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Adenosine A_{2A} Receptor as a Drug Discovery Target

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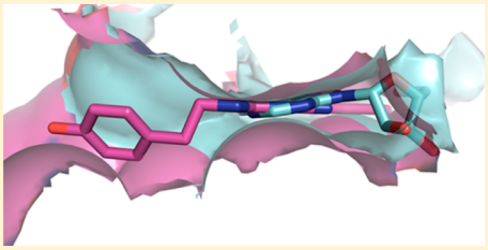
ABSTRACT: The adenosine A_{2A} receptor is a G-protein-coupled receptor (GPCR) that has been extensively studied during the past few decades because it offers numerous possibilities for therapeutic applications. Herein we describe adenosine A_{2A} receptor distribution, signaling pathways, pharmacology, and molecular structure, followed by a summary and SAR discussion of the most relevant series of adenosine A_{2A} agonists and antagonists. This review also provides an update of the A_{2A} ligands that are undergoing or have undergone clinical studies, including the two currently marketed agonists adenosine and regadenoson.



INTRODUCTION

In 1929, Drury and Szent-Györgyi discovered that adenosine (**1**, Figure 1), a naturally occurring nucleoside, can influence a wide range of physiological functions.[1] The pronounced effects of adenosine in the heart were of particular interest and inspired much research in this area. Several adenosine analogues were synthesized, and examination of the dose−response relationships suggested the presence of specific adenosine receptors (ARs).[2] In 1965, De Gubareff and Sleator documented the effect of caffeine (**7**, Figure 2) on mammalian atrial muscle,[3] and 5 years later Sattin and Rall described the effects of adenosine and adenine nucleotides on the cAMP content in the guinea pig brain.[4] In 1980, Fredholm et al. observed that in mice the naturally occurring methylxanthines caffeine and theophylline (**8**, Figure 2) have a stimulant effect and enhance locomotor activity by blocking adenosine receptors.[5] The existence of distinct types of adenosine receptors was first suggested by Van Calker et al.[6] Working with cultured glial cells from perinatal mouse brain, it was found that some adenosine derivatives were able to increase intracellular cAMP levels whereas others inhibited its accumulation. These experimental results were obtained using adenosine-based agonists with different potencies at the distinct receptor subtypes. The receptors that inhibited adenylyl cyclase were classified as A_1 receptors, and those that stimulated adenylyl cyclase were classified as A_2. That there may yet be more adenosine receptors of the A_2 subtype was suggested in 1983 by the results of Daly and co-workers, who were studying which adenosine receptors were crucial to the various central activities of caffeine.[7]

After several decades of intense research, it is well established that adenosine is one of the human body's most important neuromodulators in both the central and the peripheral nervous systems.[8] The effects of this purine nucleoside are modulated via four receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3, all of which belong to the family of G-protein-coupled receptors (GPCRs).[9] In 1989, Libert and co-workers cloned several orphan G-protein-coupled receptors from the dog thyroid, one of which was subsequently identified as an A_{2A} receptor.[10] A_{2A} receptors have thereafter been cloned from several species including rat,[11] human,[12] mouse,[13] and guinea pig.[14]

Adenosine receptors have distinct distributions and control different functions in the mammalian organism. Only receptors related to the A_{2A} receptor subtype will be included in this review. Adenosine A_{2A} receptors are highly expressed in the spleen, thymus, leukocytes, blood platelets, striatopallidal GABAergic neurons, and the olfactory bulb and expressed to a lesser extent in the heart, lung, blood vessels, and other brain regions.[9] The actions of the A_{2A} receptor are complicated by the fact that this subtype colocalizes and physically associates to other unrelated G-protein-coupled receptors, forming heterodimers such as dopamine D_2/A_{2A},[15] and D_3/A_{2A},[16] cannabinoid CB_1/A_{2A},[17] and glutamate mGluR5/A_{2A},[18] as well as $CB_1/A_{2A}/D_2$ heterotrimers.[19] The A_{2A} receptor is important in mediating vasodilation, supporting the synthesis of new blood vessels and protecting tissues from collateral inflammatory damage. In the brain, A_{2A} receptors influence the activity of the indirect pathway of the basal ganglia.

The therapeutic potential of interaction between the A_{2A} receptor and small molecules has been validated by the U.S. Food and Drug Administration's approval of regadenoson (**19**, Figure 6), a selective A_{2A} adenosine receptor agonist that increases blood flow during cardiac nuclear stress tests.[20] A number of agonists and antagonists discussed in this review are currently undergoing clinical trials. Adenosine A_{2A} receptor antagonists have emerged as an attractive approach to treat Parkinson disease (PD). In addition, investigations are being conducted on a number of compounds to treat inflammation, cancer, ischemia reperfusion injury, sickle cell disease, diabetic nephropathy, infectious diseases, and cognition and other CNS disorders. The numerous A_{2A} antagonists in the early discovery

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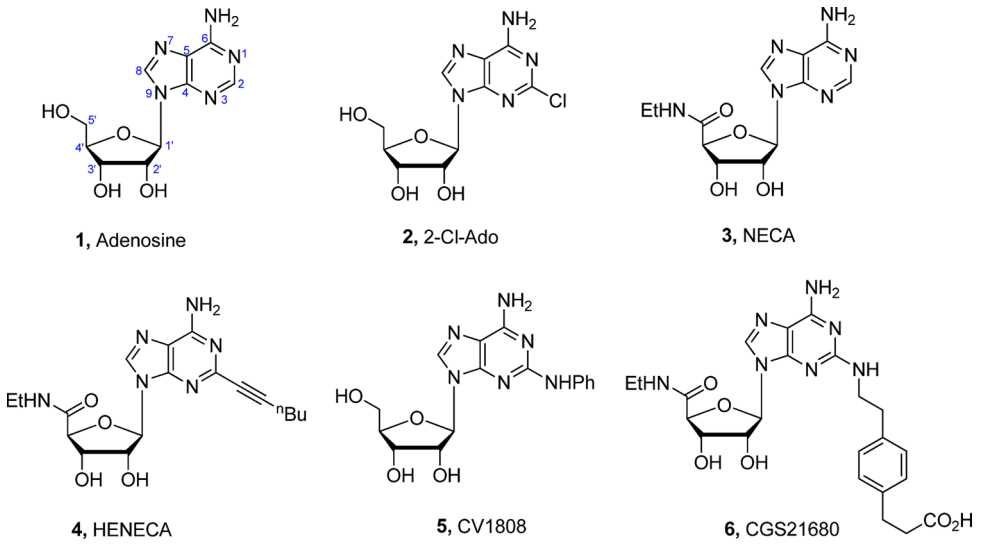

Figure 1. Adenosine A_{2A} receptor historical agonists.

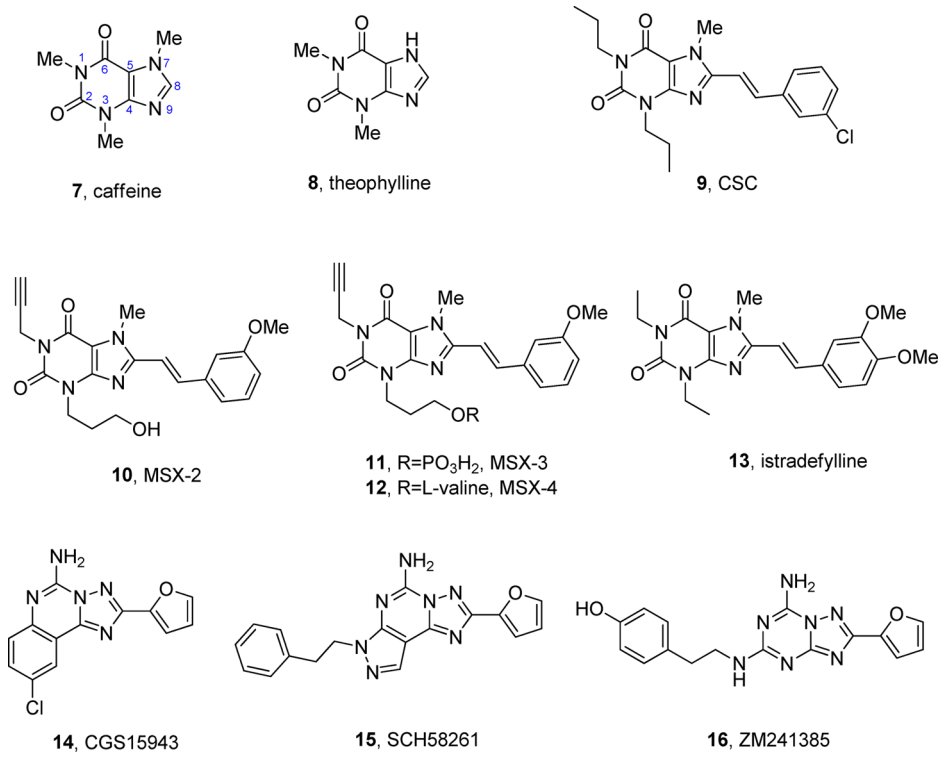

Figure 2. Adenosine A_{2A} receptor historical antagonists.

phase and the abundance of publications and patents are proof of the intense interest in this area.

RECEPTOR DISTRIBUTION AND SIGNALING PATHWAYS

The discovery of A_{2A} selective radioligands and the development of antibody, immunohistochemical, and electron microscopy techniques have made it possible to map the distribution of A_{2A} receptors. This is critical not only to determine where agonists and antagonists could interact but also to estimate receptor density in a particular area. Adenosine A_{2A} receptors are found to be concentrated in the dopamine-rich regions of the brain, in GABAergic medium-sized spiny neurons in the dorsal striatum, in neurons in the core and shell regions of the nucleus accumbens, and in the tuberculum olfactorium. They are found associated with the plasma membrane or with cytoplasmic structures in dendrites and dendritic spines, where they are primarily located at asymmetric synapses, although some receptors are present in the vicinity of symmetric, inhibitory synapses and in axon terminals. The distribution of A_{2A} receptors is not restricted to the medium-sized spiny neurons in the basal ganglia, as the A_{2A} translated protein is also expressed in numerous other tissues, such as blood vessels, endothelial and lymphoid cells, smooth muscle cells, and a number of neurons of both sympathetic and parasympathetic nervous systems.[21]

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The adenosine A_{2A} receptor is a G-protein α-s coupled receptor that induces classical second messenger pathways, such as modulation of cAMP production. Activation of the A_{2A} receptor increases the level of adenylyl cyclase, which results in an enhancement of the levels of cAMP. The signaling pathways used by the A_{2A} receptor vary, depending on the type of cell and tissue where the receptor is localized, the specific G-protein to which it is coupled, and the signaling machinery that the cell possesses. In the peripheral system, the major G-protein associated with A_{2A} receptors appears to be G_s. In the striatum, where A_{2A} receptor density is the greatest, the situation is different, and in rats it has been shown that striatal A_{2A} receptors mediate their effects predominantly through activation of G_{olf}, which is similar to G_s and like G_s couples to adenylyl cyclase.[22] Adenosine A_{2A} receptor interaction with the G protein causes the exchange of GDP for the GTP bound to the G protein α subunit and the dissociation of the β/γ heterodimer. The activated $G_{\alpha\text{-s}}$ stimulates adenylate cyclase type VI, which increases the cAMP levels in cells, and activates protein kinase A (PKA); the latter phosphorylates and stimulates cAMP responsive element binding protein 1 (CREB1). The activation of G_s and G_{olf} proteins that results in increasing the concentration of cAMP is the major general pathway of A_{2A} receptor activation; however, in monkey COS-7 cells, activation of $G_{\alpha 15}$ and $G_{\alpha 16}$ proteins stimulate the formation of phospholipid C (PLC), which induces the formation of inositol phosphates, raises intracellular calcium, and activates protein kinase C (PKC). There is good evidence that after activation of the A_{2A} receptors several other kinases of the mitogen-activated protein kinases (MAPK) and of the extracellular signal-regulated kinases (ERK) are also activated.[23] Phosphorylation of some of the kinases mentioned in this paragraph (plus others omitted for brevity) lead to specific cellular responses.[24]

