239 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher, & Tamura, 2016). HCARx, hydroxycarboxylic acid receptors; LPARx, lysophosphatidic acid receptors; OXGR1, 2-oxoglutarate receptor; SUCNR1, succinate receptor; PTAFR, PAF receptor; P2Yx nucleotide receptors. (b) Genomic clustering of P2Y-like receptors on chromosome 3 and chromosome X. Several P2Y receptors and other members of the δ group cluster at genomic loci, most prominent clusters at chromosome 3 and chromosome X. The P2Y$_{12}$ receptor-like receptors are found in a MED12L gene intron at chromosome 3. Similarly, GPR34 and GPR82 are found in a reverse-oriented CASK gene intron at Xp11.4. Phylogenetic analysis showed relation of cysteinyl-LT receptor and lysophosphatidic acid receptors (see (a)). Interestingly, CysLT$_2$ and LPA$_6$ receptors and GPR183 are closely located at chromosome 13 (13q14.2, not shown) whereas CysLT$_1$ receptors genomically cluster with LPA$_4$, P2Y10 receptors, and GPR174 at the X chromosome

In contrast to metabotropic P2Y receptors, the P2X receptors seem to appear in evolution already in invertebrates and plants (Hou & Cao, 2016). Prototypical P2Y receptors, as we know them from vertebrates, seem to be an innovation that occurred first in the Cambrian, because δ group GPCRs do not have orthologous sequences in invertebrates, hemichordates, and cephalochordates (Krishnan, Almen, Fredriksson, & Schiöth, 2013). Unfortunately, the "birth" of P2Y receptors can only be assumed at some time between the cyclostome–gnathostome split because of the lack of other recent genomes covering this evolutionary period. Because almost all P2Y- and other δ group-related sequences are present in cartilaginous and bony fish genomes, these genes must have been present in the common ancestor of both fish lineages.

Most coding regions of P2Y receptors do not contain introns, and splice variants are rare. The genomic structure of these receptors has been highly conserved during evolution. However, introns in the coding regions of several P2Y receptor genes newly appear in some fish species (Schöneberg et al., 2007). In contrast, introns in the 5′UTR seem to be common, for example, in $P2Y_{12}$ receptor-like receptors, $P2Y_2$, and $P2Y_6$ receptors. Obviously, human $P2Y_1$ and $P2Y_4$ receptors do not contain introns in their genes. Some P2Y receptors contain cryptic introns in their N terminus-coding regions ($P2Y_6$, GPR87, and GPR34).

The human $P2Y_{11}$ receptor, located at chromosome 19, is an exception in many aspects. $P2Y_{11}$ receptor orthologues are missing in murine and chicken genomes but are present in most other birds, reptiles, and amphibians sequenced to date. In humans, $P2Y_{11}$ receptor transcripts exist in several variants modifying the N terminus coding region and even producing a fusion protein with an adjacent gene (PPAN; Dreisig & Kornum, 2016). Here, a chimeric transcript, characterized by the first third of PPAN exon 12 joined to $P2Y_{11}$ receptor exon 2, has been detected. The chromosomal synteny between $P2Y_{11}$ receptors and PPAN is already established in zebrafish, and fusions at the mRNA level can be found in many birds and mammals.

4 | STRUCTURAL CHARACTERIZATION OF P2Y RECEPTORS

The availability of numerous orthologues also allows for comparing the conservation of individual amino acid positions in P2Y receptor proteins. Although P2Y receptors belong to two distinct structural and evolutionary groups (Figure 3a), as confirmed by X-ray structures (Zhang, Zhang, Gao, Paoletta, et al., 2014; Zhang, Gao, et al., 2015), several members share the same agonist (e.g., ADP). Support for different agonist binding modes between $P2Y_1$ and $P2Y_{12}$ receptors was observed in mutational analyses. While for $P2Y_1$, positions $K^{6.55}$, $Q^{7.36}$, and $R^{7.39}$ (using standard residue numbering) were shown to be critical for receptor activation following agonist binding (Jiang et al., 1997), positions $R^{6.55}$, $Y^{6.58}$, and $K^{7.35}$ are involved in $P2Y_{12}$ receptor ligand recognition (Hoffmann et al., 2009; Hoffmann et al., 2014; Schmidt et al., 2013). All these residues are conserved

within vertebrate $P2Y_1$ and $P2Y_{12}$ receptor orthologues but are not shared between both ADP receptors.

Phylogenetic and mutagenesis studies addressing the positions $R^{6.55}$, $Y^{6.58}$, and $K^{7.35}$ of $P2Y_{12}$ receptors showed that all these residues are 100% conserved among species and that most mutations of these positions interfered with receptor function (Schmidt et al., 2013). However, such residue combination is also present in some GPR87, GPR171, and GPR34 orthologues (all $P2Y_{12}$ receptor-like), which are not activated by ADP or ATP. This does not rule out that these residues are involved in nucleotide binding of, for example, $P2Y_{12}$ receptors, but it implies additional positions which determine ligand specificity.

Structural studies of P2Y receptors have made tremendous progress in recent years. Five structures of two representative receptors, $P2Y_1$ and $P2Y_{12}$, from two subfamilies were determined in 2014 and 2015, respectively (Zhang, Zhang, Gao, Paoletta, et al., 2014; Zhang, Zhang, Gao, Zhang, et al., 2014; Zhang, Gao, et al., 2015). These structures, in which the receptors are captured in complex with different ligands that vary in chemical structure and binding site, provide essential insights into ligand recognition and activation regulation of P2Y receptors.

