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ORIGINAL ARTICLE

G protein-coupled adenosine (P1) and P2Y receptors: ligand design and receptor interactions

Kenneth A. Jacobson · Ramachandran Balasubramanian · Francesca Deflorian · Zhan-Guo Gao

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Abstract The medicinal chemistry and pharmacology of the four subtypes of adenosine receptors (ARs) and the eight subtypes of P2Y receptors (P2YRs, activated by a range of purine and pyrimidine mono- and dinucleotides) has recently advanced significantly leading to selective ligands. X-ray crystallographic structures of both agonist- and antagonist-bound forms of the A_{2A}AR have provided unprecedented three-dimensional detail concerning molecular recognition in the binding site and the conformational changes in receptor activation. It is apparent that this ubiquitous cell signaling system has implications for understanding and treating many diseases. ATP and other nucleotides are readily released from intracellular sources under conditions of injury and organ stress, such as hypoxia, ischemia, or mechanical stress, and through channels and vesicular release. Adenosine may be generated extracellularly or by cellular release. Therefore, depending on pathophysiological factors, in a given tissue, there is often a tonic activation of one or more of the ARs or P2YRs that can be modulated by exogenous agents for a beneficial effect. Thus, this field has provided fertile ground for pharmaceutical development, leading to clinical trials of selective receptor ligands as imaging agents or for conditions including cardiac arrhythmias, ischemia/reperfusion injury, diabetes, pain, thrombosis, Parkinson's disease, rheumatoid arthritis, psoriasis, dry eye disease, pulmonary diseases such as cystic fibrosis, glaucoma, cancer, chronic hepatitis C, and other diseases.

Keywords GPCR structure · Adenosine receptors · P2Y receptors · Agonists · Antagonists · Clinical trials, nucleosides · Nucleotides

Abbreviations

AR	Adenosine receptor
EL	Extracellular loop
GPCR	G protein-coupled receptor
IL	Intracellular loop
TM	Transmembrane helix
NECA	Adenosine-5′-*N*-ethyluronamide
PD	Parkinson's disease
PET	Positron emission tomography
SAR	Structure–activity relationship
SPECT	Single photon emission tomography
UDPG	Uridine-5′-diphosphoglucose

Introduction

There are four subtypes of adenosine receptors (ARs, or alternately P1 receptors), i.e., A_1, A_{2A}, A_{2B}, and A_3, and eight subtypes of P2Y receptors (P2YRs), i.e., a family of G_q-coupled $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$Rs and a second family of G_i-coupled $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$Rs (Table 1) [1, 2]. The native agonists for these twelve receptors are clearly divided between purine nucleosides (ARs) and purine and pyrimidine nucleotides (P2YRs), although a high concentration of AMP (1 mM) activates the A_1AR, independent of P2YR activity [3]. The two A_2 subtypes are coupled to G_s protein to stimulate adenylate cyclase, and the

K. A. Jacobson · R. Balasubramanian · F. Deflorian · Z.-G. Gao
Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

K. A. Jacobson (✉)
Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810, USA
e-mail: kajacobs@helix.nih.gov

Table 1 Properties of ARs and P2Y receptors and key ligands (including radioligands) suitable for cell biological studies (Ado=adenosine)

Group	Subtype, gene symbol	Chromosome, human, (length, amino acids)	Native agonist (human, pEC_{50})	Selective agonist (pEC_{50})	Selective antagonist (pIC_{50})	G protein
ARs	A_1, Adora1	1q32.1 (326)	Ado **1** (6.51)	S-ENBA **13** (9.47), CCPA **8** (9.08),[d] *R*-PIA **6** (8.82),[d] CPA **7** (8.65), CHA **11** (8.64),[d] GR79236 **10** (8.51), SPA **5** (6.2)	PSB-36 **38** (9.8), KW3902 **37** (9.1), SLV320 **39** (9.0), DPCPX **36** (8.5)[d]	G_i, G_o
	A_{2A}, Adora2a	22q11.23 (412)	Ado **1** (6.14)	ATL-146e **18** (9.30), UK-432097 **19** (8.40), CGS21680 **16** (7.64)[d]	ZM241385 **44** (9.0),[d] SCH442416 **47** (8.39), SCH58261 **46** (8.3)	G_s, G_{olf}
	A_{2B}, Adora2b	17p12 (332)	Ado **1** (4.59)	Bay60-6583 **21** (8.0)	PSB-603 **57** (9.3),[d] MRS1754 **52** (8.9),[d] MRE2029-F20 **54** (8.8),[d] PSB-0788 **58** (8.6),[d] MRS1706 **53** (8.4), PSB-1115 **56** (7.7), CVT-6883 **55** (7.7), PSB-298 (7.2)[d]	G_s, G_q
	A_3, Adora3	1p13.2 (isoform 1: 347)	Ado **1** (6.53), inosine (6.60)	IB-MECA **23** (8.85), Cl-IB-MECA **24** (8.74), I-AB-MECA (8.50),[d] CP532,903 **25** (8.24)	MRE3008-F20 **62** (9.1),[d] MRS1220 **64** (8.8), MRS1334 **66** (8.6), VUF5574 **67** (8.4), PSB-11 **61** (8),[d] MRS1523 **63** (7.7), MRS1191 **65** (7.5), MRS3777 **69** (7.3)	G_i
$P2Y_1$-like	$P2Y_1$, P2RY1	3q24-25 (373)	ADP **70** (5.09)	MRS2365 **76** (9.40)	MRS2500 **95** (9.02),[d] MRS2279 **94** (8.10),[d] MRS2179 **93** (6.48),[d]	G_q
	$P2Y_2$, P2RY2	11q13.5 (377)	UTP **79** (8.10), ATP **71** (7.07)	MRS2698 (8.10), MRS2768 **90** (5.72)	PSB-716 (5.01)	G_q, G_i
	$P2Y_4$, P2RY4	Xq13 (365)	UTP **79** (5.60)[a]	MRS4062 **83** (7.64)	b	G_q, G_i
	$P2Y_6$, P2RY6	11q13.5 (328)	UDP **78** (6.52)	5-iodo-UDP **85** (7.83), PSB-0474 **84** (7.15)	MRS2578 **109** (7.43) [noncompetitive]	G_q
	$P2Y_{11}$, P2RY11	19p31 (374)	ATP **71** (4.77)	NF157 **103** (7.35), NF546 **104** (6.27)[c]	NF340 **105** (7.14)	G_q, G_s
$P2Y_{12}$-like	$P2Y_{12}$, P2RY12	3q21-25 (342)	ADP **70** (7.22)[d]	b	PSB-0739 **101** (9.8), AR-C69931MX **98** (9.40), AZ11931285,[d] PSB-0413 (8.3),[d] AZD6140 **99** (7.90)	G_i
	$P2Y_{13}$, P2RY13	3q24-25 (354)	ADP **70** (7.94)	b	MRS2211 **108** (5.97)	G_i
	$P2Y_{14}$, P2RY14	3q24-25 (338)	UDP **78** (6.80),[d]UDP-glucose **88** (6.45)	MRS2690 **89** (7.31), MRS2802 (7.20)	Compound **116** (8.7)	G_i

[a] ATP acts as an antagonist at the human $P2Y_4$ receptor and as agonist at the rat and mouse $P2Y_4$ receptors

[b] Selective ligands not yet available

[c] NF546 activates the $P2Y_{11}R$, although it belongs to a structural class of antagonists

[d] Used as a radioligand, either in [^{3}H], [^{32}P], [^{33}P] or [^{125}I] form, as appropriate. The selectivity of [^{125}I]I-AB-MECA (4-amino analogue of **23**) for the A_3AR is low, and therefore binding to A_1AR is also seen. The listed A_3AR antagonist radioligands are suitable for use in primate but not rodent species. Other (nonselective) radioligands are: agonist [^{3}H]NECA **3** for $A_{2A}AR$, $A_{2B}AR$ or A_3AR; agonist [^{125}I]I-APNEA (3-iodo analogue of **4**) for the A_3AR; agonist [^{3}H] or [^{33}P]2-MeSADP **72** for $P2Y_1R$ or $P2Y_{12}R$. [^{33}P]ADP **70** is also used for binding to the $P2Y_{12}R$. [^{3}H]UDP **78** (K_d 10 nM) has been used for binding to the $P2Y_{14}R$. Chemical names: [^{3}H]PSB-0413, 2-propylthioadenosine-5′-adenylic acid (1,1-dichloro-1-phosphonomethyl-1-phosphonyl) anhydride; [^{3}H]PSB-298, [(8-{4-[2-(2-hydroxyethylamino)-2-oxoethoxy]phenyl}-1-propylxanthine]; [^{125}I]AZ11931285 (used at 125 pM), (1*S*,2*R*,3*S*,4*R*)-2,3-dihydroxy-4-[7-[[(2*E*)-3-iodoprop-2-en-1-yl]amino]-5-(propylthio) 3H-[1–3]triazolo[4,5-d]pyrimidin-3-yl]cyclopentane-carboxylic acid

other two AR subtypes inhibit adenylate cyclase through G_i protein. In some cells, the A_{2B}AR is dually coupled to G_s and G_q and consequently elevates phosphoinositides, mobilizes calcium and activates phospholipase C and MAPK [111–115]. This signaling pathway appears to be important in mast cells, in which A_{2A} and A_{2B}ARs have opposing actions. ARs are the site of action of widely consumed alkylxanthines, which act as competitive antagonists. Knockout mice and selective ligands as pharmacological tools (Figs. 1, 2, and 3) are now available for all AR subtypes and for many of the P2Y subtypes (except for $P2Y_{11}$ that is absent in the mouse) [4]. Many ligands for these receptors are under consideration for pharmaceutical development.