HISTORIC LIGANDS

Adenosine (**1**, Figure 1, Table 1) is the natural ligand for the ARs. It is an endogenous purine nucleoside that acts as an agonist with a high affinity for the A_{2A}, A_1, and A_3 receptors (hA_{2A} K_i = 700 nM, hA_1 K_i = 310 nM, hA_3 K_i = 290 nM) and with considerably lower affinity for the A_{2B} receptor (hA_{2B} $K_i \geq$ 10 μM).[25] The unmodified molecule has been of restricted interest in studying adenosine receptors because it is readily metabolized by a number of enzymes. The main approach to discovering AR agonists has been modification of adenosine itself. Many attempts to modify the adenosine structure or its stereochemistry led to the conclusion that the adenosine scaffold must be conserved as the structural basis for agonist design. Therefore, most of the useful agonists are modified at the N6 or the 2-position of the purine and at the 5′-position of the ribose, changes that give better metabolic stability compared with adenosine. An increase in A_{2A} potency was shown by 2-chloroadenosine (2-Cl-Ado) (**2**, Figure 1, hA_{2A} K_i = 180 nM), which contains a chlorine atom at the 2-position of adenine, and a 20-fold potency increase was shown by *N*-ethylcarboxamidoadenosine (NECA) (**3**, Figure 1, hA_{2A} K_i = 20 nM) where a small alkylamide group is substituted at the 5′-position; however, both 2-Cl-Ado and NECA are nonselective agonists (Table 1). 2-Hexynyl-NECA (HENECA) (**4**, Figure 1) exhibits high affinity at A_2 adenosine receptors (hA_{2A} K_i = 6.4 nM) and 10-fold selectivity over A_1 in recombinant human receptors.[26] NECA and HENECA exhibit effective in vivo inhibitory activity on platelet function in the rabbit. Although agonist **5** (CV1808, Figure 1), which has an intact ribose structure, was the first adenosine derivative found to have some A_{2A} AR selectivity over the A_1 receptor (rA_{2A} K_i = 100 nM, rA_1 K_i = 400 nM),[27] the therapeutic potential of HENECA for treatment of cardiovascular disease prompted Cristalli et al. to synthesize a number of N6- and 2-adenine substituted analogues that culminated in the discovery of CGS21680 **6**, a moderately A_{2A} AR-selective agonist that displays binding affinities of 27 and 19 nM at the human and rat A_{2A} receptor, respectively.[28] The A_{2A} binding of CGS21680 (**6**, Figure 1) is 10-fold selective against A_1 and has a similar potency on A_3 (hA_3 K_i = 67 nM), whereas it is highly selective against A_{2B} (hA_{2B} K_i > 10 000 nM).[29]

Table 1. Affinities of A_{2A} Adenosine Receptor Agonists (Figures 1, 6 and 7) in Binding and Functional Assays at A_1, A_{2A}, A_{2B}, and A_3 Adenosine Receptors

agonist	A_{2A} K_i (nM)[a]	A_{2B} EC_{50} (nM)[b]	A_1 K_i (nM)[a]	A_3 K_i (nM)[a]
1, adenosine	700[b]	24000[b]	310[b]	290[b]
2, 2-Cl-Ado	180	ND[c]	1.39	19
3, NECA	20	330[b]	14	6.2
4, HENECA	6.4	6100[b]	60	2.4
5, CV1808	100 (r)	ND[c]	400 (r)	ND[c]
6, CGS21680	27	361000[b]	290	67
17, binodenoson (WRC0470)	270	>100000[b]	48000	903
18, apadenoson (ATL146e)	0.5	ND[c]	77	45
19, regadenoson (CVT3146)	290	10000[b]	3770	10000
20, GW328267	46	1300[b]	369	92
21, UK432097	4	ND[c]	ND[c]	ND[c]
22, sonedenoson (MRE0094)	490	10000[b]	10000	ND[c]
25	167	ND[c]	107 (r)	92

[a]Binding data (K_i) from recombinant human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors, unless rat (r) is indicated. [b]Functional assay data (cAMP) from human A_{2A}, A_{2B}, A_1 and A_3 adenosine receptors expressed as EC_{50} (nM). [c]ND: not determined or not disclosed.

Unlike A_{2A} agonists, antagonists of the A_{2A} AR lack the sugar moiety and in general possess a mono-, bi-, or tricyclic structure that mimics the adenine part of adenosine. They are classified as xanthines and non-xanthines. It is well-known that caffeine is the most widely consumed behaviorally active substance in the world. Caffeine and theophylline (Figure 2), another naturally occurring xanthine mainly found in tea, are nonselective AR antagonists. Their stimulating properties are associated with micromolar range affinities for the A_{2A} AR. Although caffeine and theophylline have similar in vitro affinities for the A_{2A} receptor (Table 2), caffeine has a higher stimulating effect due to a higher brain unbound fraction.[30]

The xanthine scaffold has been used as an important starting point for the development of selective A_{2A} antagonists. Medicinal chemistry efforts were directed not only at identifying A_{2A} selective antagonists but also at improving the poor aqueous solubility typical of xanthines. A screening of various 1,3,8-substituted xanthines led to the discovery of 3-chlorostyrylcaffeine (**9**, also named CSC), **10** (MSX-2),[31] and istradefylline[32a] (**13**, also named KW6002, Figure 2), all being potent and selective A_{2A} AR antagonists (Table 2). It is well established that the trans-styryl substituent at the 8-position of these analogues is critical to the A_{2A} selectivity. Among these compounds, **10** has been extensively studied because of a very

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Table 2. Affinities of A_{2A} Adenosine Receptor Antagonists (Figures 2 and 9−13) in Binding and Functional Assays at A_1, A_{2A}, A_{2B}, and A_3 Adenosine Receptors

antagonists	A_{2A} K_i (nM)[a]	A_{2B} K_i (nM)[a]	A_1 K_i (nM)[a]	A_3 K_i (nM)[a]
7, caffeine	23400	20500	44900	>100000 (r)
8, theofylline	25000 (r)	ND[d]	8500 (r)	ND[d]
9, CSC	54 (r)	ND[d]	28000 (r)	>10000 (r)
10, MSX-2	5, 8 (r)	2900	2500, 900 (r)	>10000 (r)
13, istradefylline	36, 12	1800	2830, 9600	>3000
14, CGS15943	0.4, 1.2 (r)	44	3.5, 6 (r)	95
15, SCH58261	1.1	1110	549	1200
16, ZM241385	0.8	50	255	>10000
26	2.4	ND[d]	406	ND[d]
27, SCH412348	0.6	ND[d]	>996	ND[d]
28, preladenant	1.1	>1700	1474	>1000
29	0.9	ND[d]	602	ND[d]
30	12.6	ND[d]	1361	ND[d]
31	2.0	ND[d]	358	ND[d]
32	1.8	ND[d]	1116	ND[d]
33	5.2	ND[d]	1398	ND[d]
34	14.2	ND[d]	1405	ND[d]
35	25	ND[d]	8775	ND[d]
36	5.0	ND[d]	2100	ND[d]
37, tozadenant	5.0	700	1350	1570
38	61	7072	244	6941
39, VER-6623	1.4	865	207	476
40, VER-6947	1.1	112	17	1472
41, VER-7835	1.7	141	170	1931
42, vipadenant	1.3	63	68	1005
43	1.7	460	42	1740
44	2.5	3185	133	366
45	115	ND[d]	270	ND[d]
46	3.5	ND[d]	31	ND[d]
47	41	ND[d]	10000	ND[d]
48	16	ND[d]	10000	ND[d]
49	12	ND[d]	ND[d]	ND[d]
50	3.0	ND[d]	ND[d]	ND[d]
51	4.0	ND[d]	ND[d]	ND[d]
52	39% at 10 nM[b]	81% at 1 μM[b]	37% at 1 μM[b]	ND[d]
	81% at 100 nM[b]	98% at 10 μM[b]	82% at 10 μM[b]	
53	30% at 10 nM[b]	21% at 1 μM[b]	4% at 1 μM[b]	ND[d]
	73% at 100 nM[b]			
54	28% at 10 nM[b]	ND[d]	ND[d]	ND[d]
	85% at 100 nM[b]			
55	4.0 (r)	ND[d]	820 (r)	ND[d]
56	(r)	ND[d]	>250 (r)	ND[d]
57	63 (r)	ND[d]	1071 (r)	ND[d]
58	12 (r)	ND[d]	40.8 (r)	ND[d]
59, ST-1535	6.6	352	79.2	>10000
60, ST-3932	8.0	ND[d]	24	ND[d]
61, ST-4206	12	ND[d]	192	ND[d]
62, ASP-5854	1.8	ND[d]	9	>557
63	0.1[c]	ND[d]	0.4[c]	ND[d]
64	4.1[c]	ND[d]	17[c]	ND[d]
65	6.5[c]	ND[d]	48.2[c]	ND[d]
66	4.4[c]	ND[d]	32.7[c]	ND[d]
67	29[c]	ND[d]	1680[c]	ND[d]
68	6.6[c]	ND[d]	290[c]	ND[d]
69	5.3[c]	ND[d]	100[c]	ND[d]
70	0.6	ND[d]	10.2	ND[d]
71	9.0	ND[d]	1998	ND[d]
72	0.4	ND[d]	40	ND[d]
73	0.4	ND[d]	77.2	ND[d]

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Table 2. continued

[a]Binding data (K_i) from recombinant human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors, unless rat (r) is indicated. [b]Percentage (%) inhibition from human A_1, A_{2A}, and A_{2B} adenosine receptors. [c]Functional assay data (cAMP) from human A_1, and A_{2A} adenosine receptors expressed as K_i (nM). [d]ND: not disclosed.

high affinity at the A_{2A} receptor (rA_{2A} K_i = 8 nM, hA_{2A} K_i = 5 nM) and a good selectivity profile. Different approaches have been explored to improve the aqueous solubility of styrylxanthines, such as the introduction of polar groups on the phenyl ring and the preparation of phosphate or amino acid prodrugs. Antagonists **11** (MSX-3) and **12** (MSX-4), the phosphate and the L-valine prodrugs, respectively, of **10**, are stable and soluble in aqueous solutions but readily cleaved (by phosphatases in the case of **11** and esterases in the case of **12**) to liberate **10**.[31] Istradefylline is the most extensively studied xanthine derivative. It has hA_{2A} K_i = 36 nM, hA_1 = 2830 nM, hA_{2B} = 1800 nM, and hA_3 K_i > 3000 nM.[32b] This compound showed anticataleptic activity at a low dose (0.03 mg/kg, po) in a mouse haloperidol model and exhibited antiparkinsonian activity without provoking dyskinesia in MPTP-treated primates.[33] However, istradefylline possesses poor photostability and undergoes dimerization via [2 + 2] cycloaddition of the styryl double bond. Furthermore, the styryl double bond in this series of compounds isomerizes in dilute solutions from the *E* to the *Z* isomer.[34]

Since xanthine derivatives present several problems such as poor water solubility and instability, a search was initiated for a different structural class based on mono-, bi-, and triheterocycles. In 1987 Williams et al. discovered CGS15943 (**14**, Figure 2),[35] a very potent A_{2A} antagonist (hA_{2A} K_i = 0.4 nM) that also has high affinity for the other ARs (hA_1 K_i = 3.5 nM, hA_{2B} K_i = 44 nM, hA_3 K_i = 95 nM). Blockade of the A_1 receptor is correlated with undesirable cardiovascular effects and has been suggested to disrupt the activity of antiparkinsonian agents.[36] In 1993, Gatta et al. synthesized a series of compounds based on the replacement of the phenyl ring of CGS15943 with a heterocycle, either pyrazole or imidazole, but their A_{2A} vs A_1 selectivity was not satisfactory.[37] Subsequent synthetic efforts by Baraldi et al. led to the major discovery that N′-substituted pyrazolotriazolopyrimidines retain A_{2A}-receptor affinity while losing affinity at the other adenosine receptors. One of the compounds synthesized was SCH58261 (**15**, Figure 2)[38] (hA_{2A} K_i = 1.1 nM, hA_1 K_i = 549 nM), which was rapidly and widely accepted as a reference A_{2A} receptor antagonist, largely due to its ability to cross the blood–brain barrier (BBB). SCH58261 has been useful as a tool to characterize the A_{2A} receptor, as well as to learn about its intracellular signaling. It shares with caffeine some stimulatory effects such as increase in locomotor activity and waking behavior in the rat. In the cardiovascular system it increases blood pressure and heart rate in rats.[39] SCH58261 showed a positive effect in the 6-hydroxydopamine (6-OHDA) lesioned rat model (5 mg/kg, po), providing support for the notion that A_{2A} receptor antagonists represent an interesting approach to the treatment of Parkinson disease. The development, SAR, and clinical status of this series will be discussed in the antagonist therapeutic applications section of this review. In general, these non-xanthines have poor water solubility, and their structures are complex and difficult to synthesize. In order to circumvent these two liabilities, the Zeneca group developed ZM241385 (**16**, Figure 2), a very potent bicyclic non-xanthine antagonist (hA_{2A} K_i = 0.8 nM) that is selective over the A_1 and A_3 receptors (hA_1 K_i = 255 nM, hA_3 K_i > 10 000 nM) although also potent at the A_{2B} AR (hA_{2B} K_i = 50 nM).[40] Compared with the tricycles discussed earlier, this compound showed a favorable aqueous solubility profile due to its bicyclic nature and the presence of two additional hydrogen donors.

The historical adenosine A_{2A} ligands described in this section have been excellent tools for examining the pharmacology and signaling pathways of these receptors. As we will see in the agonist and antagonist radioligand sections of this review, some of these compounds have been radiolabeled and successfully used to investigate receptor distribution and to calculate A_{2A} binding affinities. Efforts to optimize these ligands and use them as a basis for the design of novel therapeutic agents will be described later in this review after the A_{2A} receptor pharmacology and structure sections.