The $P2Y_1$ and $P2Y_{12}$ receptor structures share a canonical seven transmembrane (7TM) helical architecture of GPCRs. Like most other solved class A GPCR structures, the structures of $P2Y_{12}$ receptors in complex with agonists 2MeSADP and 2MeSATP and $P2Y_1$ receptors bound to inhibitors MRS2500 and BPTU exhibit a conserved disulfide bond connecting TM3 to the second extracellular loop (ECL2). However, this disulfide bond is not observed in the antagonist AZD1283-bound $P2Y_{12}$ receptor structure (Zhang, Zhang, Gao, Paoletta, et al., 2014), consistent with previous data suggesting that the ECL2 residue $Cys97^{3.25}$ may act as the covalent binding site for the active metabolites of $P2Y_{12}$ receptor drugs (Algaier et al., 2008; Ding et al., 2009). In addition to the dynamic disulfide bond, TM5 of the $P2Y_{12}$ receptor adopts a straight and elongated conformation rather than a bent helix observed in most other GPCR structures, as the highly conserved class A GPCR residue $P^{5.50}$ that leads to a helical bend in other GPCRs (Zhang, Zhang, Gao, Paoletta, et al., 2014) is substituted by an asparagine ($N201^{5.50}$) in $P2Y_{12}$ receptors.

Comparison of agonist- and antagonist-bound $P2Y_{12}$ receptor structures reveals remarkable differences in the extracellular region (Zhang, Zhang, Gao, Zhang, et al., 2014). Compared to the $P2Y_{12}$ receptor-AZD1283 structure, the extracellular tips of TM6 and TM7 shift towards the central axis of the TM bundle by over 10 and 5 Å respectively in the $P2Y_{12}$ receptor-2MeSADP structure. The inward movement of TM6 and TM7 allows extensive interactions between the agonist and the extracellular region of the receptor. As a result, the helical bundle, the $P2Y_{12}$ receptor ECLs, and N terminus undergo conformational changes, and the ligand-binding pocket shrinks to preclude antagonist binding. In contrast, the antagonist AZD1283's phenyl moiety forms a steric hindrance to impede the inward movement of TM6 in the $P2Y_{12}$ receptor-AZD1283 structure. These structural differences suggest that the receptor extracellular region may play a

role in modulating receptor activity by cooperating with the bound ligand.

The P2Y$_{12}$ receptor structures reveal different binding modes for the non-nucleotide antagonist AZD1283 and the nucleotide agonist 2MeSADP. The binding pockets of these two ligands vary in size and shape, with only partial overlap. AZD1283 binds to the P2Y$_{12}$ receptor in a large open pocket formed by residues from TM3-TM7. In contrast, the nucleotide agonist is completely enclosed within the receptor ligand binding pocket. The ligand binding cavity of P2Y$_{12}$ receptors is separated into two sub-pockets by a barrier formed by Y105$^{3.33}$ and K280$^{7.35}$. Pocket 1 is shaped by TM3–TM7, while pocket 2 is composed of TM1–TM3 and TM7. Although pocket 2 is not occupied in the solved P2Y$_{12}$ receptor structures, molecular docking studies suggested that the active metabolites of P2Y$_{12}$ receptor antagonists such as clopidogrel many bind to pocket 2 (Zhang, Zhang, Gao, Paoletta, et al., 2014), providing new clues for the development of allosteric modulators of P2Y$_{12}$ receptors as anti-cardiovascular drugs with reduced side effects.

Although P2Y$_1$ and P2Y$_{12}$ receptors share the same endogenous ligand ADP, the structures of these two receptors reveal (Figure 4) completely different recognition patterns for their nucleotide-like ligands (Zhang, Gao, et al., 2015). In the P2Y$_1$ receptor structure, the nucleotide-like antagonist MRS2500 binds to the receptor in a binding pocket bordered by the N terminus, ECL2, and TM6–TM7 within the TM bundle, close to the extracellular surface. The adenine ring of MRS2500 inserts into a sub-pocket formed by the N terminus, TM6, and TM7, while its two phosphate groups make extensive polar interactions with residues from N terminus, ECL2, TM2, and TM7. In the P2Y$_{12}$ receptor-2MeSADP structure, the adenine ring of 2MeSADP reaches deep into the ligand binding pocket, making contacts with TM3 and TM4. The negatively charged diphosphate group of 2MeSADP attracts positively charged residues and hydrogen-bonding groups from N terminus, ECL2, TM3, TM6, and TM7, forming electrostatic force to stabilize the "closed" extracellular region (Jacobson et al., 2015). These structural differences highlight the diversity of recognition by GPCRs.

The most striking finding in the recent work on the P2Y receptor structures is that the two P2Y$_1$ receptor inhibitors MRS2500 and BPTU occupy two disparate ligand binding pockets (Figure 4c; Zhang, Gao, et al., 2015). Unlike the nucleotide-like ligand MRS2500 whose binding site is within the receptor helical bundle, the non-nucleotide antagonist BPTU binds to the receptor in a shallow binding pocket on the external interface of TM1–TM3 with the lipid bilayer, which accommodates the ligand mainly through hydrophobic interactions. This hydrophobic binding environment of BPTU is consistent with the high hydrophobicity of this ligand, and previous efforts showing that addition of any polar groups to reduce its lipophilicity decreased binding affinity to P2Y$_1$ receptors (Wang, Qiao, et al., 2013). The unique binding site of BPTU indicates that this ligand acts as an allosteric modulator of P2Y$_1$ receptors . It most likely inhibits receptor function by blocking the conformational change of TM1–TM3 to lock the receptor in an inactive state, which is supported by ligand binding assays showing that BPTU can

accelerate agonist [^{35}S]2MeSADP dissociation (Zhang, Gao, et al., 2015). BPTU was the first structurally characterized selective GPCR ligand located entirely outside of the canonical ligand binding pocket of GPCRs. As such, the finding offers new opportunities to GPCR drug discovery to target novel sites.

Currently, there is no crystal structure for pyrimidine nucleotide receptors. However, recent mutational and docking studies suggest that the conserved R$^{7.39}$ in P2Y$_1$-like receptors is also involved in UDP binding in P2Y$_6$ receptors (Bruser et al., 2017) and agonist binding of P2Y$_2$ receptors (Rafehi, Neumann, et al., 2017) indicating that the binding pocked is very similar at least within the two P2Y receptor groups.