Processing of ARs and P2YRs in the cell has been studied, including posttranslational modification and trafficking, intracellular localization, and the related phenomena of agonist-induced desensitization, internalization, and degradation [5–7]. The mechanisms of release, uptake, and degradation of extracellular nucleosides and nucleotides have also been explored [8, 9]. The ubiquitous presence of pharmacologically active endogenous ligands of ARs and P2YRs warrants careful consideration in experimental design.

Structure of adenosine and P2Y receptors

The ARs and the P2YRs share the overall topological structure typical of G protein-coupled receptors (GPCRs) belonging to family A: seven α-helical domains (TM) that cross the cell membrane and are connected by three extracellular (ELs) and three intracellular (IL) loops, the N terminus in the extracellular part and the C terminus in the intracellular part of the receptor. Nevertheless, from comprehensive sequence comparisons and phylogenetic analyses, it is clear that ARs and P2YRs belong to two different groups of the rhodopsin-like family of GPCRs [10], respectively, the rhodopsin α-group and γ-group of GPCRs [11].

Within the AR family, the average sequence identity between subtypes of the same species is about 47%, which increases to an average of ~57% if only the TM domains are considered. The residues in the binding cavity involved in ligand recognition are mostly conserved among the AR subtypes and between species, with the A_3AR being the most divergent from the other subtypes, as shown in Table 2. Specific variable amino acids in the binding site are most likely involved in the ligand selectivity or the unique pharmacological behavior of each AR subtype.

For the ARs, structural information has been available since 2008 with the high resolution X-ray structure of the human A_{2A}AR in complex with the antagonist 4-(2-[7-amino-2-(2-furyl)]1,2,4] triazolo[2,3-a][1,3,5]triazin-5-yl-amino] ethyl)phenol **44** (ZM241385) [12]. The crystal structure of the A_{2A}AR in its inactive conformation gave insight into the ligand recognition mechanism, showing the key residues involved in the ligand binding and the major interactions anchoring the antagonist to the binding site. Many of the site-directed mutagenesis data previously available for the ARs were structurally explained, and new mutational experiments were guided by the knowledge gained from the A_{2A}AR structure, helping to further define the ligand binding cavity of this AR [13].

The antagonist-bound A_{2A}AR structure has since improved widely the modeling approaches to the ARs, suggesting for example the possible binding modes of agonists to the A_{2A}AR [13, 14] or aiding the modeling of the other AR subtypes [15]. Before the release of the A_{2A}AR X-ray structure, other structural templates were used for the modeling of the ARs, as detailed in a recent review by Dal Ben et al. [16]. More recently, new crystal structures of a thermostabilized (by mutagenesis) A_{2A}AR in complex with the xanthine derivative 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-l,3-dipropylxanthine (XAC, structure not shown) and caffeine **30** were made available [17]. New structural insights into the activation mechanism and the conformational changes that occur upon agonist binding to the A_{2A}AR were revealed with the recent release of new crystal structures of the A_{2A}AR in complex with different agonists, the bulky substituted agonist 2-(3-[1-(pyridin-2-yl)piperidin-4-yl]ureido)ethyl-6-*N*-(2,2-diphenylethyl)-5′-*N*-ethylcarboxamidoadenosine-2-carboxamide **19** (UK432097) [18], the native agonist adenosine **1**, and the non-selective adenosine-5′-*N*-ethyluronamide **3** (NECA) [19]. These new crystal structures lack a coupled G protein, but nevertheless, they are helpful in understanding the function of the ARs, and they will aid the drug design approaches for the AR family, thereby also improving the quality of models for other AR subtypes. The conformational changes upon activation of the A_{2A}AR resemble those of opsin, but there are other changes more specific to this receptor structure such as a see-saw movement of TM7 [18]. Homology models of A_1AR and A_3AR based on the agonist-bound structure of the A_{2A}AR, were recently proposed with docked agonists in the binding site [15].

The crystal structures of the A_{2A}AR revealed a peculiar orientation of the ligand in the binding site of the receptor when compared to the available structures of other GPCRs, e.g., bovine rhodopsin or the β-adrenergic receptors. The binding site for the ligands in the ARs is located near the extracellular portion of the domains TM3, TM5, TM6, and TM7, and the ligands are in a roughly vertical orientation with respect to the plane of the membrane. A crucial residue anchoring the aromatic core of agonists and antagonists in the binding pocket of A_{2A}AR is Asn6.55 (using the numbering convention of Ballesteros and Weinstein [20]), a residue conserved among the AR subtypes and also among different species. His6.52, Thr3.36, Ser7.42, and His7.43 play key roles in the binding of the hydrophilic ribose moiety of

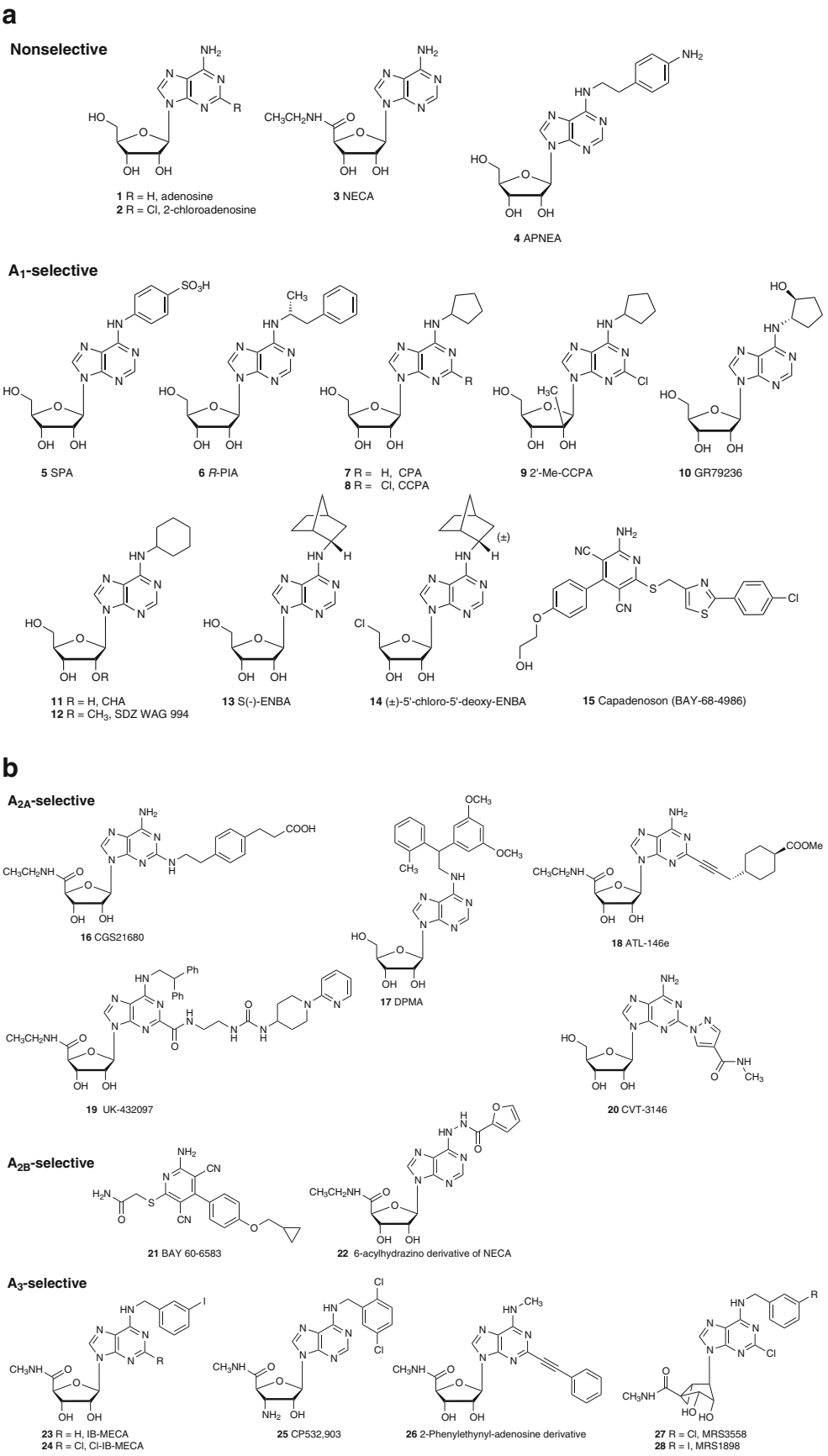

Fig. 1 **a** Nonselective AR and A_1AR selective agonists (including nucleosides and a nonnucleoside derivative **15**). **b** A_{2A}AR, A_{2B}AR, and A_3AR selective agonists (including nucleosides and a nonnucleoside derivative **21**). AR affinites (Table 1) and selectivities of many of these ligands are available [1, 55]