RECEPTOR PHARMACOLOGY

In recent years, studies using genetically modified mice have provided insights into the pharmacology of adenosine A_{2A} receptors. In 1997, Ledent et al. generated the first knockout (KO) mice, in which the first coding exon of the A_{2A} receptor was targeted. These mice showed aggressiveness, decreased sensitivity to pain, and slightly higher blood pressure.[13] Two years later, Chen and co-workers targeted the second exon and observed that the corresponding A_{2A} receptor deficiency attenuates brain injury induced by transient local ischemia in mice.[41] A_{2A} KO mice are viable, fertile, and normal in size and do not display any gross physical or behavioral abnormalities. These mice show a reduction in the volume of experimentally induced cerebral infarction and resulting impairment of neurological function compared with the wild type. Treatment with receptor agonist CGS21680 does not elicit decreased locomotor activity in the A_{2A} receptor KO mice, compared with the wild type. A reduction of spontaneous activity and increased resistance to the addictive substances amphetamine and cocaine were also observed.[42]

Adenosine is a potent biological mediator that affects numerous cell types, including neuronal cells, platelets, neutrophils, and smooth muscle cells. Although a number of nonselective A_{2A} ligands have been used as tools to understand the pharmacology of A_{2A} receptors, we are going to focus on the effects of the more A_{2A}-selective agonists CGS21680 and HENECA and the A_{2A}-selective antagonists istradefylline and SCH58261 in order to simplify the complex nature of this subject.

The A_{2A} receptor is responsible for regulating myocardial blood flow by vasodilating the coronary arteries, which increases blood flow in the myocardium but may lead to hypotension. As we mentioned earlier, A_{2A} KO mice are slightly hypertensive. Direct activation of the A_{2A} receptors by adenosine or other A_{2A} agonists results in vasodilation of different types of vessels such as coronary arteries, afferent arterioles of the kidney, mesenteric arteries, and CNS vessels. Monopoli et al. have shown that non-xanthine A_{2A} adenosine receptor antagonist SCH58261 blocks blood pressure (BP) and heart rate (HR) changes induced by the A_{2A}-selective receptor agonist HENECA but does not affect the responses evoked by

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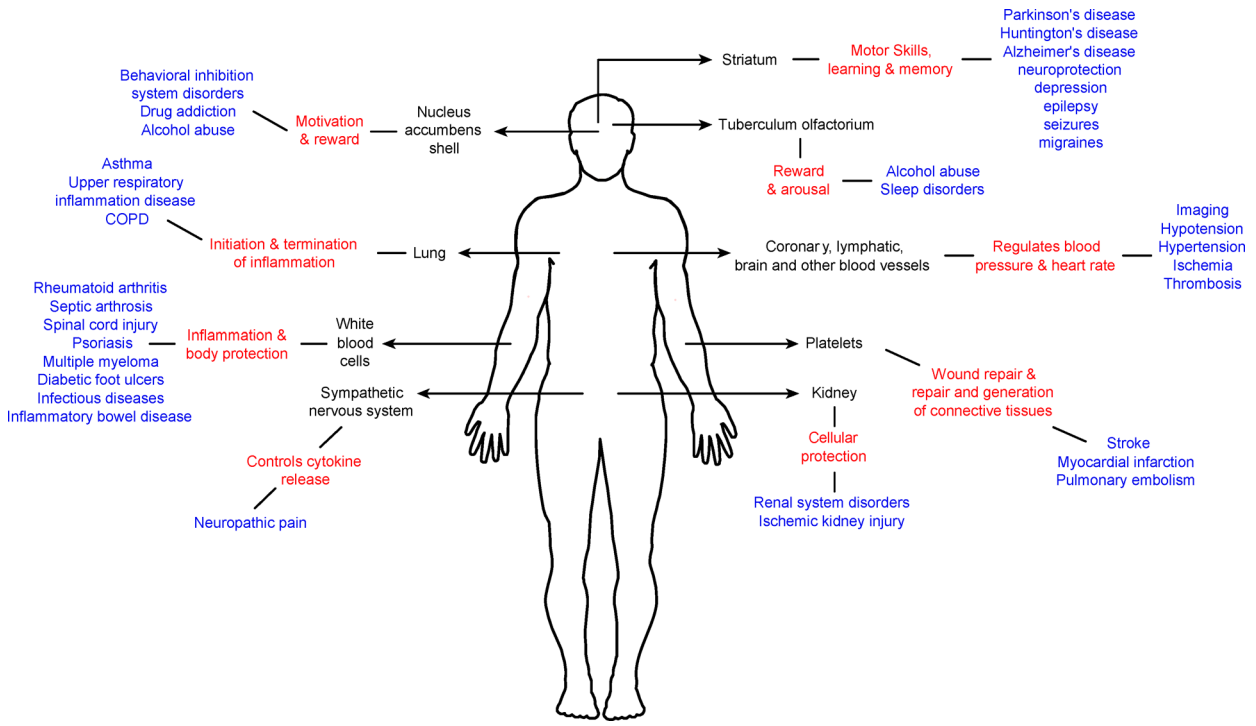

Figure 3. Adenosine A_{2A} receptor location (black), cell or tissue function (red), and possible therapeutic applications (blue).

the A_1 receptor agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA). Moreover, SCH58261 alone has been found to increase both BP and HR at a dose consistent with A_{2A} receptor antagonist activity. In conscious, spontaneously hypertensive rats, HENECA given intraperitoneally causes a dose-dependent decrease in BP. This hypotensive response is short-lasting and, as expected, is accompanied by reflex tachycardia. SCH58261 was effective in antagonizing the A_{2A} agonist-induced fall in BP and the reflex increase in heart rate.[39] All of these findings show the importance of the A_{2A} receptors in regulating blood flow. Related therapeutic applications will be discussed in the agonist therapeutic applications section of this review.

The A_{2A} receptor is also expressed in the brain, where it has important roles in the regulation of glutamate and dopamine release. In 1996, Bertorelli et al. found that SCH58261 at a 10 mg/kg dose produced stimulatory effects comparable with those induced by caffeine in rats.[43] In the striatopallidal neurons, dopamine D_2 receptors are colocalized with adenosine A_{2A} receptors.[16] The stimulation of A_{2A} receptors decreases the affinity of D_2 receptors for dopamine in rat striatal membranes[44] and in a mouse fibroblast cell line stably cotransfected with A_{2A} and D_2 receptors.[45] Adenosine A_{2A} receptor agonists inhibit, while A_{2A} receptor antagonists potentiate, the effects of the D_2 receptor agonist on motor activity, neurotransmitter release, and striatal expression of c-Fos, a transcription factor that is used as an indirect marker of neuronal activity; this expression is also increased after consumption of cocaine, methamphetamine, heroin, and other psychoactive drugs.[46] Because of the key role played by adenosine A_{2A} receptors in the regulation of striatal dopaminergic neurotransmission, drugs acting on these receptors are likely to be useful in the treatment of neurological disorders related to dopaminergic dysfunction, in particular Parkinson disease which will be described later in this review. Popoli et al. have observed the ability of the A_{2A}-selective SCH58261 to selectively potentiate D_2-dependent rotations in rats unilaterally lesioned with 6-OHDA, a rodent model of PD.[47] This finding is in line with a previous study showing that CGS21680, an adenosine A_{2A} receptor agonist, antagonized quinpirole-induced turning in 6-OHDA-lesioned rats.[48] Popoli and co-workers also observed that chronic adenosine receptor blockade does not induce tolerance to the potentiating effects of SCH58261 presumably because A_{2A} receptors are not up-regulated after chronic caffeine intake, a finding in agreement with a report by Kanda et al. showing that chronic administration of istradefylline over 21 days reversed motor defects in parkinsonian monkeys.[33]

Adenosine A_{2A} receptor knockout mice displayed reduction of immobility in functional in vivo assays, including the tail suspension and forced swim tests, that are predictive of clinical antidepressant activity. Reduction of immobility by antidepressants cannot be explained by a nonspecific behavioral stimulation, as many antidepressants tend to decrease motor activity. SCH58261 reduced immobility in the tail suspension test performed with mice that were selectively bred for their spontaneous "helplessness" in this test. Istradefylline and SCH58261 were also examined in the forced swim test, where both drugs reduced the duration of immobility in mice.[49]

In addition, blockade of striatal adenosine A_{2A} receptor by SCH58261 exerts neuroprotective effects in quinolinic acid (QA) lesioned rats. These effects are paralleled by an inhibition of QA-induced glutamate outflow. The beneficial effect of SCH58261 in this model suggests that striatal A_{2A} receptors could represent a target for Huntington disease (HD).[50]

Paterniti et al. observed that 24 h after spinal cord injury (SCI), A_{2A} receptors were expressed in neurons in the central part of the gray matter in the ventral horn of the spinal cord. Systemic and continuous administration of SCH58261 after SCI for 10 days shows protection from motor deficits up to 10 days after trauma. This A_{2A} antagonist affords protection from tissue damage, demyelination, and expression of death signals such as TNF-α, Fas-L, PAR, and Bax and from activation of Jun

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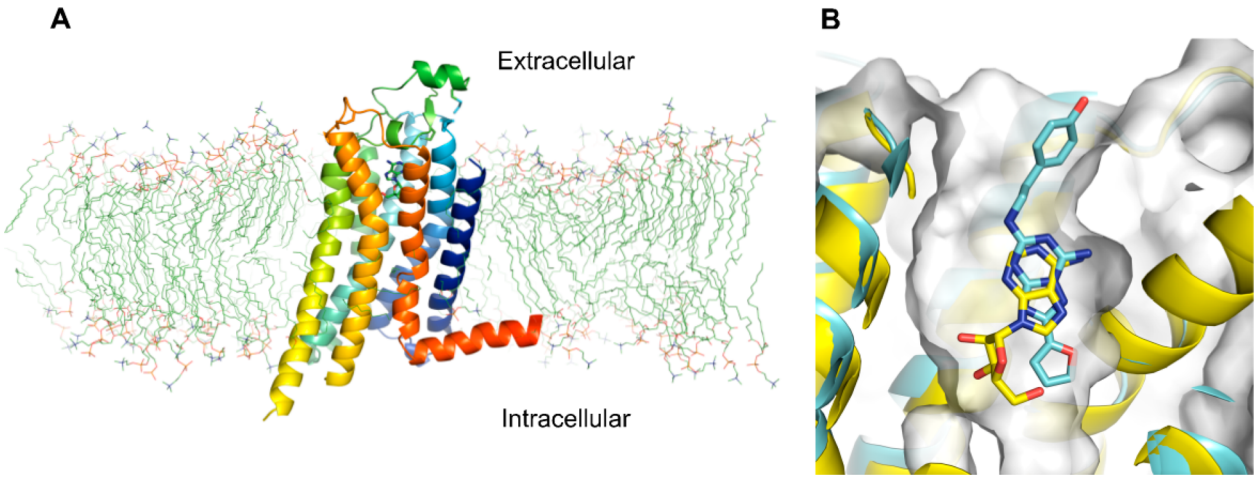

Figure 4. (A) Crystal structure of the A_{2A} receptor. (B) Overlap between the crystal structure of the agonist adenosine **1** (yellow) and the antagonist ZM241385 **16** (cyan).

N-terminal kinase (JNK) MAPK. Also, when centrally applied, SCH58261 protects from tissue damage based on evaluation 24 h after SCI. In contrast, A_{2A}-selective agonist CGS21680 centrally applied is not protective.[51] Recently Mohamed and co-workers have observed that central blockade of adenosine A_{2A} using SCH58261 significantly ameliorates hippocampal damage following ischemia reperfusion injury (IR) by halting inflammatory cascades and modulating excitotoxicity in rats. After IR, rats showed increased infarct size and lactate dehydrogenase, habituation deficit, increased anxiety and locomotor activity, increased hippocampal glutamate, GABA, glycine, and aspartate compared with their control counterparts. IR also raised myeloperoxidase, TNF-α, nitric oxide, prostaglandin E_2 but decreased interleukin-10. SCH58261, when administered intraperitoneally after carotid occlusion and before exposure to a 24 h reperfusion period, significantly reversed these effects.[52]

A summary of the adenosine A_{2A} receptor distribution, the functions of the cells or tissue, and the possible therapeutic applications is shown in Figure 3.

RECEPTOR STRUCTURE

Figure 4A shows the structure of the adenosine A_{2A} receptor. Adenosine receptors display the topology typical of GPCRs. They have in common a central core consisting of seven transmembrane helices (TM1−7), each TM being mainly α-helical and composed of 20−27 amino acids. Each TM domain is linked by three intracellular (IL1, IL2, and IL3) and three extracellular (EL1, EL2, and EL3) loops. There is also a short helix TM8 that runs parallel to the cytoplasmic surface of the membrane. Two cysteine residues (one in TM3 and one in EL2) form a disulfide link. ARs differ in the length and function of their N-terminal extracellular domain, their C-terminal intracellular domain, and their intracellular/extracellular loops. Each of these areas provides very specific properties that are critical for achieving ligand selectivity among the different receptor subtypes. Considering overall sequence identity at the amino acid level, the human A_{2A} AR shares 49% amino acid sequence identity with human A_1 AR, 58% with human A_{2B} AR, and only 41% with the human A_3 AR. Within the seven TM domains the residues critical for interaction with the ligand are located toward the extracellular part of the receptor and are highly conserved, with an average identity of 71%.[53] The primary sequence of the cloned A_{2A} ARs from various species ranges from 409 to 412 residues with the human ortholog length being 412 amino acids.[54]

Mutation studies and homology models based on other GPCRs such as bovine and squid rhodopsin and human β_2 adrenergic receptor have provided us with detailed structural information about the A_{2A} receptor. In 2008, the resolution of the crystal structure of the A_{2A} receptor bound to ZM241385 was reported,[55] and three years later crystal structures were resolved for the A_{2A} receptor bound to the agonists adenosine and NECA.[56] Figure 4B shows a picture of the overlap between the crystal structures of the agonist adenosine and ZM241385 at the orthosteric site of the A_{2A} receptor. These structures are of the utmost importance in this field, and although they reveal numerous insightful details with respect to the changes in the conformation of these receptors, we are going to focus only on analysis of the ligand-binding cavity.