5 | SIGNALLING PATHWAYS FOR P2Y RECEPTORS

The cellular sources of nucleotide agonists of P2Y receptors include co-transmission, endocytosis, release through **pannexin1 (Px1)** hemichannels and lysis in cell damage (Abbracchio et al., 2006; Nishimura et al., 2017). The principal G protein coupling of each of the P2Y receptor subtypes are indicated in Table 1. However, each of the P2Y receptors can be associated with multiple G proteins and other signalling pathways, as was shown for P2Y$_1$ receptors, including both G protein-dependent and G protein-independent, for example, β-arrestin, pathways (Gao & Jacobson, 2017). Some of the P2Y receptors additionally couple to G$_{12/13}$ (P2Y$_2$ and P2Y$_6$) or G$_s$ (P2Y$_{11}$).The P2Y receptors regulate **MAPK** pathways and consequently affect cell survival and proliferation (Miras Portugal et al., 2019). This review emphasizes the novel contribution of nucleotide receptors to maintain cell homeostasis through the regulation of MAPKs and phosphatases. P2Y receptors also undergo agonist-induced desensitization and can heterodimerize with other GPCRs, such as the vascular **angiotensin II AT$_1$ receptor** and the **A$_1$ adenosine receptor** (Nishimura et al., 2017).

6 | P2Y RECEPTORS IN THE NERVOUS SYSTEM

The cloning of the different P2Y receptors showed their presence in brain tissue. P2Y receptors are especially abundant in glial cells, astrocytes and microglia, but they are also present in central and peripheral neurons, oligodendrocytes, and cerebral microvasculature. P2Y receptors together with ionotropic P2X receptors mediate neurotransmission, neuron–glia interaction, regulation of cerebral blood flow, and neuroprotection or even contribute to neurodegeneration and pain transmission (Burnstock, 2017; Toth et al., 2015; Weisman et al., 2012). P2Y receptors are also present in adult stem cells, which suggests potential actions in neuroregeneration (Gómez-Villafuertes et al., 2015; Stefani et al., 2018).

Astroglia from different areas, cortex, *hippocampus*, *striatum*, *cerebellum*, and spinal cord, express a great variety of P2Y receptors: ADP
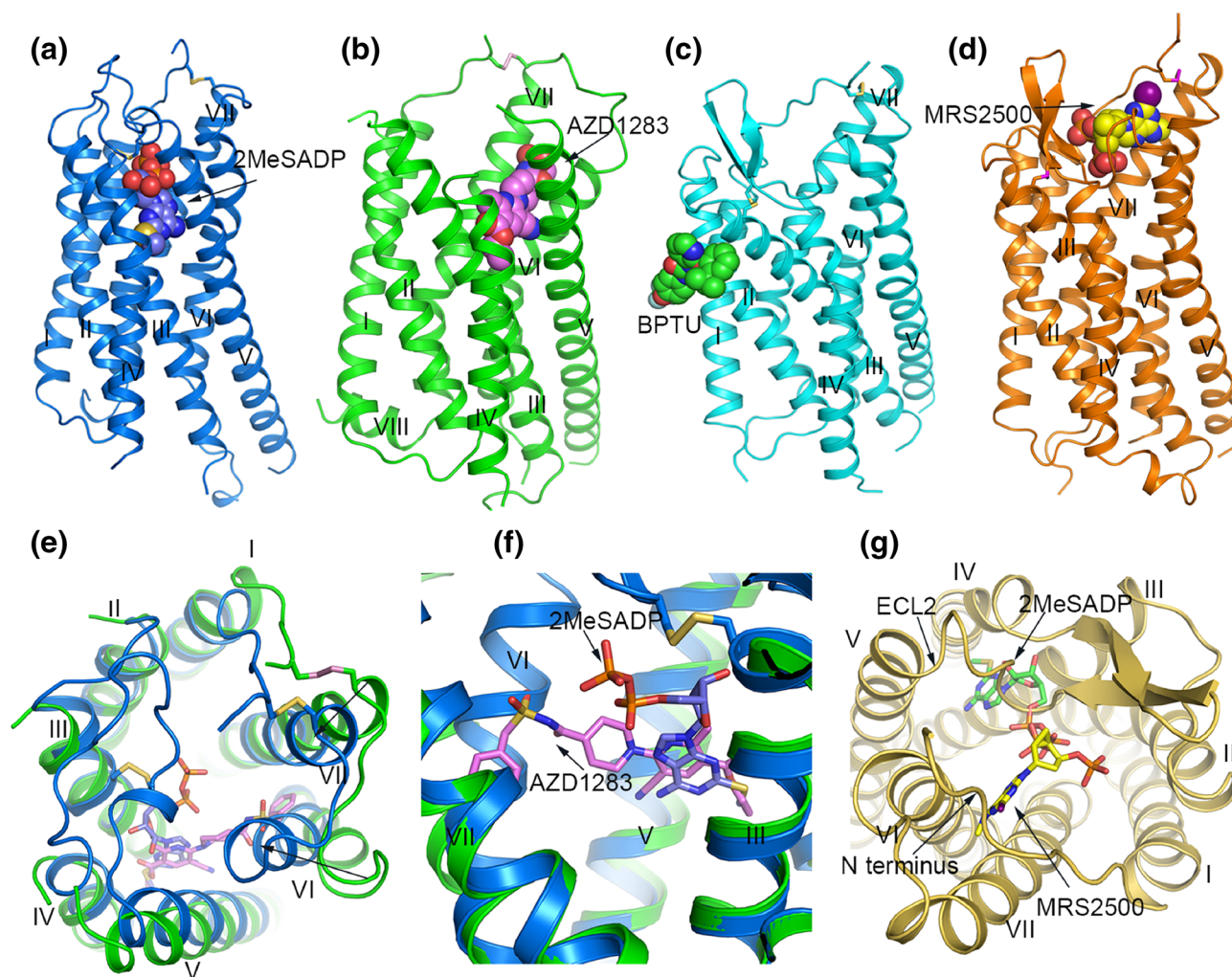
FIGURE 4 Structures of P2Y$_{12}$ receptors and P2Y$_1$ receptors. (a) Structure of the P2Y$_{12}$ receptor-2MeSADP **8** complex. The P2Y$_{12}$ receptor is shown in blue cartoon representation. 2MeSADP is shown as blue spheres. The disulphide bonds are shown as yellow sticks. (b) Structure of the P2Y$_{12}$ receptor-AZD1283 **40** complex. The receptor is coloured green and shown in cartoon representation. AZD1283 is shown as spheres with pink carbons. The disulphide bridge is shown as pink sticks. (c) Structure of the P2Y$_1$ receptor-BPTU complex. The P2Y$_1$ receptor is coloured cyan and shown as cartoon. The ligand BPTU is shown as spheres with green carbons. The disulphide bonds are shown as yellow sticks. (d) Structure of the P2Y$_1$ receptor-MRS2500 **10** complex. The P2Y$_1$ receptor is shown in orange cartoon representation. MRS2500 is shown as spheres with yellow carbons. The disulphide bonds are show as magenta sticks. (e) Structural comparison of the extracellular region in the P2Y$_{12}$ receptor-2MeSADP and P2Y$_{12}$ receptor-AZD1283 complexes. In the P2Y$_{12}$ receptor-AZD1283 structure, the receptor is shown in green cartoon representation, the ligand AZD1283 is shown as pink sticks. In the P2Y$_{12}$ receptor-2MeSADP structure, the receptor is shown in blue cartoon representation and 2MeSADP is shown as blue sticks. The black arrows indicate the movements of helices VI and VII in the 2MeSADP-bound structure relative to the AZD1283-bound structure. (f) Comparison of the binding pose of AZD1283 and 2MeSADP in P2Y$_{12}$ receptors. The colour scheme is the same as that in panel (e). (g) Comparison of the nucleotide binding modes in P2Y$_1$ receptors and P2Y$_{12}$ receptors. The P2Y$_1$ receptor is shown in yellow cartoon representation. MRS2500 and 2MeSADP are shown as yellow sticks and green carbon respectively