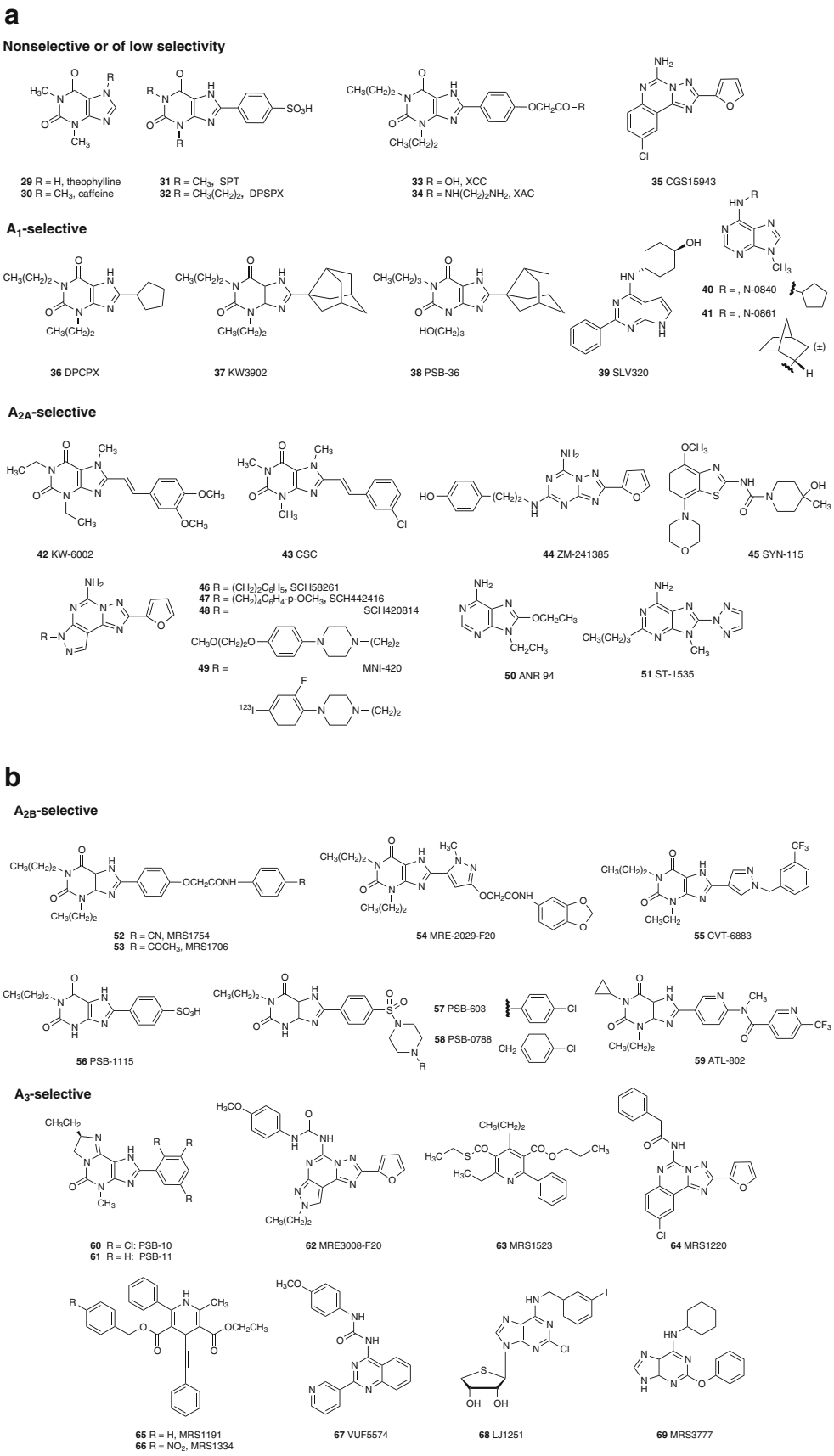

Fig. 2 **a**. Nonselective AR antagonists and A_1AR and A_{2A}AR selective antagonists (including xanthines and nonxanthine derivatives **44**–**51**). **b** A_{2B}AR (xanthines **52**–**59**) and A_3AR selective (nonxanthine derivatives **60**–**69**) antagonists. Compound **68** is a truncated nucleoside derivative that displays A_3AR antagonist properties. AR affinites (Table 1) and selectivities of many of these ligands are available [1, 42, 55]

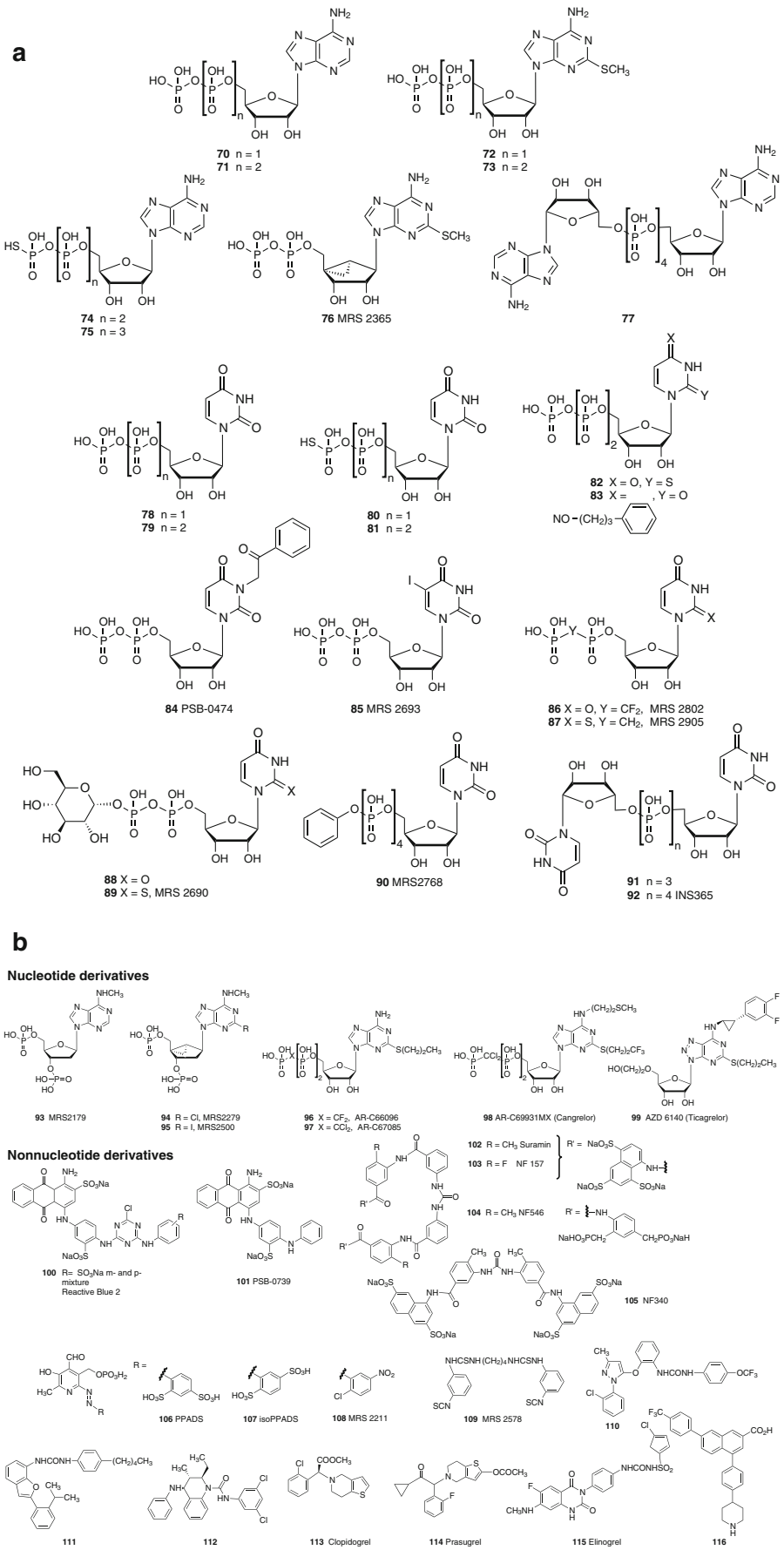

Fig. 3 **a** Nonselective and selective P2YR agonists and related substances (including nucleotide derivatives). **b** Nonselective and selective P2YR antagonists (including nucleotide and nucleotide derivatives). Note that NF546 **104** is a $P2Y_{11}R$ agonist, although it belongs to a structural class of antagonists. Compound **110** is a $P2Y_1R$ antagonist containing a novel chemotype, and **111**–**115** are $P2Y_{12}R$ antagonists. Compound **116** is a $P2Y_{14}R$ antagonist containing a novel chemotype. P2Y potencies of many of these ligands are available in Table 1 or reference [2]