X-ray analysis of the A_{2A} receptor bound to ZM241385 showed that the bicyclic triazolotriazine core of ZM241385 is anchored by an aromatic stacking interaction with Phe168 (5.29) and an aliphatic hydrophobic interaction with Ile274 (7.39). Analysis of the shape of the binding site of the A_{2A} receptor shows that it consists of a deep, planar, and narrow cavity that comfortably accommodates fused polyheteroaromatic cores.[57a] Nitrogen N17 forms a hydrogen-bond interaction with Asn253 (6.55). Adjacent to Phe168 (5.29), a polar residue (Glu169 (5.30)) interacts with the exocyclic amino group (N15 atom) linked to the bicyclic core of ZM241385. The phenolic hydroxyl group extending from the ethylamine chain forms a hydrogen bond with an ordered water molecule, while the phenyl ring forms hydrophobic interactions with Leu267 (7.32) and Met270 (7.35). A ZM241385 derivative with a cycloalkyl substituent (LUF5477)[57b] instead of the phenylmethylene also has high affinity for the A_{2A} adenosine receptor, suggesting that the nature of this interaction is hydrophobic and not π-stacking and demonstrating furthermore the tremendous substituent flexibility that exists in this area of the pharmacophore. The phenylethylamine chain in ZM241385 is directed toward the more solvent-exposed extracellular region (EL2 and EL3). These interactions appear to be important in designing synthetic A_{2A} selective antagonists, since different ARs have different residues located in this area. The furan ring of ZM241385 is situated deep in the

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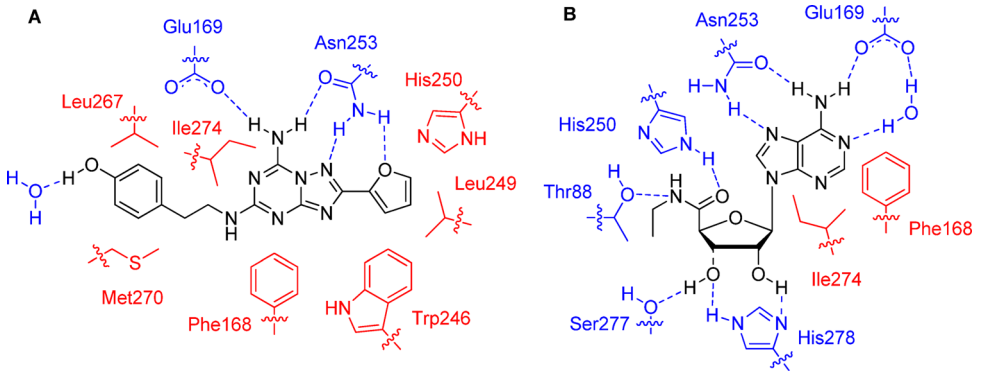

Figure 5. Binding interactions at the adenosine A_{2A} receptor: (A) antagonist ZM241385; (B) agonist NECA.

ligand-binding cavity. Its oxygen atom forms a hydrogen bond to Asn253 (6.55), and the furan ring has hydrophobic interactions with His250 (6.52) and Leu249 (6.51). The furan ring is approximately 3 Å away from the highly conserved Trp246 (6.48), limiting the motion of this tryptophan residue, which is believed to act as the "toggle switch" of this receptor, so that hydrophobic interactions between the furan ring and Trp246 hinder the structural rearrangements necessary for activation and constrain the receptor in the inactive state.

The crystal structures of A_{2A}-bound agonists adenosine and NECA show that both of these ligands bind to the stabilized receptor A_{2A}R-GL31 (vide infra) in a virtually identical fashion. Figure 5 shows the binding interactions of the antagonist ZM241385 (Figure 5A) and the agonist NECA (Figure 5B) at the orthosteric site of the A_{2A} receptor. The interaction of the adenine ring of adenosine and NECA with the A_{2A} receptor is similar to that of the chemically related triazolotriazine ring of the antagonist ZM241385. Thus, a similar hydrogen bond network joins the adenine scaffold to both Glu169 (5.30) and Asn253 (6.55), and the π-stacking and hydrophobic interactions with Phe168 (5.29) and Ile274 (7.39) are maintained. The main difference between agonists and antagonists is that agonists have a ribose moiety that forms hydrogen bonds with Ser277 (7.42) and His278 (7.43). These strong, attractive noncovalent interactions pull the extracellular ends of TM3, TM5, and TM7 together, which is believed to be the necessary prerequisite for receptor activation. In addition, because of the presence of the hydrogen donors at the ribose moiety, amino acids Val84 (3.32) and Trp246 (6.48) have to significantly shift their positions, a change that seems to be critical to achieving the conformation required to activate this receptor.

The structural information just summarized suggests alternative receptor-based approaches to finding new A_{2A} ligand chemotypes. For example, since the A_{2A} receptor has a deep and well-defined pocket, Katritch et al. developed a homology model and virtually screened more than 4 million commercially available "druglike" and "leadlike" compounds, looking for antagonists of novel structural types. This screen resulted in the identification of 23 high ligand efficiency (0.3–0.5 kcal/mol per heavy atom) hits with affinities under 10 μM in A_{2A} AR binding assays, 11 of those had submicromolar affinities, and two compounds had affinities under 60 nM.[58]

Despite being membrane-bound, these receptors are very dynamic structures that can adopt numerous thermodynamically stable conformations. To obtain diffraction-quality crystals, it is necessary to considerably stabilize the receptor. For example, the structure of the A_{2A} receptor bound to ZM241385 (A_{2A}-T4L) was modified by T4 lysozyme fusion in cytoplasmic loop 3 and the deletion of the carboxy-terminal tail (Ala317-Ser412). The A_{2A} structure bound to NECA is a thermostabilized construct (A_{2A}R-GL31) that contains four point mutations. Although none of the thermostabilizing mutations occurred in the binding pocket, these variations have an impact on the affinity values with respect to the wild type. As we have seen before, NECA is an agonist that activates the receptor; however, analysis of its crystal structure with the A_{2A} receptor shows a conformation between the inactive state (R) and the active state (R*). Although the X-ray crystal structures mentioned here represent the best tools to inspire rational ligand design and a platform to build homology models for virtual screening, unexpected results are sometimes obtained, due mainly to the conformational differences between the stabilized nature of the crystallized receptor and the dynamic nature of the wild type.

THERAPEUTIC APPLICATIONS OF A_{2A} RECEPTOR AGONISTS

The therapeutic value of adenosine was first considered and investigated in the late 1980s,[59] and the development of potent and selective A_{2A} agonists has been a subject of medicinal chemistry research for the ensuing 3 decades. The SAR of adenosine-based ligands has been recently reviewed,[60−62] and it has been widely accepted that the basic adenosine scaffold must be maintained.[63,64] As we discussed in the receptor structure section of this review, these adenosine-based ligands have little or no oral bioavailability and short half-lives due to the presence of three hydrogen bond donors in the sugar moiety which are critical for A_{2A} receptor activation but are subject to extensive metabolism. Research in medicinal chemistry has focused on improving the pharmacokinetic and A_{2A} selectivity profiles of these agonists by performing chemical modifications at the ribose and/or the purine moiety. Adenosine (Figure 1) shows the standard numbering of positions of these agonists to facilitate the understanding of the SAR discussed below.

Ribose-Modified Adenosine Derivatives. Structural modifications of the ribose ring have been extensively explored. Most of these analogues are devoid of adenosine agonist activity because they lack the 2′- and 3′-hydroxyl groups essential for activity.[63,65] Replacement of the ribose furan ring with cyclopentane results in carbocyclic analogues with very weak A_{2A} activity.[8] The most promising position for structural changes in the ribose unit is the primary 5′-hydroxyl group. In 1980, Prasad and co-workers discovered that *N*-alkylcarboxamide analogues showed increased agonist activity for all adenosine receptors.[66] As previously seen, the *N*-ethylcarbox-

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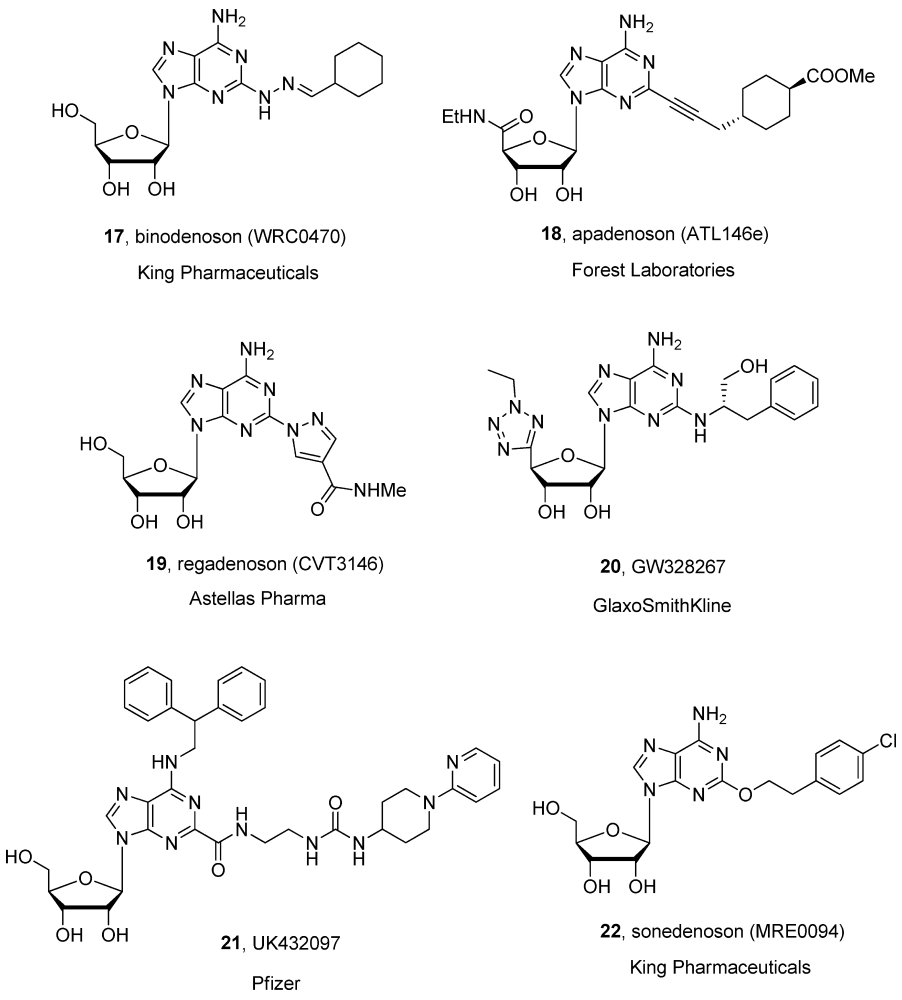

Figure 6. Structures of therapeutically relevant A_{2A} agonists.

amide derivative NECA shows good binding affinity at A_{2A} receptors (hA_{2A} K_i = 20 nM). Apparently the carbonyl group of NECA is involved in an important hydrogen bonding interaction with Ser277 on the seventh transmembrane domain of the activated conformation of A_{2A} receptors.[67] *N*-Alkylthiocarboxamides are also agonists, although these analogues are less active.[68] Among the most active A_{2A} agonists are 2-alkynyl NECA derivatives. Many compounds from this class have A_{2A} affinities in the nanomolar range. The alkylalkynyl derivatives generally exhibit higher potencies in binding and functional assays than those with aryl or heteroarylalkynyl groups.[69] In addition, good levels of potency and selectivity are shown by a number of different derivatives with a heteroaryl group as a bioisostere replacement of the amide moiety at the 5′-position.[70]

Purine-Modified Adenosine Derivatives. Substitution at C8 of the adenine ring of adenosine led to a decreased affinity for all adenosine receptors, presumably due to a conformational change of the nucleoside from an *anti*-conformation to a less favorable *syn*-conformation. The presence of nitrogen at the 3 and 7 positions is important for activity with all receptor subtypes.[63,71] A number of SAR studies have revealed that modifications at C2 and N6 of the adenine can be tolerated. Agonist **5**, a C2 aniline derivative briefly discussed in the historical agonists section of this review, displays 10-fold selectivity for A_2 versus A_1 and as such was the first reported A_2 selective agonist.[27] Since then, a variety of other A_{2A}-selective C2-substituted analogues have been discovered, including ethers,[72,73] thioethers,[73−75] amines such as CGS21680,[76] alkynes such as HENECA,[69,77,78] and hydrazines.[79−81] 2-(*N*′-Alkylidenehydrazino)adenosines and 2-(*N*′-arylalkylidenehydrazino)adenosines have been reported to be potent and selective agonists that have been used as coronary vasodilators.[79,80] The cyclohexylmethylene, cyclohexylethylidene, and benzylidene analogues all have EC_{50} values of less than 1 nM. Of particular interest are 2-(*N*′-cyclohexymethylidenehydrazino)adenosines where the hydrazone moiety has an *E*-conformation. Potency at A_2 receptors increases when bigger alkyl groups at the hydrazone are introduced and is higher for cycloalkyl than for linear alkyl groups. The size of the alkyl group is irrelevant for binding to A_1 receptors. Substitutions that limit the flexibility of this part of the molecule by conjugation with the −CH═N− bond (e.g., 1-cyclohexene) result in a reduction of potency. The most relevant compound of this series is binodenoson (**17**, Figure 6) which will be discussed in the agonist therapeutic applications part of this review.