selective receptors, P2Y$_1$ receptors and P2Y$_{13}$ receptors, the receptor equally activated by UTP and ATP, receptors, and pyrimidine selective receptors P2Y$_4$ and P2Y$_6$ receptors (Franke, Verkhratsky, Burnstock, & Illes, 2012). Astroglial P2Y$_1$ receptors mediate neuron–glia interaction and calcium waves contributing to neuronal activity synchronization (Shigetomi, Hirayama, Ikenaka, Tanaka, & Koizumi, 2018). P2Y$_1$, P2Y$_2$ and P2Y$_{13}$ receptors are also present in neurons. P2Y$_1$ and P2Y$_{13}$ receptors together with the ionotrotropic **P2X7 receptors** regulate neuronal differentiation and are also involved in neuroprotection and

neurodegeneration (Miras Portugal et al., 2019; Perez-Sen et al., 2015). Some P2Y receptors - P2Y$_1$, P2Y$_2$, and P2Y$_4$ - appear to be up-regulated in pathological conditions such as brain injury, Alzheimer's disease, and epilepsy (Franke et al., 2012). P2Y$_1$ receptor antagonism is associated with cerebroprotection and might improve cognition in Alzheimer's disease (Reichenbach et al., 2018) and gluta-mate mediated hippocampal neurodegeneration (Simões et al., 2018). P2Y$_1$ receptors could also be novel candidates for the treatment of epilepsy (Alves et al., 2019). However, P2Y$_1$ and P2Y$_{13}$ receptor

agonists could also display neuroprotective actions against oxidative stress and glutamate excitotoxicity as reported in other experimental models (Miras Portugal et al., 2019).

The $P2Y_{12}$ receptors exhibit a more restricted distribution, mainly located in microglia, where it plays relevant role in inflammation and neuropathic pain (Tozaki-Saitoh et al., 2008). Microglial $P2Y_{12}$ and $P2Y_{13}$ receptors and astroglial $P2Y_1$ receptors are new players in the complex microglia–astrocyte interaction after brain damage. After brain injury, ATP and ADP increase at extracellular space, and ADP acts as a chemotactic signal recruiting microglia to damaged area via $P2Y_{12}$ receptor activation. Activation of microglial $P2Y_{12}$ and $P2Y_{13}$ receptors releases cytokines and TNF-α, which down-regulate $P2Y_1$ receptors in astrocytes, avoiding astroglial proliferation. Down-regulation of astroglial $P2Y_1$ receptors by microglia changes astrocyte phenotype from pro-inflammatory to neuroprotective phenotype, increasing reactive astrogliosis (enhances glial fibrillary acidic protein expression), glial scar formation, restoring brain blood barrier functions, and suppressing leukocyte infiltration (Shinozaki et al., 2017). $P2Y_{12}$ receptors are also present in oligodendrocytes. Loss of these receptors could contribute to the demyelination process in multiple sclerosis (Amadio et al., 2010).

As previously mentioned, P2Y and P2X receptors modulate pain transmission (Burnstock, 2017). $P2Y_1$, $P2Y_2$, $P2Y_{12}$ and $P2Y_{13}$ receptors are expressed in nociceptive neurons and surrounding glial cells, and both pro-nociceptive and pro-analgesic effects have been proposed (Malin & Molliver, 2010). A complex regulatory mechanism for $P2Y_1$ and $P2Y_{13}$ receptors of glycine transporter function in glutamatergic and GABAergic synapsis confirms the important role of these receptors as targets to modulate different pain states at the spinal cord (Jiménez et al., 2011).

Concerning the intracellular mechanisms mediating P2Y receptor effects in neurons and glial cells, a great variety of intracellular routes have been unravelled that include the PI3K/Akt axis, glycogen synthase kinase 3, NF-κB, JAK-STAT, MAPK, transactivation of growth factor tyrosine-kinase receptors, and crosstalk with prostanoid receptors. Among them, MAPK activation deserves special attention. ERKs are responsible for astrocyte proliferation and migration (Franke et al., 2012; Paniagua-Herranz et al., 2017). ERK activation is also required for neuroprotective actions displayed by $P2Y_{13}$ receptors in cerebellar granule neurons and astrocytes (Miras Portugal et al., 2019). Novel players in P2Y receptor-activated signalling network encompass dual specificity protein phosphatases (DUSPs), which connect P2Y receptors to the inactivation mechanisms and fine-tuning of MAPK signalling. P2Y receptor-mediated DUSP regulation ensures proper intensity and duration of MAPK signalling required to preserve cell survival and to avoid the harmful impact of MAPK over-activation that can compromise cell viability and promote aberrant proliferation and differentiation rates (Miras Portugal et al., 2019). $P2Y_{13}$ receptors counteract genotoxic stress by inducing the nuclear DUSP2, which is responsible for the dephosphorylation of p38 in the nuclear compartment (Miras Portugal et al., 2019).