Table 2 List of the key residues in the binding pocket of the A_{2A}AR compared with the corresponding residues in other AR subtypes and in ARs of different species (*h*, human; *m*, mouse; *r*, rat)

Rec/Res	TM2 2.61	TM3 3.32	TM3 3.33	TM3 3.36	TM3 3.37	EL2	EL2	TM5 5.38	TM5 5.42	TM6 6.48	TM6 6.51	TM6 6.52	TM6 6.55	TM7 7.35	TM7 7.39	TM7 7.42	TM7 7.43
h_A_1	A66	V87	L88	T91	Q92	F171	E172	M180	N184	W247	L250	H251	N254	T270	I274	T277	H278
m_A_1	A66	V87	L88	T91	Q92	F171	E172	M180	N184	W247	L250	H251	N254	I270	I274	T277	H278
r_A_1	A66	V87	L88	T91	Q92	F171	E172	M180	N184	W247	L250	H251	N254	I270	I274	T277	H278
h_A_{2A}	A63	V84	L85	T88	Q89	F168	E169	M177	N181	W246	L249	H250	N253	M270	I274	S277	H278
m_A_{2A}	A60	V81	L82	T85	Q86	F163	E164	M172	N176	W241	L244	H245	N248	M265	I269	S272	H273
r_A_{2A}	A60	V81	L82	T85	Q86	F163	E164	M172	N176	W241	L244	H245	N248	M265	I269	S272	H273
h_A_{2B}	A64	V85	L86	T89	Q90	F173	E174	M182	N186	W247	V250	H251	N254	M272	I276	S279	H280
m_A_{2B}	A64	V85	L86	T89	Q90	F173	E174	M182	N186	W247	V250	H251	N254	M272	I276	S279	H280
r_A_{2B}	A64	V85	L86	T89	Q90	F173	E174	M182	N186	W247	V250	H251	N254	M272	I276	S279	H280
h_A_3	A69	L90	L91	T94	H95	F168	V169	M177	S181	W243	L246	S247	N250	L264	I268	S271	H272
m_A_3	A70	L91	L92	T95	H96	F169	R170	M178	S182	W244	L247	S248	N251	M265	I269	S272	H273
r_A_3	A71	L92	L93	T96	H97	F170	R171	M179	S183	W245	L248	S249	N252	M266	I270	S273	H274

Residues are denoted in the top row by a numbering convention as described [20]

Images displaying the role in ligand binding of amino acids in the binding site of various ARs are found in references [12–19, 62]

nucleoside agonists, while they are less critical in the binding of antagonists. Residues from the extracellular domain EL2 are also involved in anchoring ligands in the binding pocket. Phe168 in A_{2A}AR, a residue conserved among the ARs, interacts through a strong π–π stacking with the aromatic core of agonists and antagonists. Glu169, conserved in A_1AR and A_{2B}AR but substituted with a hydrophobic valine in human A_3AR, interacts with both agonist and antagonist H-bond donor groups, i.e., the exocyclic amino group of ZM241385, the exocyclic amino groups of NECA and adenosine, or the urea moiety at the C2 position of UK432097. Trp6.48, the so-called "toggle switch" of GPCR activation, is conserved among the ARs and was found in close proximity to the ligands in the A_{2A}AR complexes. While the residues surrounding the ligand core in the A_{2A}AR structure are mostly conserved in the binding pocket of the AR subtypes, other residues are less conserved, and possibly, they might be involved in the selectivity of the receptors for different substituted ligands. Those less conserved residues are located mainly in the most extracellular part of the binding cavity embedding the substitutuent groups projecting from the core of the ligands. For example, Leu267(7.32) in A_{2A}AR is substituted with a serine in A_1AR, with a lysine in A_{2B}AR, and a glutamine in A_3AR. Met270(7.35) in A_{2A}AR is a threonine in A_1AR and a leucine in A_3AR. Also in EL3, there are some nonconserved residues, such as His264 of A_{2A}AR, which is substituted with an asparagine in A_{2B}AR and a glutamate in A_3AR (Table 2).

Sequence alignments, phylogenetic analysis, and effector coupling of the P2YRs have distinguished two P2YR subfamilies [2, 21]. The $P2Y_1$-like family activates the phospholipase C signaling pathway through coupling with G_q protein. $P2Y_{11}$R also couples with G_s protein to activate adenylate cyclase. The other family of P2YRs is the $P2Y_{12}$-like family, which couples to G_i protein to inhibit the adenylate cyclase pathway [23, 24]. The sequence identity between the two subfamilies is quite low, with only 20% identity between $P2Y_1$R and $P2Y_{12}$R, while the sequence identity is higher between the members within the same subfamily, for example, with a 45% identity between $P2Y_{12}$R and $P2Y_{14}$R.

Unlike the ARs, no experimentally determined structural information is yet available for the P2YR family, and so far, the only structural characteristics of the P2YRs have come from structural modeling [21, 25–28]. Mostly, the modeling of P2YRs has focused on identifying the putative binding site and the analysis of the residues involved in the ligand binding and receptor specificity, with the aim to gain information on the ligand recognition mechanism. Site-directed mutagenesis and structure–activity relationship (SAR) analysis have been used to support and guide the modeling of the P2YRs [29–36], which has been used to identify new key residues important for the ligand binding and receptor activation [25, 37–39]. Several models based on different structural templates have been published for many of the P2YRs. The bovine rhodopsin crystal structure was used to build models for $P2Y_1$R [21, 39–41], $P2Y_2$R [31, 35, 42], $P2Y_4$R [42], $P2Y_6$R [43, 44], $P2Y_{11}$R [45], $P2Y_{12}$R [21], and $P2Y_{14}$R [46].

The putative binding pocket of the $P2Y_1$R, suggested by the modeling and supported by the many available mutagenesis data, is located near the extracellular region of TM3, TM6, and TM7. The positively charged residues of $P2Y_1$R Arg3.29, Lys6.55, and Arg7.39, conserved among the $P2Y_1$-like receptors (Table 3), appear to be involved in the coordination of the negatively charged phosphate groups of

Table 3 List of the key residues in the putative binding pocket of the human P2YRs

Rec/Res	TM3 3.29	TM3 3.32	TM3 3.33	TM3 3.37	TM5 5.47	TM6 6.48	TM6 6.51	TM6 6.52	TM6 6.55	TM6 6.58	TM7 7.35	TM7 7.36	TM7 7.39
$P2Y_1$[b]	**R128**	F131	H132	Y136	F226	**Y273**	F276	**H277**	**K280**	N283	Y306	**Q307**	**R310**
$P2Y_2$[b]	R110	F113	Y114	**Y118**	F207	F258	F261	H262	R265	**Y268**	Y288	K289	R292
$P2Y_4$	R112	F115	Y116	Y120	F209	F258	F261	H262	R265	Y268	Y288	K289	R292
$P2Y_6$	R103	F106	Y107	H111	F201	F252	F255	H256	K259	Y262	Y283	K284	R287
$P2Y_{11}$	R106	F109	T110	L114	C214	**Y261**	Y264	H265	**R268**	N271	Y303	Q304	**R307**
$P2Y_{12}$	S101	F104	Y105	Y109	**F198**	F249	F252	**H253**	**R256**	**Y259**	**K280**	E281	L284
$P2Y_{13}$	S99	F102	Y103	Y107	F217	F247	F250	H251	R254	Y278	K278	E279	L282
$P2Y_{14}$	A98	F101	Y102	Y106	F195	F246	F249	H250	R253	Y256	K277	E278	L281

Residues are denoted in the top row by a numbering convention as described [20]. Residues in bold have been shown using site directed mutagenesis to be important in ligand recognition

Images displaying the role in ligand binding of amino acids in the binding site of various P2YRs are found in references [25–27, 31, 33, 35, 38, 40, 42–47, 49, 50]

Residues in the ELs that affect ligand recognition include: D204, E209, and R287 (in $P2Y_1R$) [31]; R177, R180, and R272 (in $P2Y_2R$) [31]

nucleotide ligands. Hydrophilic residues in the binding pocket were suggested to surround the ribose moiety of the nucleotides, while hydrophobic residues created a favorable environment for the aromatic nucleoside core of the nucleotides derivatives. Position 6.52 is a histidine residue that is conserved across the P2YRs, and mutagenesis studies on $P2Y_1R$, $P2Y_2R$, and $P2Y_{12}R$ have implicated this residue in ligand recognition [28, 33, 35, 47]. Mutagenesis studies on the $P2Y_1R$ also showed the crucial role of two disulfide bridges for the correct function of the receptor: a disulfide bridge between TM3 and EL2, conserved among family A GPCRs, and a second disulfide bridge between the N terminus and EL3 [32].