Although N6 substitution is tolerated, it generally decreases A_{2A} potency. In many cases, N6 substituents can actually enhance A_1 and A_3 affinity and selectivities.[78,82] This is exemplified by *N*6-(tetrahydrofuryl)adenosine (tecadenoson), which is a potent A_1-selective agonist.[83,84]

Ribose and Purine-Modified Adenosine Derivatives. As we have seen already, the 2-position of the adenine moiety

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and the 5′-position of the ribose offer us two opportunities for structural modification. These can both be modified simultaneously to prepare active and selective analogues. One example is CGS21680, a 5′-NECA derivative bearing a 2-(2-phenylethyl)amino group at the adenine 2-position, a compound already discussed in the historical ligand section of this review.[28b] Bulky groups can be incorporated at the terminal carboxylate group of such analogues without compromising A_{2A} potency.[85] In the following section we will discuss other A_{2A} active and selective agonists in which adenosine has been successfully modified at C2 and 5′, such as apadenoson (**18**, Figure 6) and antagonist **20** (GW328267, Figure 6).

A_{2A} Agonist Therapeutic Applications. *Myocardial Perfusion Imaging (MPI).* In cardiac stress tests, either physical exercise or a pharmacologic vasodilating agent is used to stimulate the heart and achieve maximum myocardial hyperemia. Pharmacologic agents are generally used when a patient is unable to achieve an adequate work level with treadmill exercise or has poorly controlled hypertension.[86,87] Almost 50% of myocardial perfusion imaging (MPI) is performed with a pharmacologic stress agent.[86] The endogenous agonist adenosine has a vasodilating effect that is mediated primarily through stimulating A_{2A} receptors on arteriolar vascular smooth muscle cells. Adenosine, marketed by Astellas Pharma under the trade name Adenoscan, is widely used in clinical practice to induce coronary arterial vasodilation after being intravenously administered.[88,89] Because of its nonselective nature adenosine activates the A_1, A_{2B}, and A_3 receptors as well as the A_{2A}, and so has limited therapeutic applications. To be useful in stress tests, the activity of an agonist needs to be sufficiently short to minimize side effects, while long enough (ideally 2−4 min) to allow maximal extraction of the radiotracer during the vasodilation. Although adenosine's extremely short half-life minimizes many of its side effects such as bronchospasms, dyspnea, and high-grade atrioventricular (AV) block, an A_{2A} selective agonist would be preferable.[88]

Binodenoson (Figure 6, also known as WRC-0470 or MRE-0470) was a clinical candidate for myocardial perfusion imaging. Binodenoson has moderate binding affinity at the human A_{2A} receptor (hK_i A_{2A} = 270 nM), with a 370-fold selectivity for A_{2A} versus A_{2B} and more than 170-fold selectivity versus A_1 (Table 1).[79,80] The hydrazone double bond has a *E*-configuration.[90] Binodenoson was developed by King Pharmaceuticals as a potential coronary vasodilator and an adjunct to a SPECT imaging agent for myocardial perfusion imaging in the diagnosis of coronary artery disease (CAD).[91] In a rat study, binodenoson produced systemic vasodilation (ED_{50} = 0.31 mg) and a decrease in heart rate (A_1 mediated ED_{50} = 620 mg). Intravenous infusion of binodenoson (0.6 mg kg^{-1} min^{-1}) was equipotent to adenosine (300 mg kg^{-1} min^{-1}) in increasing coronary flow. Blood pressure was markedly reduced by adenosine but remained unchanged with binodenoson.[91] King Pharmaceuticals filed an NDA in 2008, but the application was rejected by FDA. Binodenoson development appears to have been halted after Pfizer acquired King Pharmaceuticals, since binodenoson is not listed in Pfizer's pipeline.

Apadenoson (also named ATL164e or stedivaze, **18**, Figure 6) is a derivative with a propynylcyclohexanemethylester group at the 2-position of adenine. Apadenoson displays a remarkable binding potency with recombinant human A_{2A} receptors (hA_{2A} K_i = 0.5 nM), with 150-fold and 90-fold selectivity versus A_1 and A_3, respectively (Table 1).[92] In 2000, Adenosine Therapeutics (since acquired by Forest Laboratories) licensed apadenoson from the University of Virginia. In 2009, the first phase III trial in myocardial perfusion imaging (MPI) was initiated, followed by a second phase III trial in June 2011. However, by May 2012, further development was discontinued, and no clinical data were reported.

Regadenoson (also known as CVT3146, **19**, Figure 6)[93,94] is an adenosine derivative bearing a *N*-pyrazole at its 2-position. The *N*-pyrazole is designed as a constrained mimetic of the *E*-hydrazone moiety in binodenoson. A large variation of hydrophilic and lipophilic substitutuents can be placed on the pyrazole ring while retaining activity at the A_{2A} receptor. Regadenoson was approved by FDA in 2008 for MPI and is marketed by Astellas Pharma under the trade name Lexiscan. Regadenoson has a relatively low binding affinity (hA_{2A} K_i = 290 nM) for human A_{2A} receptors and greater than 30-fold selectivity versus the A_{2B} and A_3 AR subtypes and 13-fold over the A_1 AR.[94] Regadenoson can produce a response of equivalent magnitude and a more rapid termination of action than other higher affinity agonists (e.g., CGS21680). This drug is administered as an iv bolus 30 s before the radionuclide.[86,95]

Inflammation. Because of its potentially severe side effects, including hypotension and bradycardia, as a result of nonselective activation of all four widely expressed subtypes of adenosine receptors, systemic administration of adenosine has limited clinical potential for treating inflammation. The activation of the A_{2A} receptors regulates the activity of the inflammatory cells involved in innate and adaptive immune responses and plays a role in terminating inflammation.[96] A_{2A} agonists modulate the activity of neutrophils, macrophages, and T lymphocytes, as well as various other inflammatory cells including fibroblasts, monocytes, platelets, and mast cells.[97] In vivo studies showed that **18** reduces joint destruction due to septic arthrosis, and CGS21680 regulates HIV-1 transactivating regulatory protein (Tat) induced inflammatory responses.[98] Although A_{2A} agonists are vasodilators, they inhibit inflammation at lower doses which produce few or no cardiovascular side effects. The wide distribution of the A_{2A} receptor, however, suggests that the therapeutic potential of A_{2A} agonists is likely to reside in topical treatments to avoid systemic side effects associated with oral administration.

Agonist **20** has good A_{2A} binding affinity (A_{2A} K_i = 46 nM) with a selectivity of 28-fold, 8-fold, and 2-fold versus A_{2B}, A_1, and A_3 receptors, respectively.[99,100] In functional assays, it shows high potency and efficacy in cAMP formation (EC_{50} = 9 nM, $E_{max(NECA)}$ = 78%) and in the isolated rat aorta assay (EC_{50} = 10 nM, $E_{max(NECA)}$ = 90%).[100] Studies showed that **20** improved lung function after acute lung injury in rats, and it was developed by GlaxoSmithKline for the treatment of allergic rhinitis and asthma.[101,102]

The C2- and N6-modified adenosine analogue UK432097 (**21**, Figure 6) was developed by Pfizer for the treatment of chronic obstructive pulmonary disease. It has a very potent A_{2A} binding affinity (hA_{2A} K_i = 4 nM),[102] but its development was discontinued because of poor efficacy results. The use of A_{2A} receptor agonists as potential agents to treat rheumatoid arthritis has also been proposed, but there are no clinical reports yet available.[103]

Neuropathic Pain. Studies show that glial proinflammatory cytokines have been identified as important contributors to neuropathic pain and that interleukin 10 (IL-10) can suppress such pain.[104] Activation of A_{2A} receptors decreases proinflammatory cytokine release and increases release of the potent

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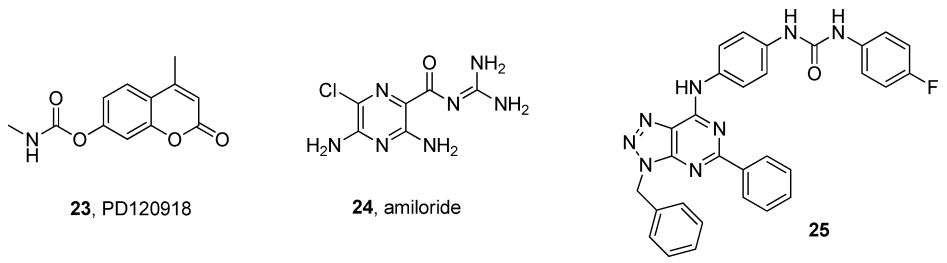

Figure 7. A_{2A} allosteric modulators.

anti-inflammatory cytokine IL-10. Activation of A_{2A} receptors after intrathecal administration of A_{2A} agonists may be a novel therapeutic approach for the treatment of neuropathic pain by increasing IL-10 in the immune cells of the CNS. However, to the best of our knowledge, there is no report in clinical trial where intrathecal administration is being used as a viable route of administration. One in vivo study shows CGS21680 (Figure 1) produced a long-duration reversal of mechanical allodynia and thermal hyperalgesia for prolonged time.[105] Cambridge Biotechnology and Ergomed are developing BVT115959 (structure not disclosed) as a new therapy for neuropathic pain. A phase I study demonstrated that BVT115959 is safe and well tolerated, and results of a phase II trial initiated in March 2012 are expected in mid-2013.

Other Therapeutic Areas. In vivo studies with **21** indicate that A_{2A} receptor agonists promote wound healing and reduce ulcer formation in normal and diabetic animals.[25] A C2-ether derivative, 2-[2-(4-chlorophenyl)ethoxy]adenosine, also known as sonedenoson (also named MRE0094, **22**, Figure 6, hA_{2A} K_i = 490 nM and 20-fold selective against A_{2B} and A_1) was under development by King Pharmaceuticals as a potential new therapy for diabetic foot ulcers.[25,106] However, clinical trials failed to demonstrate the desired clinical efficacy, and its development was discontinued. In addition, Forest Laboratories Inc. has been investigating A_{2A} receptor agonists for the potential treatment of *Clostridium difficile* infection.[107] Zalicus Inc. has been using combinations of an A_{2A} agonist with a β_2-adrenergic receptor agonist for the potential treatment of B-cell malignancies such as multiple myeloma,[108] and Inotek Pharmaceutical Corporation investigated PJ1165 as a potential topical treatment for psoriasis and atopic dermatitis.[109]

Partial Agonists. Regadenoson behaves as a weak partial agonist causing cAMP accumulation in PC12 cells but as a full and potent agonist causing coronary vasodilation.[110] In 2003, van Tilburg and co-workers reported that 2,8-disubstituted adenosine derivatives were adenosine receptor partial agonists.[65] Although both 2-(1-hexynyl)adenosine and 2-[(*E*)-1-hexenyl]adenosine have high binding affinities (K_i in the nanomolar range), they show submaximal levels of cAMP production in Chinese hamster ovary (CHO) cells expressing human A_{2A} receptors, compared to the reference compound CGS21680. Introduction of 8-alkylamino substituents in most cases further reduces intrinsic activity. Most of these 8-alkylamino derivatives behave as partial agonists, with 8-propyl and 8-butylamino derivatives having the best intrinsic activity and behaving as full agonists.

Agonist Radioligands. Both [^{3}H]NECA and [^{3}H]-CGS21680 are used extensively as agonist radioligands to characterize A_2-adenosine receptors in a variety of tissues. As we have seen previously, NECA is a highly potent agonist, but it is nonselective with regard to the four adenosine receptor subtypes. [^{3}H]NECA has been successfully used to localize A_{2A} adenosine receptors in rat striatal membranes.[111] CGS21680 displays high A_{2A} binding affinity (rA_{2A} K_i = 11 nM) and has more than 140-fold selectivity versus A_1 in rat striatal membranes.[28b] In humans, it is highly selective versus A_{2B} and exhibits a 10-fold selectivity versus A_1, but it has similar potency at the A_3 receptor. The high affinity and degree of selectivity over A_{2B} makes [^{3}H]CGS21680 the current radiolabeled agonist of choice as a tool for many different experimental studies involving A_{2A} receptors.