7 | P2Y RECEPTORS IN ENDOCRINE AND EXOCRINE FUNCTION

Endocrine and exocrine tissues/organs secrete hormones, cytokines, enzymes, and electrolytes and fluid, though in different direction—towards blood (endo-system) or through ducts to epithelial surfaces (exo-system). These two systems influence a whole spectrum of functions ranging from metabolism, digestion, to electrolyte/fluid homeostasis, development, growth, and reproduction. The role of purinergic signalling in major endocrine glands has been recently reviewed (Bjelobaba, Janjic, & Stojilkovic, 2015). Here, we focus on tissue/cells regulating metabolism: pancreatic beta-cells, adipocytes, and the liver.

Extracellular ATP affects insulin secretion from pancreatic beta-cells, and the positive or negative outcome depends on glucose levels and a particular P2 receptor expressed in a given species (Burnstock & Novak, 2013). ATP originates from nerves, insulin granules, and by transport through Panx1 (Tozzi et al., 2018). In rodent beta-cells and pancreas, $P2Y_1$ and $P2Y_6$ receptors stimulate insulin secretion (Balasubramanian, Maruoka, Jayasekara, Gao, & Jacobson, 2013), while in mouse, this effect is overshadowed by $P2Y_{13}$ receptors that inhibit secretion (Amisten et al., 2010). In human beta-cells, P2X receptors were thought to be more important, but recently, interest in the $P2Y_1$ receptors has been renewed (Khan et al., 2014), not the least because one single nucleotide polymorphism (SNP) in the 3′UTR of P2YR1 is associated with disturbance in glucose homeostasis (Todd et al., 2015). In addition to classical PLC/Ca^{2+} signalling stimulating insulin release, one study revealed alternative signalling. $P2Y_1$ receptor agonist ADP-βS stimulated cAMP/EPAC, which via PI3K inhibited Kv channels, but it is not clear whether this was mediated through the $P2Y_1$ and/or $P2Y_{11}$ receptors (Zhang, Wang, et al., 2015). Another study showed that the $P2Y_6$ receptors activated calmodulin-AMPK to increase insulin secretion in murine MIN6 cells (Balasubramanian et al., 2013). Regarding regulation of beta-cells mass, $P2Y_6$ receptors are cytoprotective, while $P2Y_{13}$ receptors are pro-apototic (Burnstock & Novak, 2013). Taken together, targeting P2Y receptors to increase insulin secretion and beta-cell mass would be a good strategy to ameliorate the course of Type 1 and Type 2 diabetes.

Adipose tissue releases a number of hormones and cytokines, which contribute to the regulation of appetite and satiety, fat distribution, insulin secretion and sensitivity, energy expenditure, and inflammation. White adipocytes express P2Y receptor subtypes that often have opposite effects on adipogenesis, lipogenesis, lipolysis, glucose transport, as well as on leptin and adiponectin production, reflecting adipocyte origin; therefore, the organ or body impact may differ (Tozzi & Novak, 2017). Most illustrative are studies on mice. $P2Y_1$ receptor deletion or its inhibition by MRS2500 reduced leptin production and secretion in lean mice, but this effect disappeared in mice on high-fat diet (Laplante, Monassier, Freund, Bousquet, & Gachet, 2010). Inhibition or deletion of $P2Y_4$ receptors increased adiponectin secretion in cardiac adipocytes and was cardioprotective (Lemaire et al., 2017). In $P2Y_2$ receptor knockout (KO) mice on high-fat diet, there was decreased immune cell infiltration and inflammation of adipose tissue, as well as a lower fat and body weight (Merz

et al., 2018). In addition, hepatic steatosis was improved, and animals were protected against insulin resistance and hypercholesteremia, all indicating that the P2Y$_2$ receptor is a promising target to combat the initial phases of metabolic syndrome. Brown adipocytes are specialized in energy expenditure, they express P2Y$_2$, P2Y$_6$, and P2Y$_{12}$ receptors and P2X receptors, but it is not clear yet how they regulate adipocyte function (Tozzi & Novak, 2017).

Recent work shows that plasma uridine levels are influenced by adipocyte-biliary clearance (Deng et al., 2017). Potentially, uridine levels could affect UDP/UTP levels, which is the case in the hypothalamus, where UDP acts on the P2Y$_6$ receptors of AgRP neurons and promotes feeding behaviour (Steculorum et al., 2015). This offers a challenging opportunity to target this receptor for the treatment of diseases associated with energy balance.

The major exocrine glands include pancreas, salivary glands, liver, sweat, sebaceous and tear glands and associated epithelia. They contribute to digestion and fluid secretion, contain various ions, enzymes, and mucus, and can be affected in a number of diseases, such as cystic fibrosis, Sjögren's syndrome (SS), and cancer. Here, we focus on the first three glands.

Liver, the metabolic hub, also performs endocrine function (secreting, e.g., IGF-1) and exocrine function (secreting bile). Not so much is known about the physiological role of purinergic signalling in endocrine function, but the role in liver diseases is well-reviewed, including the role of P2Y receptors on stellate cells and their relation to liver fibrosis (Lu & Insel, 2014). One key component is the P2Y$_2$ receptor , as KO mice are protected from liver damage and necrosis in experimental liver injury models (Ayata et al., 2012). Cholangiocytes, epithelial cells lining intrahepatic ducts, produce bile containing bile acids/salts and bicarbonate. The bile secretion requires a number of Ca^{2+}-activated ion channels that are stimulated via P2Y receptors , and one of these, the P2Y$_{12}$ receptor, resides on the primary cilia (Masyuk et al., 2008).