The P2YR models have been improved and refined continually using updated information from studies of their structural biology and mutagenesis. Recent advances in the structural biology of GPCRs have provided alternative templates to the bovine rhodopsin structure as a basis for the homology modeling. For example, the sequence identity between rhodopsin and human $P2Y_{12}R$, the site of action of the active metabolite [37] of the blockbuster antithrombotic Clopidogrel [2], is only 16% overall and 19% for only the TM regions, while the sequence identity between human $P2Y_{12}R$ and the chemokine receptor CXCR4 is 22% overall and 26% for the TM domains. The X-ray structures of the human CXCR4 in its inactive state in complex with a small antagonist and a long peptide were released in late 2010 [48]. A sequence comparison between $P2Y_{12}R$ and CXCR4 suggested other structural features of CXCR4 that might be shared by $P2Y_{12}R$, making the crystal structure of CXCR4 a more suitable template than other available GPCR crystal structures for the modeling of $P2Y_{12}$ and other P2YRs [49, 50]. A model of $P2Y_{12}R$ based on the CXCR4 crystal structure and guided by the mutagenesis data and SAR studies available on the $P2Y_{12}R$ was recently published [49]. The homology models of $P2Y_2R$ and $P2Y_4R$ based on the CXCR4 crystal structure were used to explain the selectivity of agonists toward these two P2YR subtypes [50]. The modeling studies of $P2Y_{12}R$ showed how key residues in the binding pocket in TM6 and TM7, Arg6.55, Lys7.35, and Tyr6.58, were involved in the anchoring of the negatively charged phosphate groups of the nucleotide ligands. Those residues are conserved across the $P2Y_{12}$-like subfamily of P2YRs, as shown in Table 3. Other hydrophobic or aromatic residues from TM1, TM3, TM6, and TM7 were suggested to form a suitable environment for the aromatic core of nucleotides derivatives, while the ribose moiety was surrounded by hydrophilic residues.

Key ligand tools for studying adenosine and P2Y receptors

AR agonists and antagonists

Selective agonist and antagonist ligands for each of the four AR subtypes are now available as pharmacological tools. The medicinal chemistry of the $A_{2B}AR$ is the least developed of the four subtypes, with selective antagonists and a few selective agonists reported only since 2000 [51, 52]. The optimal binding features of AR ligands have also been predicted on the basis of quantitative SAR (QSAR) approaches, such as comparative molecular field analysis (CoMFA) [116–119], although the use of X-ray structural data is now able to provide greater insight than earlier approaches. The selective AR ligands now include compounds that are stable in vivo, high affinity radioligands for binding assays or in vivo imaging by positron emission tomography or single photon

emission tomography [53], that bind irreversibly to the receptor affinity probes, fluorescent and other spectroscopic probes, and multivalent conjugates that retain high potency. An overview of SAR is provided below for AR agonists and antagonists.

1. Agonists: The SAR of adenosine derivatives as AR agonists has been well explored [1, 54], and selective agonists and antagonists for all four subtypes have been reported (Figs. 1 and 2 and affinities of selected compounds listed in Table 1). Data on selectivity of AR ligands have been collected [1, 55]. Typically, these agonists are nucleoside derivatives substituted at one or more of the following positions: ribose 5′, adenine C2, and adenine N^6. Hydrophobic groups substituted at the adenine C2 position (linked by NH or S) often provide selectivity for the A_{2A}AR (e.g., **16**, **18**, **20**), and hydrophobic groups substituted at the adenine N^6 position often provide selectivity for the A_1AR (e.g., **5**–**14**). An N^6-(4-aminophenylethyl) derivative, APNEA **4**, is a nonselective AR agonist with high affinity for both A_1 and A_3ARs, and has been used as a radioligand in its [^{125}I] 3-iodo form. In exceptional cases, this selectivity pattern may be altered to display A_{2A}AR selectivity, as in **17**, or with combined modifications, as in **19**. Nonnucleoside agonists of the A_1AR are also known, including the clinical candidate Capadenoson **15** [56].

   The most common ribose modification that enhances AR potency is a small *N*-alkyl-uronamide at the 5′ position, as in the potent nonselective agonist NECA **3**, an *N*-ethyl-uronamide. The presence of an *N*-ethyl-uronamide is typical of A_2AR-selective agonists (A_{2A}: **16**, **18**, and **19**; A_{2B}: **22**), and a *N*-methyl-uronamide is typical of A_3AR-selective agonists (**23**–**28**). The ribose moiety of nucleoside ligands having high AR affinity could also be substituted with a limited set of other modifications [54, 55, 57–60], for example: carbocyclics, including a ring-constrained methanocarba (fused cyclopropyl and cyclopentyl rings as in A_3AR-selective **27** and **28**), 4′-thio in place of oxo (**68**), 2′-methyl (**9**), 2′-methoxy (**12**), and 3′-amino-3′-deoxy (**25**). By comparing the AR binding affinities of isomeric bicyclic methanocarba adeonsine analogues that maintain either a North (*N*), as in **27** and **28**, or a South (*S*) conformation, it was determined that there is a strong preference for the (*N*) conformation in binding to the A_3AR. This bicyclic modification of ribose often enhances the affinity, as well as selectivity, at the A_3AR [54]. The (*N*)-methanocarba modification is also preferred over the (*S*)-methanocarba modification at the A_1AR, but affinity enhancement was not observed. The preference of the (*N*) over (*S*) conformation of the ribose moiety was also determined using C methylation at the 2′ and 3′ positions [59].

   It is to be noted that some nucleoside derivatives act as full agonists at certain AR subtypes and antagonists or partial agonists at other subtypes. Typically, the efficacy at the A_3AR is particularly sensitive to structural modification of the nucleoside derivative. Thus, reducing the flexibility or H-bond donating ability of the ribose moiety, especially around the 5′-amide group, or introducing certain sterically bulky hydrophobic substituents at the N^6 or C2 position tends to lower the relative efficacy at the A_3AR [61]. Various 8-cycloalkylamino adenosine derivatives or those modified at the ribose hydroxyl positions have reduced efficacy at the A_1AR or A_{2A}AR [60]. The introduction of bulky groups at the 5′ position has been shown to reduce efficacy at the A_1AR [56].

   Selective agonists for the A_1AR [54, 57] include: R-PIA **6**, CPA **7** and its more selective 2-chloro analogue CCPA **8**, and CHA **11** (all of which have been radiolabeled as tracers for binding experiments); SPA **5** (excluded from crossing the blood brain barrier). CPA, CCPA, and CHA are more selective for the A_1AR in mouse than in human, in comparison to the A_3AR. (S)-ENBA **13** displays high A_1AR selectivity (human, rat) in comparison to both A_{2A}AR and A_3AR, but also has reduced water solubility. A 4′-truncated (N)-methanocarba nucleoside containing an N^6-dicyclopropylmethyl group (not shown) fully activated the A_1AR with moderate selectivity [15]. CGS21680 **16** and DPMA **17** are A_{2A}AR selective in binding to the rat and mouse A_{2A}ARs, but in the human, they bind with similar affinity to the A_3AR. The nonnucleoside 3,5-dicyanopyridine derivative **21** and the nucleoside derivative **22** are moderately A_{2B}AR selective. A_3AR-selective agonists typically have combined N^6 and ribose 5′ modications (**23**–**28**). Introduction of certain bulky groups at the 5′ position of A_1AR reduced the efficacy in functional assays, to provide partial agonists [57]. N^6-Benzyl substitution tends to provide greater between-species consistency in A_3AR binding affinity [61], while small N^6-alkyl groups, such as methyl (**26**) are often more potent at the human A_3AR than at rat and mouse A_3ARs. However, N^6-benzyladenosine derivatives are variable in their A_1AR binding affinity depending on the substitution pattern, which can reduce A_3AR selectivity. The product of enzymatic action of adenosine deaminase, inosine, also activates the A_3AR in the micromolar range [1].
2. Antagonists: The prototypical AR antagonists are theophylline **29**, caffeine **30** and other naturally occurring xanthines, but these are of micromolar affinity and not subtype-selective antagonists. Both synthetic purine and nonpurine (e.g., nonselective **35**, A_1AR-selective **39**, A_{2A}AR-selective **44**–**49**, A_3AR-selective **60**–**67**) heterocycles have been extensively explored as subtype-selective AR antagonists [55]. Purine derivatives as

selective AR antagonists include xanthines (e.g., high affinity 8-phenylxanthines **31**–**34** (including water-soluble and peripherally selective sulfophenyl derivatives **31** and **32**), A_1AR-selective 8-cycloalkylxanthines **36**–**38**, A_{2A}AR-selective 8-styrylxanthines **42** and **43**, A_{2B}AR-selective 8-arylxanthines **52**–**59**) and adenines (e.g., A_1AR-selective **39**–**41**, A_{2A}AR-selective **50** and **51**, A_3AR-selective **69**). A_3AR-selective nucleoside **68** behaves as an antagonist in functional assays, which is related to the absence of the 5′-hydroxymethyl group that is associated with the conformational change needed to activate the A_3AR [15].

The in silico screening of chemical libraries of diverse structure by docking to an X-ray structure or even a homology model is now an accepted method of discovering new chemotypes that bind to a given GPCR. Non-nucleotide antagonists of ARs and P2YRs have been discovered in this manner [62].