Allosteric Modulators. Allosteric modulators bind at a distinct site other than the natural ligand binding site (orthosteric site). They exert their effect only in the presence of the orthosteric ligand. A positive allosteric modulator (PAM) induces an enhancement of effects of the orthosteric ligand, while a negative allosteric modulator (NAM) attenuates those effects. Coumarin **23** (PD120918, Figure 7) enhances agonist radioligand binding to rat striatal A_{2A} adenosine receptors, but no functional difference in activity was observed.[112,113] A_{2A} adenosine receptors are allosterically modulated by sodium ions and the potassium-sparing diuretic amiloride (**24**, Figure 7).[114] In rat striatal membranes, both amiloride and its analogues increase, while sodium ions decrease, the dissociation rate of the antagonist [^{3}H]ZM241385 from the A_{2A} adenosine receptors in a concentration-dependent manner. However, amiloride, amiloride analogues, and sodium ions do not show any effect on the dissociation rate of the agonist [^{3}H]-CGS21680. In 2008, Giorgi and co-workers reported that the *N*6-1,3-diphenylurea derivative of 2-phenyl-9-benzyl-8-azadenine (**25**, Figure 7) acts as a positive binding-enhancer of agonist and antagonist radioligands at the A_{2A} receptors (hA_{2A} K_i = 167 nM, Table 1).[115] The agonist-enhancing activity of **25** was demonstrated by a significantly higher vasodilating effect of CGS21680 when allosteric modulator **25** was present in the rat aortic ring assay. Although most of the efforts to modulate A_{2A} receptors have been focused on the use of orthosteric ligands, the facts mentioned in this paragraph show that pharmacologic responses can also be fine-tuned using allosteric modulators.

THERAPEUTIC APPLICATIONS OF A_{2A} RECEPTOR ANTAGONISTS

Parkinson's Disease. Parkinson's disease (PD) is a neurodegenerative disorder named after James Parkinson, the English doctor who in 1817 first described its symptoms.[116] PD is caused by a progressive loss of dopaminergic neurons in the substantia nigra region of the basal ganglia, which results in progressive impairment in motor functions (i.e., bradykinesia, resting tremor, muscle rigidity, and postural instability).[117] It is currently estimated to affect ~1.5% of the world population over the age of 60.[118] The current treatment for PD is primarily based on dopamine replacement therapy. Levodopa (L-DOPA), a metabolic precursor of dopamine (DA), has been

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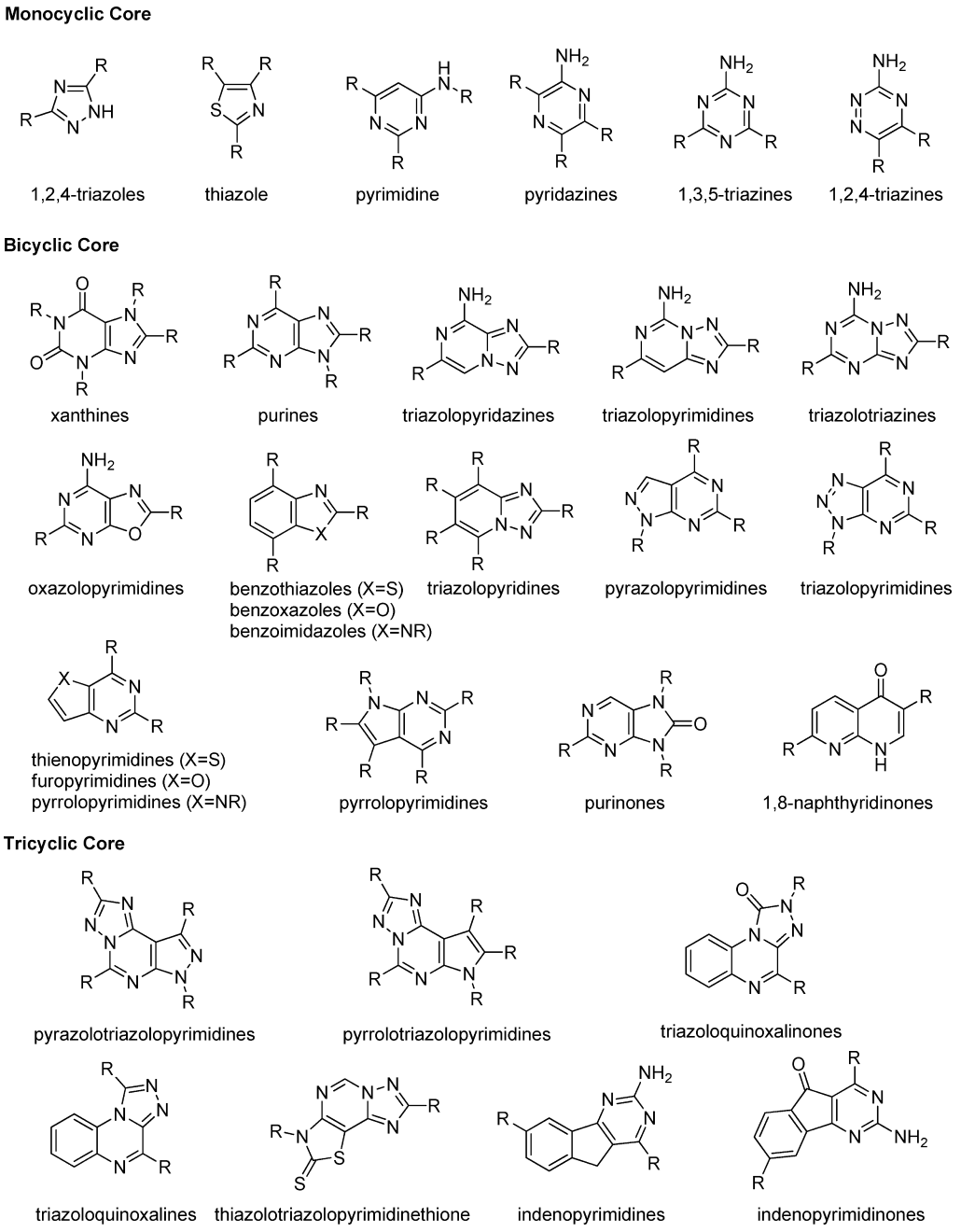

Figure 8. Overview of general core structures of A_{2A} antagonists for the treatment of PD.

the gold standard treatment for decades.[119] Other strategies used to elevate or maintain DA brain levels involve the use of DA agonists,[120] inhibitors of DA reuptake,[121] or inhibitors of DA metabolizing enzymes such as monoamine oxidase B (MAO-B)[122] and catechol-*O*-methyltransferase (COMT).[123] However, with long-term treatment, these dopamine-targeted drugs carry the risk of undesirable side effects, including motor fluctuations (e.g., wearing-off, "on−off" phenomena, dyskinesia) and hallucinations.[124] Because of these significant limitations of dopamine replacement therapy, nondopaminergic strategies have been explored for potential PD treatment.[125] Among nondopaminergic strategies, selective adenosine A_{2A} and dual A_1/A_{2A} receptor antagonists have emerged in recent decades as potential therapeutic agents to treat the symptoms of PD.[126]

As we mentioned earlier in this review, in the striatopallidal neurons at the striatum, adenosine A_{2A} receptors are colocalized with dopamine D_2 receptors, and these two receptors exert opposite effects on motor behavior.[127] For example, stimulation of the dopamine D_2 receptors with dopamine or other dopamine D_2 receptor agonists enhances motor activity, while activation of A_{2A} receptors reduces this effect by inhibiting dopamine D_2 receptor signaling.[46] Therefore, antagonism of A_{2A} receptors enhances D_2-dependent signaling and improves motor disabilities in animal models of PD (e.g., 6-OHDA treated animal model (dopamine depleted);[128] 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated non-human primate model).[129] Furthermore, it has been reported that blockade of A_{2A} receptors results in remarkable therapeutic advantages, not only potentiating the effects of L-DOPA but

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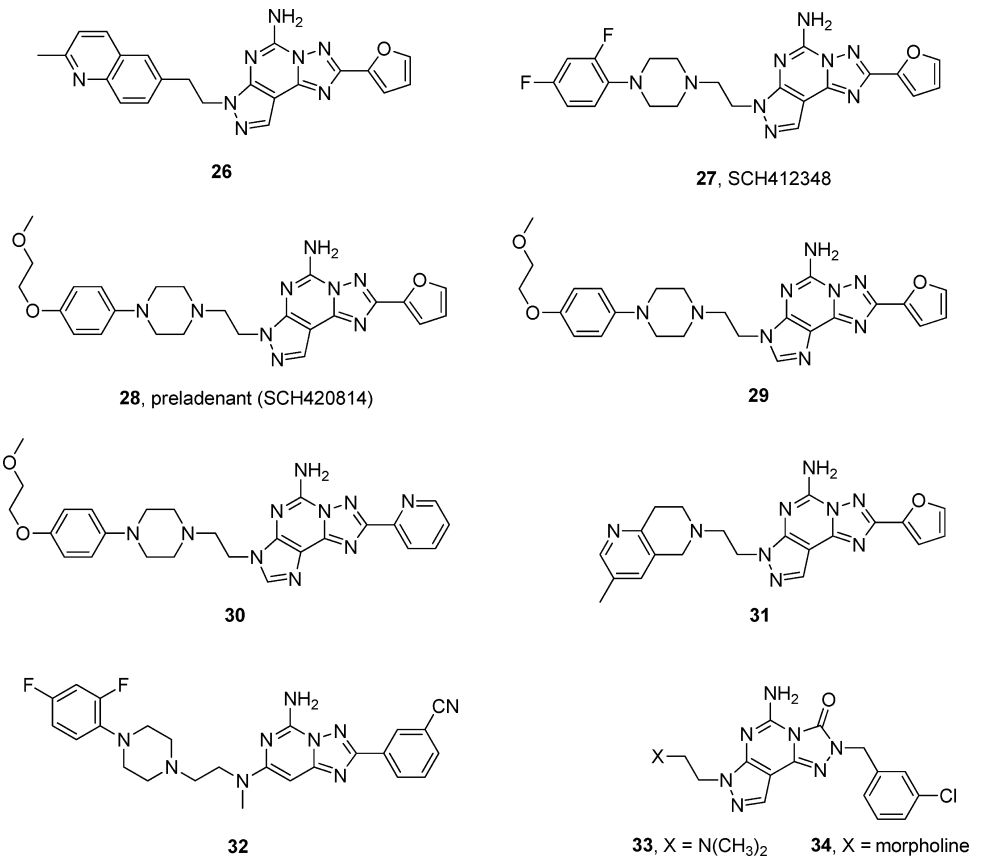

Figure 9. Selected A_{2A} antagonists from Merck/Schering-Plough.

also alleviating development of the dyskinesia normally associated with long-term L-DOPA treatment.[130]

Recently, a number of reviews related to A_{2A} receptor antagonists have been published.[131] As discussed in the historic A_{2A} ligands section of this review, adenosine A_{2A} receptor antagonists have been traditionally divided into xanthine-based and non-xanthine-based derivatives. Xanthine derivatives have several limitations as pharmacologic tools, such as poor water solubility.[132] In addition, rapid photoisomerization of the side chain olefin of istradefylline after exposure to daylight in dilute solutions have been observed.[133] Consequently, the discovery of xanthine-based A_{2A} receptor antagonists with desirable pharmacologic and physicochemical properties has remained a challenge, and research has become focused on the search for alternative non-xanthine-based heterocyclic derivatives.

Non-xanthine-based adenosine A_{2A} receptor antagonists have been generally classified, on the basis of their core structures, as monocyclic, fused bicyclic, and fused tricyclic derivatives (Figure 8). A number of monocyclic core derivatives are currently being evaluated as potential adenosine A_{2A} receptor antagonists,[134] and a variety of fused bicyclic[135] and tricyclic compounds[136] structurally related to adenosine have been identified as A_{2A} receptor antagonists. These antagonists contain an exocyclic amino group, and their potency and selectivity have been explored by the installation of various substituents onto each of these heterocyclic templates.

It is not clear yet which is a better approach toward treating the symptoms of PD: a selective A_{2A} antagonist or a dual A_1/A_{2A} antagonist. On one hand, most research organizations are looking for selective A_{2A} antagonists to minimize possible cardiovascular effects caused by interaction with the A_1 receptor.[36] On the other hand, companies such as Astellas Pharma and Johnson & Johnson consider A_1 antagonism as a desirable feature because it enhanced cognition in rodents.[137,138] Herein, we provide a summary of the most relevant compounds organized by the different companies that have been actively working in the area of PD.