Pancreatic acini secrete ATP as well as digestive enzymes but do not express active P2 receptors (Haanes et al., 2014). In contrast, pancreatic ducts express various P2 receptors (Novak, 2011), where in particular, the luminal P2Y$_2$ and P2Y$_4$ receptors activate Ca^{2+}-activated Cl$^-$ and K$^+$ channels (TMEM16A, cystic fibrosis transmembrane conductance regulator, and K$_{Ca}$3.1) and thereby potentiate bicarbonate and fluid secretion (Wang, Haanes, & Novak, 2013), thus fine-tune pancreatic function. In contrast, distension of ducts via ATP release and P2Y$_2$ receptor inhibition of K$_{Ca}$1.1 channels on the basolateral membrane can inhibit secretion (Wang, Haanes, et al., 2013). Potentially, it would be important to increase pancreatic secretion, in, for example, cystic fibrosis or in pancreatitis, but access of drugs to the pancreatic lumen is not feasible.

Salivary gland acini secrete amylase and fluid, and here, P2X receptors are important. Ducts modify saliva to become hypotonic, and P2Y$_2$ receptor agonists increase ductal Cl$^-$ reabsorption via cystic fibrosis transmembrane conductance regulator (Novak, 2011). In addition to ion channel regulation via Ca^{2+} signalling, P2Y$_2$ receptors trans-stimulate EGFR that promotes gland repair and regeneration

(Ratchford et al., 2010). However, the P2Y$_2$ receptor is up-regulated in salivary gland inflammation, as in SS, and genetic ablation of P2Y$_2$ receptors in SS mouse models reduced gland inflammation (Woods et al., 2018). Other exocrine glands and epithelia of relevance are the tear glands and corneal epithelium. Secretion of tear glands is mainly regulated via P2X receptors, but minor glands and corneal epithelium express the P2Y$_2$ receptor that stimulates fluid and mucin secretion. This receptor has been a successful target of the drug diquafosol in treating dry eye diseases. A similar approach to target airway epithelia hydration in cystic fibrosis patients with the related drug denufosol (INS37217) did, however, not continue past the second phase III clinical trial (Kellerman et al., 2008).

8 | P2Y RECEPTORS IN THE CARDIOVASCULAR SYSTEM

P2Y receptors regulate or participate in all the physiological functions of the cardiovascular system, including heart contractility and cardiac frequency, vascular tone, release of endothelial factors, angiogenesis, smooth muscle cell proliferation, haemostasis, and immunity. As such, they are involved in pathological processes such as acute vascular inflammation, atherosclerosis, arterial thrombosis including myocardial infarction, and cerebrovascular stroke. All the eight P2Y receptor subtypes have been found in the cardiovascular system, that is, in the heart, the vasculature, including smooth muscle cells and endothelial cells, or in blood cells (Nishimura et al., 2017). Studies using combined techniques, such as selective antagonists, or tissue selective or global P2Y receptor subtype deficient mice, helped to elucidate the role of these receptors in most aspects of the cardiovascular system. Also, the role of P2YRs in renal function was recently reviewed (Vallon, Unwin, Inscho, Leipziger, & Kishore, 2019).

The earliest papers on the role of adenine compounds by Drury and Szent-Györgyi in 1929 reported on their effect on the mammalian heart. P2Y receptors , namely, P2Y$_1$ and P2Y$_{11}$ receptors, among other P2 receptor subtypes, including P2X receptors, are involved in cardiac function both indirectly via blood vessels and nerves and directly via the cardiomyocytes (Nishimura et al., 2017). These receptors have been reported to exert positive and negative inotropic effects as well as positive and negative chronotropic effects. The nucleotides also play a role in ischaemic preconditioning. All known receptor subtypes have been found, by RT-PCR, in cardiomyocytes. However, the cardiac function and vascular tone were evaluated in P2Y$_1$ receptor-deficient mice without detectable differences from those in wild-type mice (Gachet, unpublished work). These data do not mean that these receptors are not involved in these functions, but clearly, they either only participate in the regulation of these functions or the deficient mice compensated for receptor deficiency. Studies on isolated tissues from these mice indicate a role for P2Y$_1$ and P2Y$_{11}$ receptors in calcium signalling in the cardiomyocytes (Nishimura et al., 2017).

More is known concerning the vascular function, and one has to subdivide it in endothelial function and smooth muscle cell function.

In the endothelium, $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are responsible for nucleotide-induced vasodilation through release of **nitric oxide** and prostacyclin (Nishimura et al., 2017). In smooth muscle cells, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are responsible for vasoconstriction. $P2Y_{12}$ receptors are found in rat capillary endothelial cells (Simon et al., 2002), while other researchers found these receptors on smooth muscle cells where it may trigger vasoconstriction (Wihlborg et al., 2004) and smooth muscle cell proliferation during atherosclerosis (Rauch et al., 2010).

Indeed, in addition to their short-term effects on vascular tone, nucleotides and P2 receptors are also involved in long-term trophic effects on cell growth, proliferation, and death which has great implications for diseases such as atherosclerosis and restenosis (Nishimura et al., 2017). The $P2Y_1$ receptors especially play a key role during acute vascular inflammation (Zerr et al., 2011) and in atherosclerosis in apolipoprotein E-deficient mice (Hechler et al., 2008). Similar results have been reported concerning the $P2Y_2$ (Stachon et al., 2014) and $P2Y_6$ receptors (Garcia et al., 2014) in vascular inflammation.

P2Y receptors are expressed in all blood cells including monocytes, granulocytes, dendritic cells, red blood cells, and platelets. ATP and ADP are major agonists for platelets where they act on **P2X1**, $P2Y_1$, and $P2Y_{12}$ receptors in a coordinated fashion (Gachet, 2006). The main role of blood platelets is to ensure primary haemostasis, which is the maintenance of vessel integrity and cessation of bleeding upon injury. While playing a major part in acute arterial thrombosis, platelets are also involved in inflammation, atherosclerosis, and angiogenesis. ADP and ATP play a crucial role in platelet activation, and their receptors are potential targets for antithrombotic drugs. $P2Y_1$ and $P2Y_{12}$ receptors selectively contribute to platelet aggregation and formation of a thrombus. Owing to its central role in the growth and stabilization of a thrombus, the $P2Y_{12}$ receptor is an established target of anti-thrombotic drugs such as clopidogrel, prasugrel, and ticagrelor (Nishimura et al., 2017).