P2YRs

Progress in the development of selective agonist and antagonist ligands for P2YRs (Fig. 3, and potencies of selected compounds listed in Table 1) has accelerated in recent years. Detailed SAR analyses are available for activation by nucleotides of most of the P2YRs [2, 63]. One must keep in mind that extracellular nucleotides can be interconverted in situ to different phosphate forms or to the corresponding nucleoside, which may complicate pharmacological studies. In some cases, the addition of an inhibitor of ectonucleotidases or of other enzymes involved in this conversion, or even addition of a purified enzyme, aids in the interpretation of pharmacological data. A challenge is to design P2YR ligands that are stable in vivo. Nevertheless, there are now nucleotide agonists selective for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{14}$Rs and nucleotide antagonists selective for $P2Y_1$ and $P2Y_{12}$Rs. The diastereoselectivity of binding of the phosphate groups of nucleotide agonists selective at the $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_{11}$ Rs has been characterized [29, 41, 42]. Also, subtype-selective non-nucleotide antagonists have been introduced for $P2Y_1$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$Rs. Isolated reports have suggested non-nucleotide antagonists of the $P2Y_2$R, but these so far are weakly binding. Chemically diverse library screening is now being applied to the problem of identifying new structural leads for receptor antagonists, e.g., the A_{2A}AR, $P2Y_{12}$R and $P2Y_{14}$R [49, 62, 64]. A general description of SAR is provided below for each of the P2Y subtypes.

$P2Y_1$R One of the earliest potent agonists of the $P2Y_1$R identified was 2-MeSADP **72** (Fig. 3a). However, like the native agonist ADP **70**, it also activates the $P2Y_{12}$R and $P2Y_{13}$R. There has been a question about the ability of 5′-triphosphate derivatives such as 2-MeSATP **73** to activate the $P2Y_1$R; some studies show it to be an agonist while others demonstrate low efficacy [2].

The introduction of conformationally restricted (i.e., rigid) ribose substitions has established the favored ribose-ring conformation for each of the subtypes of the $P2Y_1$-like subfamily [38, 43]. Principally, this approach has made use of the methanocarba ring system consisting of fused cyclopropane and cyclopentane, as applied earlier to the ARs, in exploring the biologically active conformations of nucleoside and nucleotide derivatives. Thus, the North (*N*)-methanocarba analog of 2-MeSADP, i.e., MRS 2365 **76** is a selective, high affinity agonist of the $P2Y_1$R that does not appreciably activate the other ADP-preferring subtypes, i.e., $P2Y_{12}$ and $P2Y_{13}$Rs [65]. The (*N*)-methanocarba modification is also known to improve the stability of the phosphate esters toward nucleotidases, especially the 5′-monophosphate toward the hydrolytic action of the ectonucleotidase CD73. Borano analogues of the phosphate group have been found in some cases to preserve potency and to enhance selectivity of P2YR agonists, e.g., $P2Y_1$R agonists [66].

Many nucleotide antagonists of the $P2Y_1$R have been introduced. Usually, these are adenine nucleotides containing bisphosphate groups, for example, a ribose 3′,5′-bisphosphate moiety. N^6-methyl 2′-deoxyadenosine bisphosphate derivatives MRS 2179 **93** and its 2-chloro analogue MRS 2216 (not shown) are selective $P2Y_1$ antagonists [38]. In both agonist and antagonist series, only limited substitution of the N^6 position of ADP and other nucleotides, i.e., methyl and ethyl, is tolerated at the $P2Y_1$R. The same (*N*)-conformational constraint of the ribose moiety that enhances $P2Y_1$R agonist action also favors potency and selectivity in nucleotide antagonists. For example, the ring-constrained (*N*)-methanocarba nucleotide bisphosphates MRS 2279 **94** and MRS 2500 **95** are selective, high affinity antagonists of the $P2Y_1$R [67]. Antagonists of the $P2Y_1$R of moderate affinity may also be derived from acyclic nucleotides, such as the bisphosphate derivative MRS 2298 (not shown) [67].

A representative antagonist of the $P2Y_1$R discovered through optimization of a high throughput screening hit is a substituted 1-phenyl-3-methyl pyrazol-5-one **110**, which has a K_i of 90 nM and is orally bioavailable [68]. Other structurally diverse antagonists of the $P2Y_1$R have been reported.

$P2Y_2$ and $P2Y_4$Rs UTP **79** is a native agonist of both $P2Y_2$R and $P2Y_4$R. Another native ligand, ATP **71**, activates the $P2Y_2$R, but at the $P2Y_4$R its action is species-dependent, i.e., it acts as an antagonist at the human homologue and agonist at the rat $P2Y_4$R. Synthetic UTP analogues with selectivity for the $P2Y_2$R have been reported, e.g., UTPγS

77, 2-thioUTP **82** and MRS 2698 (not shown), which is 300-fold $P2Y_2R$-selective in comparison to the $P2Y_4R$ [35]. Recently, an N^4-alkoxyimino derivative of CTP, MRS 4062 **83**, was found to be a full agonist of the $P2Y_4R$ with ~30-fold selectivity in comparison to the $P2Y_2R$ and $P2Y_6R$ [50]. Molecular modeling and docking of N^4-alkoxyimino derivatives of CTP defined a new subpocket facing the exterior of the $P2Y_4R$ that could accommodate steric bulk.

Dinucleoside tetraphosphates, e.g., INS 365 (Diquafosol) **92** and Up_4-2′-deoxyC (structure not shown, INS 37217, Denufosol) are moderately potent agonists of both $P2Y_2R$ and $P2Y_4R$. In general, dinucleotides are more stable to hydrolysis by nucleotidases than are nucleotides bearing a free terminal phosphate group [69]. MRS 2768 **90** (uridine tetraphosphate δ-phenyl ester) is somewhat selective for the $P2Y_2R$ but is less potent than other $P2Y_2R$ agonists [70].

Several weak antagonists of the $P2Y_2R$ that are uracil derivatives, e.g., AR-C126313 and AR-C 118925 (not shown), have been reported but full pharmacological characterization is still lacking [2]. For lack of better antagonists, the anti-infective drug suramin **102** and the large anthraquinone dye Reactive blue 2 **100** (RB2), which is a mixture of isomers, are used as partially selective antagonists of the $P2Y_2R$ and $P2Y_4R$, respectively. It should be noted that suramin and many other weak P2YR antagonists typically display other activities, such as inhibition of ectonucleotidases, which may complicate the interpretation of experiments [2].

$P2Y_6R$ UDP **78** is the native agonist of the $P2Y_6R$, but was recently found to also activate the $P2Y_{14}R$ [71]. UDPβS **80**, 3-phenacyl UDP (PSB 0474) **84**, 5-iodo-UDP (MRS 2693) **85** and dinucleoside triphosphates, such as Up_3U **91** and INS 48823 (not shown) [2], have been used as moderately selective agonists of the $P2Y_6R$ [44]. Probing the conformation of the ribose ring at the $P2Y_6R$ by molecular modeling and chemical synthesis of ring-constrained analogues has clearly identified the South (*S*)-conformation as the receptor-preferred conformation at this subtype [43]. Thus, a rigid bicyclic (*S*)-methanocarba-UDP (not shown) was more potent than UDP, and the corresponding ring-constrained isomer with a (*N*)-conformation was inactive. The di-isothiocyanate derivative MRS 2578 **109** is a non-competitive $P2Y_6R$ antagonist that has limited stability in aqueous medium and presumably reacts irreversibly with the receptor.

$P2Y_{11}R$ ATPγS **75** is usually used as a potent but nonselective $P2Y_{11}R$ agonist. Few $P2Y_{11}R$-selective agonists have been reported, but an atypical agonist NF546 **104** of the suramin class of antagonists was reported to activate this receptor selectively [72]. However, several reported $P2Y_{12}R$ antagonists, such as 2-propylthio-β,γ-dichloromethylene-ATP (AR-C 67085 **97**), also act as potent $P2Y_{11}R$ agonists. The suramin derivative NF 157 **103** is an antagonist of the $P2Y_{11}R$, but it is not selective with respect to the nucleotide-gated ion channels $P2X_1R$, $P2X_2R$, and $P2X_3R$. NF340 **105** related to suramin is a selective $P2Y_{11}R$ antagonist.

$P2Y_{12}R$ ADP **70** is the native agonist of the $P2Y_{12}R$, and another native ligand, ATP **71**, acts as a competitive antagonist. Many nucleotide (**96**–**98**) and non-nucleotide (**111**–**115**) antagonists of the $P2Y_{12}R$ have been reported, because of commercial interest. The thienopyridine Clopidgrel **113** is a blockbuster antithrombotic agent, which must be first activated in two steps by cytochrome P450 in the liver to subsequently irreversibly inhibit the $P2Y_{12}R$ [2, 37, 70]. The recently approved antithrombotic Prasugrel **114** belongs to this thienopyridine family of $P2Y_{12}$ antagonists. Competitive $P2Y_{12}R$ antagonists that do not require preactivation are also under development, for example, the antithrombotic nucleotide derivative AR-C 69931MX **98** (Cangrelor). An uncharged nucleoside derivative that binds potently to the $P2Y_{12}R$, AZD 6140 **99** (Ticagrelor) was recently approved to reduce cardiovascular death and heart attack in cases of acute coronary syndrome. A major metabolite of **99** that is formed by oxidative loss of the hydroxyethyl side chain also acts as a potent $P2Y_{12}R$ antagonist [22]. A sulfonate derivative related to RB2, PSB-0739 **101**, is a representative nonnucleotide antagonist of the $P2Y_{12}R$ that displays high affinity and was used in the characterization of the effects of site directed mutagenesis of the receptor and in molecular modeling [34, 49].