Merck/Schering-Plough. Merck & Co. (following its acquisition of Schering-Plough) has been very active in the adenosine A_{2A} antagonist field, having advanced the compound preladenant (also named SCH420814, **28**, Figure 9)[141] to phase III in clinical trials. The core scaffold of preladenant had its origin in SCH58261 (Figure 2), a pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine described in the historic A_{2A} antagonists section of this review. Because of poor water solubility and the low oral bioavailability of SCH58261, researchers at Schering-Plough explored the SAR of the phenethyl side chain, replacing the phenyl ring with biaryl and fused heteroaryl substituents. The resulting methylquinoline analogue **26** (Figure 9) exhibited a potent A_{2A} binding affinity (hA_{2A} K_i = 2.4 nM) and moderate selectivity over human A_1 (169-fold).[139] Notably, fused heteroaryl analogue **26** demonstrated a superior pharmacokinetic profile in rats (AUC = 1405 ng·h/mL at 3 mg/kg, po) compared with SCH58261, having sustained plasma levels over 4 h and efficacy in the rat catalepsy model at 1 h (86% inhibition at 3 mg/kg, po) and 4 h (38% inhibition at 3 mg/kg, po) after dosing.[139] In addition, efforts to replace the phenethyl side chain with an arylpiperazine scaffold delivered SCH412348 (**27**, Figure 9), a very potent A_{2A} antagonist (hA_{2A} K_i = 0.6 nM), with high selectivity over the human A_1 receptor (>1660-fold).[140] Despite its excellent anticataleptic effect in rats (75% and 80% inhibition at 1 and

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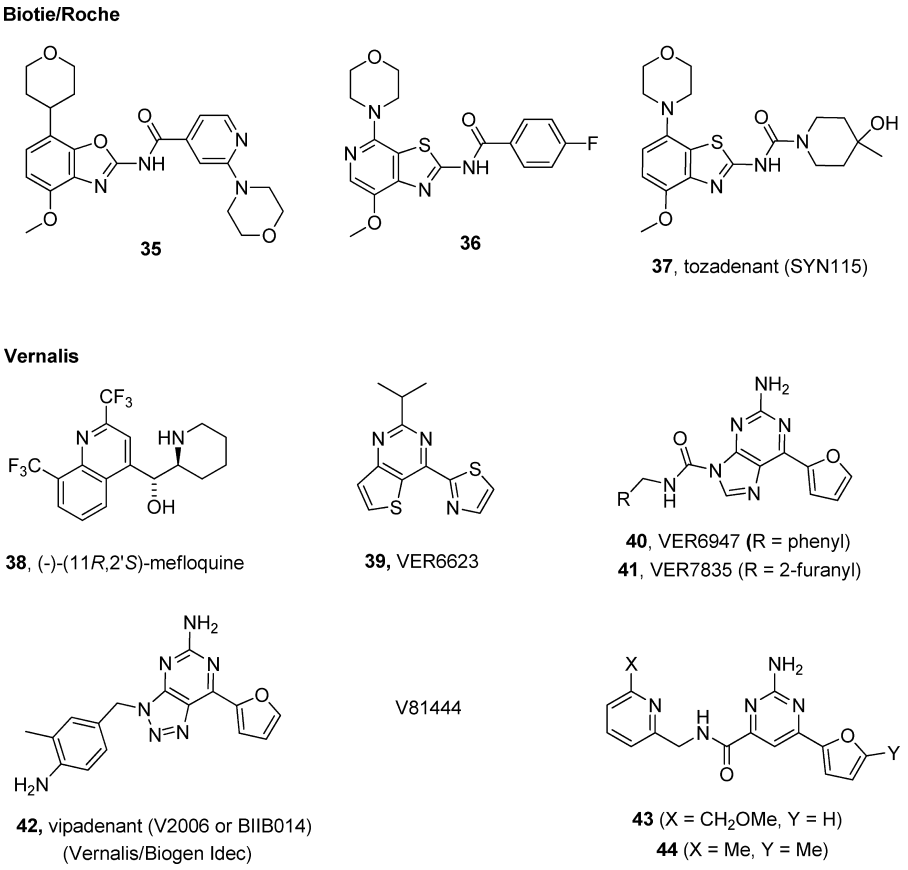

Figure 10. Selected A_{2A} antagonists from Biotie/Roche and Vernalis.

4 h, respectively, at 1 mg/kg dose, po), **27** was not developed further because of its poor water solubility. In order to improve solubility, a polar substituent (methoxyethoxy) was introduced at the para position of the aryl group to give preladenant, a compound that exhibited excellent A_{2A} binding affinity (hA_{2A} K_i = 1.1 nM) and human A_1 selectivity (1340-fold). In the rat, preladenant displayed good plasma levels (AUC = 1560 ng·h/mL at 3 mg/kg, po), adequate oral bioavailability (F = 57%), a relatively short half-life ($t_{1/2}$ = 2.1 h at 1 mg/kg dose, iv), moderate clearance (Cl_p = 37 mL min^{-1} kg^{-1}), and a brain-to-plasma ratio of 1. Preladenant demonstrated excellent dose-dependent in vivo efficacy in the haloperidol-induced rat catalepsy assay (77% and 70% inhibition at 1 and 4 h, respectively, at 1 mg/kg dose, po), with a minimum efficacious dose (MED) of 0.3 mg/kg at both 1 and 4 h time-points. Furthermore, preladenant reversed the hypolocomotion induced by treatment with the A_{2A} agonist CGS21680 in rats and showed substantial striatal A_{2A} receptor occupancy after oral doses of more than 0.1 mg/kg. In addition, preladenant showed a dose-dependent potentiation of L-DOPA-induced contralateral rotations in unilaterally 6-OHDA-lesioned rats (0.03 to 1 mg/kg, po).[141] Although preladenant showed efficacy in one phase II clinical trial (P4501) at 2, 5, and 10 mg/kg (po,), on May 13, 2013, Merck announced that development of preladenant was discontinued because it failed to demonstrate efficacy versus placebo in three phase III clinical trials for PD.[142]

Further optimization of the preladenant structure was investigated by modifying the tricyclic core, replacing the furan ring, and modifying the side chains. Replacing the pyrazole ring with an imidazole on the tricyclic core provided compound **29** (Figure 9), a potent and highly selective analogue (hA_{2A} K_i = 0.9 nM, 669-fold over hA_1). Compound **29** reversed haloperidol-induced rat catalepsy with 55% and 50% inhibition (1 mg/kg, po) at 1 and 4 h, respectively, but this imidazolopyrimidine series showed inferior selectivity and pharmacokinetic profiles and a lower in vivo efficacy than the pyrazolopyrimidine series.[143] Numerous efforts have been made to replace the furan moiety; however, substitution of this critical structural functionality is a major challenge, and the corresponding aryl and heteroaryl analogues show a reduction in A_{2A} potency and selectivity versus A_1 receptors (e.g., compound **30**, Figure 9, hA_{2A} K_i = 12.6 nM, 108-fold over hA_1).[143] As mentioned previously, analogues having an arylpiperazine-based tail are potent and selective A_{2A} receptor antagonists, but all of the reported lead compounds possess poor water solubility. To address this issue, researchers at Schering-Plough have explored a variety of fused heterocyclic side chains containing amines. Compound **31** (Figure 9, hA_{2A} K_i = 2 nM, 179-fold over hA_1) shows excellent water solubility (100 μM at pH 7.4), a good plasma level (AUC = 7980 ng·h/mL at 3 mg/kg dose, po), a long half-life ($t_{1/2}$ = 11.3 h), high oral bioavailability (F = 71%), and a low plasma clearance (4.7 mL min^{-1} kg^{-1}). It also showed a potent oral anticataleptic activity at doses of 3 mg/kg (80% inhibition at 1 h) and 1 mg/kg (65% inhibition at 1 h, ~35% inhibition at 4 h) in the rat catalepsy model. Unfortunately, compound **31** was not further developed because of a lower selectivity versus the human A_1 receptor (179-fold) with respect to other compounds in this series.[144]

During SAR exploration of A_{2A} antagonists, researchers at Schering-Plough investigated a 1,2,4-triazolo[1,5-*c*]pyrimidine

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series that lacks the pyrazole ring of the preladenant series and possesses the optimized arylpiperazine side chain. In general, these compounds demonstrate a high A_{2A} binding affinity, good selectivity versus the A_1 receptor and significant rat plasma levels. Replacement of the furan moiety attached to the bicyclic scaffold by a 3-cyanophenyl group provided compound **32**, a very potent antagonist (hA_{2A} K_i = 1.8 nM) with good selectivity over the human A_1 receptor (620-fold) and desirable plasma exposure in rats (AUC = 1295 ng·h/mL at 3 mg/kg dose, po).[145] Nevertheless, this series, including compound **32**, showed significantly lower rat catalepsy inhibiting activity compared with the corresponding analogues in the tricyclic series (**7**, 35% and 14% inhibition at 3 mg/kg, po). More recently, Merck/Schering-Plough has disclosed a series of novel pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-one (core of compounds **33** and **34**) derivatives, which were developed to find a suitable replacement of the furan moiety and improve the solubility of preladenant. As discussed previously, incorporation of a basic nitrogen in the side chain of the preladenant series led to compounds with acceptable binding affinities and selectivity. On the basis of this observation, a morpholine and various other amines were introduced in the pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-3-one core containing a 3-chlorobenzyl group instead of the furan. Relevant examples of this series are the dimethylamino analogue **33** (Figure 9, hA_{2A} K_i = 5.2 nM, selectivity 269-fold over hA_1) and the morpholine derivative **34** (Figure 9, hA_{2A} K_i = 14.2 nM, selectivity 99-fold over hA_1). As we have noted with other non-furan analogues, compounds in this series showed a significant lack of anticataleptic activity in the rat at 1 and 4 h (10 mg/kg, po).[146]

Biotie/Roche. A research group from Hoffmann-La Roche has reported in the patent literature a series of potent and selective A_{2A} antagonists derived from benzoxazole, thiazolopyridine, and benzothiophene cores. Earlier SAR studies of these series were highlighted in a preceding review and references therein.[147] Although the benzothiazole appears to be the most interesting core in this series, there are also patents claiming benzoxazoles and thiazolo[5,4-*c*]pyridines as adenosine A_{2A} ligands.[148,149] Compounds **35** (Figure 10, hA_{2A} K_i = 25 nM, 351-fold over hA_1) and **36** (Figure 10, hA_{2A} K_i = 5 nM, 420-fold over hA_1) are representative examples of the benzoxazole and thiazolopyridine cores, respectively. However, no rat catalepsy data for these two compounds have been disclosed. More recent patent applications have claimed 4-morpholino-6-methoxybenzothiazoles containing a variety of urea and amide side chains as selective A_{2A} antagonists. Among these analogues, Roche has identified tozadenant (also named SYN115, **37**, Figure 10), which exhibited potent A_{2A} binding affinity (hA_{2A} K_i = 5 nM) and 270-fold selectivity over human A_1.[150] In a functional assay (cAMP), tozadenant showed over 4000-fold selectivity versus the human A_1 receptor. In the 2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-ethylcarboxamidoadenosine (APEC) induced hypolocomotion rat model, tozadenant significantly reduced motor deficits with ID_{50} of 0.5 mg/kg and ID_{90} of 3.4 mg/kg when dosed orally. In addition, tozadenant showed very good pharmacokinetic parameters in rat and dog [$t_{1/2}$ = 4 (rat), 2.2 (dog) h; Cl_p = 11 (rat), 8 (dog) mL min^{-1} kg^{-1}, V_d = 1.4 (rat), 1.2 (dog) L/kg; F = 77% (rat, 5 mg/kg, po), 88% (dog, 5 mg/kg, po)].[151] Biotie Therapies (formerly Synosia Therapeutics) has a license agreement for tozadenant with Roche as a potential treatment of PD. Tozadenant is currently in phase IIb trials to evaluate its safety and efficacy. Biotie has granted UCB Pharma S.A. a license for exclusive, worldwide rights to tozadenant. Pending evaluation of the results of the ongoing study UCB Pharma will be responsible for conducting the phase III program and commercializing tozadenant (clinicaltrials.gov identifier NCT01283594).[152]

Vernalis. While investigating side effects associated with the antimalarial drug mefloquine, researchers at Vernalis unexpectedly found that the (−)-(*R*,*S*)-isomer of mefloquine (**38**, Figure 10) displayed moderate A_{2A} receptor binding affinity (hA_{2A} K_i = 61 nM).[153] Although mefloquine had poor selectivity versus human A_1 receptor (4-fold) and was ineffective in rodent models in vivo, it inspired medicinal chemistry efforts to develop novel adenosine A_{2A} receptor antagonists for the treatment of PD. From the initial screening of over 2000 compounds, a number of interesting series emerged. Thieno-[3,2-*d*]pyrimidine **39** (also named VER6623, Figure 10) showed a high affinity for the human A_{2A} receptor (hA_{2A} K_i = 1.4 nM) with moderate selectivity over the human A_1 receptor (148-fold) but low oral bioavailability (F = 5.8%).[154] Further optimization of this series led to the novel triazolo[4,5-*d*]pyrimidine derivatives **40** and **41** (also named VER6947 and VER7835, respectively, Figure 10), which showed extremely high affinities for the human A_{2A} receptor (hA_{2A} K_i = 1.1 nM and hA_{2A} K_i = 1.7 nM, respectively), and 15- and 100-fold selectivity, respectively, over human A_1. In particular, compound **41** was orally active in a haloperidol-induced hypolocomotion mouse model at a dose of 10 mg/kg.[155] Further development of these series was discontinued, however, because of low oral bioavailability and poor in vivo stability of these compounds in the rat. This liability was circumvented by substituting the urea moiety with a benzyl group to yield vipadenant (also named V2006/BIIB014, **42**, Figure 10).[156] Vipadenant exhibited high binding affinity for the human A_{2A} receptor (K_i = 1.3 nM), moderately low selectivity versus human A_1 and A_{2B} receptor subtypes (52- and 48-fold, respectively), and a high selectivity over the human A_3 receptor (773-fold). In haloperidol-induced hypolocomotion rodent models, vipadenant was highly efficacious with a MED of 0.1 mg/kg. In addition, vipadenant increased the number of contralateral rotations in 6-OHDA-lesioned rats when administered orally in combination with a subthreshold dose of the dopamine D_1 and D_2 agonist apomorphine at 3 and 10 mg/kg. Furthermore, in MPTP-lesioned marmosets pretreated with L-DOPA, vipadenant was efficacious with a MED of 5 mg/kg and showed none of the dyskinesias usually observed when L-DOPA is the sole treatment. In partnership with Biogen Idec, Vernalis initated clinical development of vipadenant for the treatment of PD. However, in spite of positive phase II results, development was stopped in July 2010 because of adverse findings in preclinical toxicology studies.[157]