9 | P2Y RECEPTORS IN IMMUNE AND PULMONARY FUNCTION

As with P2X receptors, nucleotides acting at P2Y receptors are generally pro-inflammatory, and their antagonists may eventually be used to treat chronic inflammatory and painful conditions. However, a duality has been noted in which the same P2Y receptor subtype is associated with both damaging (or proinflammatory) and beneficial effects.

Immune cells all express various subtypes of P2Y receptors, which have been shown to be involved in the modulation of inflammation and in immune responses (Idzko, Ferrari, & Eltzschig, 2014). $P2Y_2$ receptor antagonists might be useful in treating pulmonary diseases, other chronic inflammations, or cancer. The $P2Y_2$ receptor contributes to inflammatory responses and fibrotic remodelling in allergic and inflammatory diseases, and thus, $P2Y_2$ receptor antagonists might prove useful in pulmonary and other chronic inflammatoroy diseases.

$P2Y_2$ receptor activation on neutrophils and eosinophils induces the release of IL8 and other inflammatory cytokines. $P2Y_2$ receptors on macrophages and neutrophils, activated by damaged cells, induces phagocytosis of bacteria and apoptotic cells to promote wound healing. Migrating neutrophils release ATP from the leading edge, which can induce chemotaxis of neutrophils, dendritic cells, and other immune cells. Platelets that are activated by cancerous tumours release ATP which can open the endothelial barrier to tumour cell extravasation and thus enabling metastasis, by activating an endothelial $P2Y_2$ receptor (Schumacher, Strilic, Sivaraj, Wettschureck, & Offermanns, 2013).

However, there is also reason to assess $P2Y_2$ receptor agonists for therapeutic application. $P2Y_2$ receptors on the lung epithelial surface induce Cl^- secretion and improve mucociliary clearance, raising the possibility of using an inhaled $P2Y_2$ receptor agonist for treating cystic fibrosis. Unfortunately, efforts to gain approval of denufosol failed due to the lack of demonstrating long-term efficacy in patients, possibly due to the simultaneous pro-inflammatory effects of activated $P2Y_2$ receptors (Kellerman et al., 2008).

On one hand, $P2Y_6$ receptor activation causes release of inflammatory chemokines from immune cells and from epithelial and endothelial cells. On the other hand, $P2Y_6$ receptors induce phagocytosis by microglial cells in the brain, which is beneficial in the removal of dying cell debris. Thus, like the $P2Y_2$, $P2Y_6$ receptors are associated with both beneficial and detrimental effects in disease conditions.

Microglial $P2Y_{12}$ receptors respond to ADP as a "find me" signal and induces activation and chemotaxis, suggesting a role of this receptor in neurodegeneration and pain signalling (Förster & Reiser, 2015; Tozaki-Saitoh et al., 2008). Platelets, which express $P2Y_1$ and $P2Y_{12}$ receptors, themselves play an important role not only in thrombosis but also in modulating inflammatory responses through release of inflammatory mediators or compounds with trophic activity and exposure of **P-selectin**, **CD40**, and **CD40 ligand**. These molecules allow interaction of platelets with immune cells and their subsequent activation with release of a range of inflammatory cytokines and exposure of platelet tissue factor (Morrell, Aggrey, Chapman, & Modjeski, 2014). Therefore, in addition to acting as antithrombotics, antagonists and inhibitors of platelet P2Y receptors could have anti-inflammatory effects.

$P2Y_{14}$ receptors activation promotes chemotaxis in human neutrophils, as studied in cystic fibrosis (Sesma et al., 2016), the release of proinflammatory cytokines from renal intercalated cells in sterile inflammation (Azroyan et al., 2015), and the degranulation of mast cells (Gao, Wei, Jayasekara, & Jacobson, 2013). Thus, $P2Y_{14}$ receptor antagonists are being developed for anti-inflammatory applications (Junker et al., 2016).

10 | P2Y RECEPTORS IN THE MUSCULOSKELETAL SYSTEM

P2Y receptors are expressed throughout the musculoskeletal system and mediate a variety of pharmacological responses (Burnstock,

Arnett, & Orriss, 2013). Their physiological and pathophysiological functions in skeletal muscle cells and cartilage chondrocytes, and how they might be targeted therapeutically, are still unclear, so the focus here is on bone, which not only provides support and protection for the body but also plays a central role in Ca^{2+} homeostasis. It is a dynamic tissue that turns over continually throughout life, and so requires a balance, termed bone remodelling, between formation, controlled by osteoblasts, and resorption, controlled by osteoclasts, and coordinated by osteocytes and stem cells (Burnstock et al., 2013; Wang & Gartland, 2014).

Musculoskeletal disorders (MSDs) encompass numerous conditions that are characterized by pain and reduced mobility (Burnstock et al., 2013). Furthermore, their incidence is increasing worldwide. MSDs are also a huge financial burden on health care systems, and many treatments are only moderately efficacious and/or expensive, so MSDs are an unmet health need. Thus, P2Y receptors represent potentially novel therapeutic targets for treating MSDs.

10.1 | P2Y receptors in osteoporosis

ATP, the main P2Y receptor agonist released in bone, has been implicated in osteoarthritis, rheumatoid arthritis, and cancer-induced bone diseases, but the P2X7 receptor is the main site of action studied (Wang & Gartland, 2014). In contrast, there is evidence for the involvement of multiple P2Y receptor subtypes in osteoporosis. This is the most common MSD, particularly in post-menopausal women. Oestrogen normally inhibits bone resorption by osteoclasts, and when this action is lost post-menopause, bone remodelling becomes unbalanced. Resorption exceeds formation, making bones fragile and more likely to break. As well as stimulating P2Y receptors per se, ATP is dephosphorylated to ADP in the extracellular space by ectonucleotidases, and together, the two agonists stimulate most of the eight P2Y receptor subtypes (Abbracchio et al., 2006, 2019). The limited availability of potent, subtype-selective antagonists has hindered progress in determining which P2Y receptor subtype(s) are stimulated in bone. Consequently, most advances have arisen from mouse P2Y receptor gene KO studies.