$P2Y_{13}R$ ADP **70** is a native agonist of the $P2Y_{13}R$, while ATP **71** at high concentrations is at best a weak partial agonist. The pyridoxal phosphate derivative MRS 2211 **108**, is a selective antagonist of the $P2Y_{13}R$ and related to the nonselective P2 antagonists PPADS **106** and iso-PPADS **107** [73]. However, MRS2211 and other pyridoxal phosphate derivatives also inhibit protein interactions of the 14-3-3 family of intracellular phosphoserine/threonine-recognition proteins [74].

$P2Y_{14}R$ UDP-glucose **88**, other UDP-sugars and UDP **78** are native agonists of the $P2Y_{14}R$ [71]. A synthetic 2-thio analog of UDP-glucose, i.e., MRS 2690 **89**, is a more potent and selective agonist at the $P2Y_{14}R$. α,β-difluoromethylene-UDP, MRS 2802 **86**, and the more potent α,β-methylene-2-thio analogue MRS2905 **87** are inactive at the $P2Y_6R$ and fully activate the human $P2Y_{14}R$.

Allosteric modulation of ARs and P2YRs

In addition to orthosteric agonists that bind at the same site on the receptor as the native agonist, allosteric modulators for ARs and P2YRs have been studied. The structure and

action of positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) for ARs and P2YR have been recently reviewed [75]. This includes both heterocyclic derivatives and nucleotide analogues that resemble a native P2Y agonist. Some of the PAMs have no action of their own and require the presence of an agonist, either the endogenous ligand or a synthetic agonist, and other PAMs are allosteric agonists that act in the absence of orthosteric ligands. The SAR of PAMs of the A_1AR (e.g., the tetrahydrobenzothiophene derivative T-62) and A_3AR (e.g., the imidazoquinolinamine derivative LUF6000 and the quinoline derivative LUF6096, structures not shown) has been extensively explored. Recently, AEA061 was reported as a PAM of the A_{2A}AR [76].

Ligands in the clinic and in current clinical trials

The biological role of adenosine and P2YRs has been extensively explored, contributing to the entry of certain selective ligands on a clinical pathway [54, 70, 77, 78]. Table 4 lists those AR and P2YR ligands in the clinic for therapeutic and diagnostic applications, including those currently in clinical trials for chronic diseases, such as inflammatory, ischemic, and neurodegenerative diseases, and for other conditions.

AR ligands as clinical candidates and approved drugs

Adenosine may be released from intracellular sources or generated by the action of ectonucleootidases on ATP that is released under stress conditions. Therefore, depending on pathophysiological factors, in a given tissue, there is often a tonic activation of one or more of the ARs that can be modulated by exogenous agents. AR agonists are currently in clinical trials for various conditions, including cardiac arrhythmias, neuropathic pain, myocardial perfusion imaging, cardiac ischemia, autoimmune inflammatory diseases, and cancer [57, 79–84].

The first AR agonist to be approved was adenosine **1** itself (as Adenocard), used as a rapidly metabolized therapeutic treatment of cardiac arrhythmias, specifically paroxysmal supraventricular tachycardia (PSVT), by slowing atrioventricular (AV) nodal conduction, an A_1AR effect. Its short half-life upon intravenous infusion (seconds) avoids some side effects, although A_{2A}AR-related side effects still may occur. Other agonists of the A_1AR have been in clinical trials for pain and cardiac arrythmias, including atrial fibrillation, supraventricular arrythmias, paroxysmal supraventricular tachycardia, and atrial flutter [54, 57]. CVT-3619 (GS9667, not shown), a partial A_1AR agonist, has been in clinical trials for type 2 diabetes [54]. Side effects associated with A_1AR agonists applied to cardioprotective and cardiovascular regeneration may be overcome by using partial A_1AR agonists such as Capadenoson **15** [85].

Adenosine (as Adenoscan) is used as a pharmacological stress agent for cardiovascular imaging based on its A_{2A}AR-dependent vasodilatory effect in the coronary artery. Also, more advanced A_{2A}AR-selective agonists [79] are either already approved for this purpose (CVT-3146, **20**) or in clinical trials (ATL-146e, Apadenson, **18**). A_{2A}AR agonists also have anti-inflammatory and anti-ischemic effects, and have been in clinical trials for related conditions, including sickle cell disease by targeting iNKT cells [58, 84]. Selective agonists of A_{2A}, A_{2B} or A_3ARs have been shown to have anti-inflammatory effects due to inhibition of the release of pro-inflammatory cytokines and other mechanisms [80, 86, 87]. This also led to former clinical trials of A_{2A}AR agonists for the treatment of chronic and neuropathic pain and diabetic foot ulcers. However, an A_{2A}AR agonist was found ineffective for treating foot ulcers. A_{2A}AR agonists also show beneficial effects in wound healing, because A_{2A} and A_{2B}ARs stimulate granulation tissue formation by inducing new matrix production and angiogenesis [88, 89]. A_{2B}AR agonists have been proposed for the treatment of hyperlipidemia and atherosclerosis [90].

A_{2A}AR antagonists (e.g. **42**, **45**, **48**, and **51**) are being developed for treatment of Parkinson's disease (PD) and other disorders of the central nervous system including addiction [79, 91], and several clinical candidates have been radiolabeled for in vivo imaging [53]. In the striatum, a heterodimer of the A_{2A}AR and the D2 dopamine receptor is thought to establish the inverse action of dopamine and adenosine agonists; thus, an A_{2A}AR antagonist would have a net effect similar to a D2 agonist. A_{2A}AR antagonists could also be of interest in preventing fibrosis in the liver and elsewhere [88] or in the treatment of cancer [92]. A_{2B}AR antagonists are under consideration for treating inflammatory diseases, diabetes, and asthma [81, 82], although trials of CVT-6883 **55** were unsuccessful.

Native adenosine acting at various AR subtypes has anti-ischemic activities in multiple organs, for example, a cardioprotective action, either as a preconditioning agent or during ischemia reperfusion. Adenosine and more selective AR agonists, e.g. A_3AR agonists such as CP532,903 **25**, have been considered for treating acute myocardial infarction [80]. One of the first actions discovered for A_3AR agonists administered *in vivo* was cerebroprotection. Also noted were paradoxical effects in which nM concentrations of A_3AR agonists prevented apoptosis and high μM concentrations induced apoptosis. The relative lack of cardiovascular side effects of A_3AR agonists in comparison to other AR agonists is considered an advantage in application to ischemia. The orally active A_3AR agonist CF101 (IB-MECA) **23** is in clinical trials for rheumatoid arthritis, psoriasis, keratoconjunctivitis sicca (dry

Table 4 Ligands of ARs or P2Y receptors currently in clinical use or trials (previous clinical trials with selective adenosine and P2Y receptor ligands are listed in refs. [1, 2, 57, 70])