10.2 | P2Y$_2$ receptor

The P2YR2 genotype might be a useful prognostic marker for osteoporosis. Analysis of SNPs in Danish post-menopausal women found an association between the Arg312Ser SNP, which causes a $P2Y_2$ receptor gain-of-function, and higher bone mineral density (BMD) and lower rates of bone loss (Wesselius et al., 2011). A subsequent study in Dutch female fracture patients identified a higher incidence of another SNP, Leu46Pro (Wesselius et al., 2013). It is not known yet, however, how this SNP affects $P2Y_2$ receptor function.

Deleting the mouse $P2Y_2$ receptor gene has produced conflicting results. Higher bone mineral content and BMD compared to wild-type animals was seen by Orriss et al. (2017), but Xing et al. (2014) reported lower bone volume and strength. Consistent with the latter, overexpression of $P2Y_2$ receptors in female rats increased femoral length and strength of the femoral neck but had no effect on BMD (Ellegaard et al., 2017). That these disparities are due to differences in the mouse genetic background or in methodology used cannot, at present, be discounted. The recent commercial availability of AR-C118925XX, a selective, potent, and competitive $P2Y_2$ receptor antagonist (Rafehi, Neumann, et al., 2017), may help clarify these issues.

10.3 | P2Y$_{12}$ receptor

ADP, but not ATP, stimulates $P2Y_{12}$ and $P2Y_{13}$ receptors, and both subtypes appear to modulate bone remodelling. $P2Y_{12}$ receptor-KO mice showed lower pathological and age-related bone loss (Su et al., 2012). Consistent with this, treating wild-type mice with the selective $P2Y_{12}$ receptor antagonist, clopidogrel, which is widely prescribed as an antithrombotic agent, inhibited osteoclast formation and increased bone mass. However, a contemporaneous cohort study of osteoporotic fracture in Danish patients prescribed clopidogrel found a dual effect. Those receiving the clinically recommended high dose had an increased risk of fracture, whereas a low dose was associated with a lower risk than people who had not been exposed to clopidogrel (Jørgensen, Schwarz, Iversen, & Vestergaard, 2017). Thus, clopidogrel may potentially increase the risk of osteoporosis, though it should be noted that, while patients with stroke have an increased risk of osteoporotic fractures, clopidogrel does not appear to increase the fracture risk (Jørgensen et al., 2017).

10.4 | P2Y$_{13}$ receptor

Several studies using $P2Y_{13}$ receptor-KO mice indicate that this receptor may be protective towards bone growth. First, the mice showed reduced bone trabecular volume and rate of formation, reduced number and activity of trabecular osteoblasts, and less bone loss due to lack of oestrogen after ovariectomy (Wang, Rumney, et al., 2013). In addition, there was greater bone formation in response to mechanical loading, possibly because the receptor mediates negative feedback of the osteogenic agonist, ATP, release from osteoblasts (Wang, Rumney, et al., 2013). Finally, mesenchymal stem cells tended to differentiate towards adipocytes rather than osteoblasts (Biver et al., 2013). Thus, exercise combined with a $P2Y_{13}$ receptor antagonist is potentially a novel treatment for osteoporosis.

10.5 | P2Y$_6$ receptor

$P2Y_6$ receptors are activated by UDP, which appears to be released endogenously, as $P2Y_6$ receptor-KO mice had increased bone mineral

content, cortical bone volume, and cortical thickness in the long bones and spine, but trabecular bone was unaffected (Orriss et al., 2011). Thus, $P2Y_6$ receptor antagonists have potential for treating osteoporosis.

11 | CONCLUSIONS

Discovering biomedical applications of agonists or antagonists of P2Y receptors has proven challenging, due to difficulties in using inherently unstable mononucleotides and dinucleotides as pharmacological probes and the complex biological nature of ubiquitous P2Y receptors. Purinergic signalling pioneered by Burnstock and colleagues includes the involvement of P2Y receptors in all physiological systems, including (but not limited to) the following systems: CNS and peripheral nervous, endocrine and exocrine, cardiovascular, immune, and musculoskeletal. Selective agonists and antagonists are important for studying the roles of P2Y receptor subtypes in physiology and pathophysiology, and their structure–activity relationship has been developed in detail through extensive chemical efforts. Nevertheless, there are not yet selective and versatile agonists and antagonists for all of the P2Y receptor subtypes.

Phylogenetic studies have analysed the evolutionary history of P2Y receptors. To summarize, P2Y receptors evolved in early vertebrate evolution, and an expansion of P2Y receptors and P2Y receptor-related sequences is found in vertebrates. X-ray crystallographic structures have been determined for members of the two P2Y receptor subfamilies: the G_q-coupled $P2Y_1$ receptor (with orthosteric and allosteric antagonists) and the G_i-coupled $P2Y_{12}$ receptor (with orthosteric agonists and antagonists), and these structures are surprisingly structurally distinct. Phylogenetic, mutational, and crystallographic studies suggest that $P2Y_1$ and $P2Y_{12}$ receptors evolved independently by convergent evolution presenting the same agonist specificity but structurally different binding sites.

Nucleotides acting at P2Y receptors are generally pro-inflammatory, and their antagonists may eventually be used to treat chronic inflammatory conditions. However, a duality has been noted in which the same P2Y receptor subtype is associated with both damaging (or proinflammatory) and beneficial effects. Targeting of specific P2Y receptor subtypes has already been shown to be therapeutically useful in case of $P2Y_{12}$ receptor antagonists as antithrombotics, and $P2Y_2$ receptor agonists for dry eye disease. Current research data indicate that both the clinically validated P2Y receptor subtypes and the other six subtypes have great potential for the development of new pharmacotherapeutic strategies and novel future drugs.

12 | NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Fabbro et al., 2019a, 2019b; Alexander, Mathie et al., 2019).

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

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