Ligand	Subtype action	Route	Application	Phase	Company
Adenosine **1** (Adenocard)	A_1 agonist	iv	Paroxysmal supraventricular tachycardia	Approved	Astellas
INO-8875	A_1 agonist	Topical	Glaucoma	I–II	Inotek
Capadenoson **15,** Bay68-4986	A_1 agonist	Oral	Atrial fibrillation	II	Bayer-Schering
Adenosine **1** (Adenoscan)	A_{2A} agonist	iv	Myocardial perfusion imaging	Approved	Astellas
Apadenoson **18**, ATL146e (Stedivaze)	A_{2A} agonist	iv	Myocardial perfusion imaging	III	Forest Laboratories
Regadenoson **20**, CV-3146 (Lexiscan)	A_{2A} agonist	iv	Myocardial perfusion imaging	Approved	Astellas/Gilead
Regadenoson **20**, CV-3146 (Lexiscan)	A_{2A} agonist	iv	Sickle cell disease	I	Dana-Farber Cancer Institute
IB-MECA **23**, CF101	A_3 agonist	Oral	Rheumatoid arthritis, psoriasis, dry eye, glaucoma	II/III	Can-Fite
Cl-IB-MECA **24**, CF102	A_3 agonist	Oral	Hepatocellular carcinoma, chronic hepatitis C (genotype 1)	II	Can-Fite
Caffeine **30**	AR antagonist	iv or oral	Sleep apnea, cancer pain, PD	II/III	Univ. of Texas, McMaster Univ., Nobelpharma, Korea Research, McGill University
Theophylline **29**	AR antagonist	Oral	Asthma, COPD	Approved	–
Istradefylline **42**, KW-6002	A_{2A} antagonist	Oral	PD	III	Kyowa Hakko
KW-6356	A_{2A} antagonist		PD		Kyowa Hakko (in Asia), Lundbeck (non-Asia)
Preladenant **46**, SCH-420814	A_{2A} antagonist	Oral	PD	III	Schering
Tozadenant **45**, SYN-115	A_{2A} antagonist	Oral	PD, cocaine dependence	IIB	Biotie, NIDA (Synosia Therapeutics)
ST-1535 **51**	A_{2A} antagonist	Oral	PD	I	Sigma-Tau
V81444	A_{2A} antagonist	Oral	PD	I	Vernalis[a]
DT1133	A_{2A} antagonist	Oral	PD	Pre-clinical	Domain Therapeutics
[^{11}C]-SCH442416 **47**	A_{2A} antagonist	iv	PET imaging of PD	I	Institute for Neurodegenerative Disorders
[^{123}I]MNI-420 **49**[c]	A_{2A} antagonist	iv	SPECT imaging of PD, Huntington's disease	I	Institute for Neurodegenerative Disorders
CVT-6883 **55**, GS 6201	A_{2B} antagonist	Oral	Chronic pulmonary and inflammatory diseases[d]	I	Gilead
Diquafosol **92** (Diquas)	$P2Y_2$ agonist	Local	Dry eye disease	Approved (Japan)	Santen (Inspire)
Clopidogrel **113** (Plavix)	$P2Y_{12}$ antagonist	Oral	Acute coronary syndrome, atherosclerosis	Approved	BMS/Sanofi
Prasugrel **114** (Effient)	$P2Y_{12}$ antagonist	Oral	Acute coronary syndrome, angioplasty	Approved	Lilly/Daiichi Sankyo
Ticagrelor **99**, AZD6140 (Brilinta)	$P2Y_{12}$ antagonist	Oral	Acute coronary syndrome	Approved	AstraZeneca
Cangrelor **98**, AR-C69931MX	$P2Y_{12}$ antagonist	iv	Coronary artery bypass[b]	III	The Medicines Co.
Elinogrel **115**, PRT-060128	$P2Y_{12}$ antagonist	Oral or iv	Acute coronary syndrome	II	Portola/Novartis

PD Parkinson's disease

[a] Clinical trials of another A_{2A}AR antagonist, vipadenant (V2006/*BIIB014*), for PD were recently halted by Vernalis and partner Biogen Idec

[b] Effective at maintaining platelet inhibition in patients on thienopyridines who required bypass surgery

[c] Reference [110]

[d] Clinical trials discontinued or unsuccessful (see also references [1, 2, 57, 70])

eye syndrome), and glaucoma [80]. The closely related CF102 (Cl-IB-MECA) **24** is in clinical trials for advanced hepatocellular carcinoma and for patients with chronic hepatitis C genotype 1.

P2YR ligands as clinical candidates and approved drugs

Although the ARs are a mature field of medicinal chemistry, the P2YRs generally lag behind in the development of

selective ligands, radioligands and other affinity probes, imaging agents, and clinical candidates. The most successful application in that area is the use of $P2Y_{12}R$ antagonists as antithrombotics, but other disease areas are potentially amenable to treatment using selective P2YR agonists or antagonists [70]. Since some P2Y subtypes have a widespread distribution, there might be substantial side effects, such as those noted to occur in bone [4].

Nucleotides, such as ATP **71** and UTP **79**, are readily released from intracellular sources under conditions of injury and organ stress, such as hypoxia, ischemia, or mechanical stress, and through channels and vesicular release. One of the consequences of this release is a proinflammatory effect [2], for example from ATP that accummulates in asthmatic airways. Consistently, antagonists and other ligands of the P2YRs could serve as therapeutic targets for a variety of conditions, including cardiovascular diseases and inflammatory diseases such as asthma and neurodegeneration [70, 93]. It has been suggested that antagonists of $P2Y_2R$, $P2Y_6R$, or $P2Y_{11}R$ might be beneficial in asthma and inflammatory bowel disease [70]. Beneficial effects of P2 receptor antagonists have been observed in a stroke model [94]. The effects of various P2YR ligands on apoptosis in cell culture and in the central nervous system have been explored [95–98], suggesting application to a variety of diseases, from cancer to diabetes to ischemia.

P2YRs are widespread in hematopoietic cells, and therefore the effects of extracellular nucleotides and their antagonists are being studied in the immune/inflammatory system. The platelet expresses two P2YRs, i.e., $P2Y_1R$ and $P2Y_{12}R$, both of which have to be activated in order for ADP to have a prothrombotic effect [23]. Therefore, blocking either of these receptors produces an antithrombotic effect. $P2Y_{12}R$ antagonists, three of which are already approved as agents for acute coronary syndrome and for prevention of secondary thrombotic events, have been described above. The antithrombotic action of MRS 2500 **95** by selectively blocking the $P2Y_1R$ is evident in vivo in the mouse and other species, suggesting this receptor subtype as a clinical target. Furthermore, genetic deletion of the $P2Y_1R$ is associated with fewer atherosclerotic lesions in $ApoE^{-/-}$ mice. Bone marrow reconstitution has demonstrated the involvement of non-hematopoietic-derived cells, probably the endothelial cells [99].

Several agonists of the $P2Y_2R$ have been in clinical trials for cystic fibrosis and other pulmonary conditions. Activation of the $P2Y_2R$ on epithelial cells in the airways and the eye promotes chloride secretion, independently of the genetically defective transporter in cystic fibrosis. However, the $P2Y_2R$ agonist Up_4-2′-deoxyC (Denufosol) was denied approval for the treatment of cystic fibrosis due to the failure to reproduce the positive results of the TIGER-1 study in the longer duration TIGER-2 trial. A $P2Y_2R$ agonist of low selectivity, Up_4U **92** (Diquafosol), has been approved in Japan but not the U.S. for the treatment of dry eye disease [100]. $P2Y_2R$ activation has also been shown to protect rat fetal cardiomyocytes against ischemia [101]. $P2Y_4R$ activation by UTP promotes chloride and water secretion by intestinal epithelial cells, suggesting the use of agonists of this subtype in treating chronic constipation [102].

Pancreatic islets express both the $P2Y_1R$ and $P2Y_6R$, both of which are coupled to G_q and promote insulin release. The use of $P2Y_1R$ agonists in diabetes has been proposed, and relatively stable nucleotide analogues that activate this subtype have been applied in vivo [69]. Furthermore, agonists of the $P2Y_6R$ have been shown to have beneficial antiapoptotic effects on pancreatic islets cells in culture, suggesting their possible application to diabetes [95] Endogenous UDP activating the $P2Y_6R$ is involved in the autocrine potentiation of insulin secretion [103]. However, there are significant side effects of activation of the $P2Y_6R$, such as a proinflammatory effect, atherosclerotic plaques, cardiac fibrosis and possibly a loss of bone mass [4, 70, 104].

$P2Y_{11}R$ activation mediates ATP-induced semi-maturation of human monocyte-derived dendritic cells and increases the release of interleukin-8 from human monocyte-derived dendritic cells, suggesting use of ligands of this subtype in immune modulation [72, 105]. Semi-maturation of dendritic cells is characterized by an increased expression of co-stimulatory molecules with no stimulation of interleukin-12 secretion, leading to a Th2 response or tolerance.

The activation and migration of microglia in the brain are modulated by P2YRs [106, 107]. ADP activating the microglial $P2Y_{12}R$ induces a “find-me” signal (to induce migration), and UDP activating the microglial $P2Y_6R$ induces an “eat-me” signal (to induce phagocytosis). These findings suggest application of $P2Y_{12}R$ or $P2Y_6R$ ligands to neuropathic pain and neurodegenerative diseases. Indeed, intrathecal administration of $P2Y_{12}R$ antagonist AR-C69931MX **98** prevented the development of tactile allodynia [106].

Activation of the $P2Y_{13}R$ by ADP promotes reverse cholesterol transport in hepatocytes with the endocytosis of HDL particles [108]. Thus, activation of $P2Y_{13}R$ might be a new target for treatment of dyslipidemia and atherosclerosis.

Modulation of the $P2Y_{14}R$ has potential for the treatment of immune and inflammatory disorders, pain, asthma, gastric disorders, central nervous system diseases, and glaucoma. Non-nucleotide antagonists of the $P2Y_{14}R$, e.g., **116**, and prodrug derivatives to increase their bioavailability have been proposed [64]. Intracellular UDP sugars, many of which would activate the cell-surface $P2Y_{14}R$, are substrates for protein glycosylation, and are released as the proteins are trafficked to the surface [71], where they may fulfill a cell signaling role. The role of P2Y receptors in stem cell differentiation has been explored; $P2Y_4$ and $P2Y_{14}Rs$ appear to regulate the onset of mesenchymal differentiation, and the downregulation of $P2Y_1$ and $P2Y_2Rs$ are markers for early osteogenic differentiation [109].

Conclusions

There have been significant recent advances in the structural biology of purine receptors and in the medicinal chemistry of selective ligands and their pharmacology. Potent purine and pyrimidine analogues have aided in the characterization of regulation of many physiological and pathophysiological processes. It is apparent that this ubiquitous cell signaling system has implications for understanding and treating many diseases. Thus, this field has provided fertile ground for pharmaceutical development.

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