Vernalis is developing V81444 (structure not disclosed), an alternative compound that was also in development for the potential treatment of PD and other CNS disorders and that is currently in phase I trials. In December 2012, Vernalis announced successful positive results from a receptor occupancy study with this antagonist and plans for a phase IIa study to begin in the first half of 2013.[158]

In addition to the development of the series just mentioned, Vernalis also explored pyrimidine-4-carboxamide, pyridine-4-carboxamide, and triazine-4-carboxamide derivatives as monocyclic adenosine A_{2A} antagonists, in hope of obtaining improved aqueous solubility in comparison to the bicyclic and tricyclic analogues while maintaining A_{2A} potency and selectivity.[159,160]

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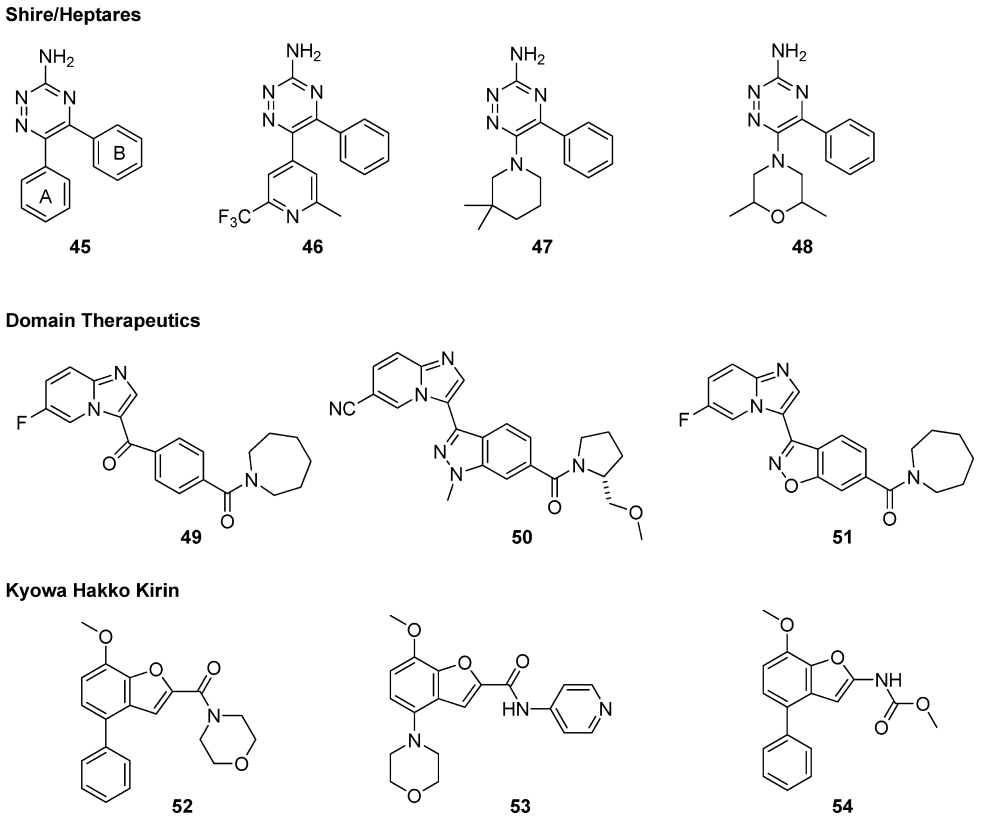

Figure 11. Selected A_{2A} antagonists from Shire Therapeutics, Domein Therapeutics, and Kyowa Hakko Kirin.

Most of the compounds in this series showed a high in vitro A_{2A} affinity, modest selectivity against A_1 receptors, and acceptable selectivity against A_{2B} and A_3 receptors. Pyridine and triazine-based compounds had considerably lower binding affinities for the A_{2A} receptor compared with the pyrimidine analogues. The 6-methoxymethyl-2-pyridyl analogue **43** (Figure 10, hA_{2A} K_i = 1.7 nM, selectivity 25-fold over hA_1) displayed excellent aqueous solubility (>450 μM at pH 7.4), very high oral bioavailability in the rat (F = 90%), and a good brain/plasma ratio. It was efficacious in the haloperidol-induced hypolocomotion mouse model with a MED of 0.1 mg/kg. The 6-methyl-2-pyridyl analogue with a 5-methyl-2-furanyl moiety **44** (Figure 10, hA_{2A} K_i = 2.5 nM, selectivity 53-fold over hA_1) was also orally active in the hypolocomotion mouse model at 1 mg/kg dose, although it had a very low oral bioavailability (F = 3%). As was mentioned earlier, the structure of V81444 has not been disclosed, and it is not known if V81444 corresponds to the vipadenant series or whether Vernalis has been able to discover a monocyclic analogue with good in vivo efficacy in animal models and the superior aqueous solubility profile expected for monocyclic compounds.

Shire/Heptares Therapeutics. Shire, under license from Heptares Therapeutics, is currently investigating A_{2A} receptor antagonists for the treatment of Parkinson disease and cognition and other CNS disorders. By performing a virtual screening of over 500K compounds, researchers at Heptares Therapeutics identified several novel compounds with high in vitro A_{2A} antagonism, with CNS druglike properties, and lacking structural alerts such as the furan ring.[161] The most interesting derivatives were those with the 1,3,5-triazine core (Figure 8) which upon optimization led to the discovery of 1,2,4-triazine analogues **45**–**47** (Figure 11) where the central ring mimics adenine, and ring A accesses the water pocket that is occupied by the ribose group of the natural ligand adenosine. Commercially available 5,6-diphenyl-1,2,4-triazine-3-amine **45** (Figure 11) exhibited an antagonism at human A_{2A} receptor (K_i = 115 nM) and basically no selectivity over human A_1 (2-fold). Additional SAR exploration in this series, such as replacement of the phenyl ring (ring A) of compound **45** with a 4-pyridyl group, resulted in the discovery of analogue **46**, which showed a substantial increase in potency at the human A_{2A} receptor (K_i = 3.5 nM); however, the human A_1 selectivity continued to be low (8.8-fold). Heptares has reported that compound **46** displayed moderate clearance (Cl_p = 42 mL min^{-1} kg^{-1}), a relatively high volume of distribution (4.6 L/kg), an acceptable half-life ($t_{1/2}$ = 1.1 h), high bioavailability (F = 100%), and good plasma exposure (AUC = 846 ng·h/mL at 2 mg/kg, po) in the rat. Compound **46** also demonstrated excellent brain penetration (ratio of brain/plasma of 3.2 at 0.5 h after iv dose). Moreover, compound **46** was found to significantly inhibit rat catalepsy induced by haloperidol, with ED_{50} values of 0.2 mg/kg at both 1 and 2 h time-points. In a recent patent application, Heptares has claimed a variety of 5,6-disubstituted-1,2,4-triazine-3-amine derivatives as dual A_1/A_{2A} receptor antagonists.[162] Analysis of the X-ray crystal structure of **46** showed a hydrogen bond interaction between the 4-pyridyl nitrogen and water molecules in the A_{2A} receptor binding pocket, and this inspired the synthesis of other ring-A-substituted analogues.[163] Substitution at the para- and meta-positions of ring A can afford compounds with a slightly improved A_1 selectivity profile that also retain desirable A_{2A} potency, especially when the aromatic A-ring contains a fluorine atom at the para-position. Compounds **47** and **48** (Figure 11) are representative examples in which ring A is a cyclic amine. Replacement of the 3-dimethylpiperidine ring of **47** (Figure 11, hA_{2A} K_i = 41 nM, 245-fold selective over hA_1) with a 2,6-

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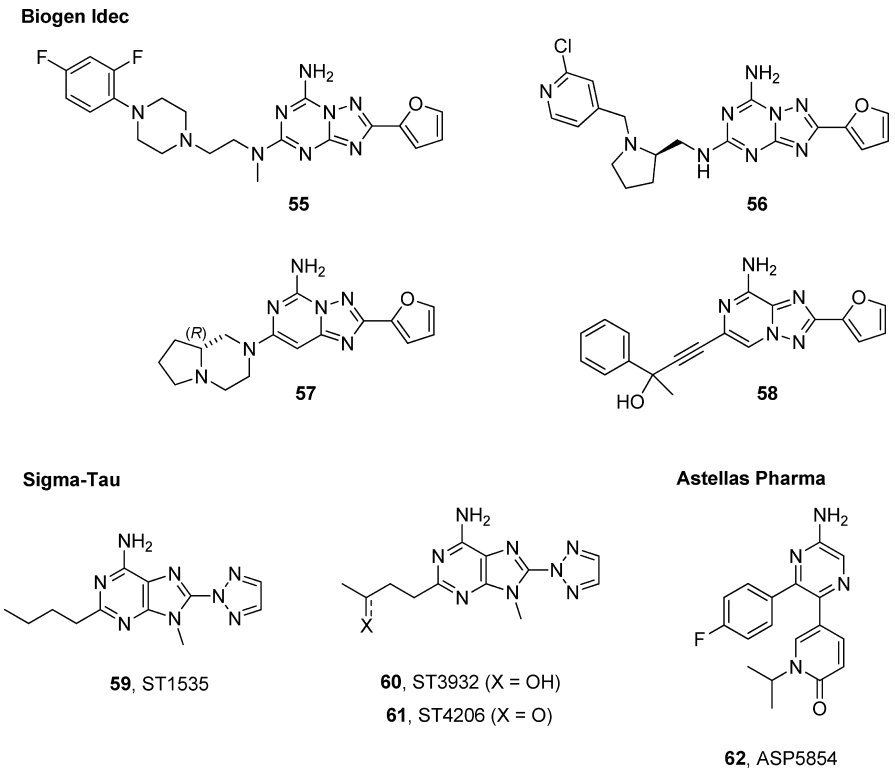

Figure 12. Selected A_{2A} antagonists from Biogen Idec, Sigma-Tau, and Astellas Pharma.

dimethylmorpholine gave analogue **48** in which the morpholine oxygen atom is believed to be responsible for its higher binding affinity (Figure 11, hA_{2A} K_i = 16 nM) with respect to analogue **47**. In addition, compound **48** showed excellent selectivity over the human A_1 receptor (617-fold). Compounds **47** and **48** possess an attractive preliminary in vitro profile, and it would be interesting to learn if these or similar monocyclic structures can be efficacious in in vivo animal models of PD.

Domain Therapeutics. According to the company's pipeline, Domain Therapeutics (formerly Faust Pharmaceuticals) is currently investigating the selective adenosine A_{2A} receptor antagonist DT1133 (also named FP1133, structure not disclosed).[164] In a recent patent application containing very limited biological data, a variety of imidazo[1,2-*a*]pyridine derivatives are claimed.[165] Only A_{2A} receptor binding affinity is reported for selected compounds, and no A_1 receptor data have been released. Compound **49** (Figure 11) displayed good human A_{2A} receptor binding affinity (hA_{2A} K_i = 12 nM). When administered orally in the haloperidol-induced catalepsy mouse model, compound **49** showed a significant anticataleptic activity in a dose-dependent manner at 10 and 30 mg/kg doses. Replacement of the central phenyl ring of **49** with an indazole or a benzoxazole scaffold gave compounds **50** and **51**, which exhibited very high affinities at the human A_{2A} receptor (hA_{2A} K_i = 3 nM and hA_{2A} K_i = 4 nM, respectively).

Kyowa Hakko Kirin. As discussed in the historic A_{2A} receptor antagonists section of this review, investigators at Kyowa Hakko Kirin (formerly Kyowa Hakko Kogyo) developed the xanthine derivative istradefylline (Figure 2). Despite completion of phase III clinical trials in 2008, it was not approved in the U.S. by the FDA because of a lack of efficacy; however, in March of 2013, Kyowa Hakko Kirin received approval to market istradefylline in Japan as Nouriast (20 mg tablets).[166] Recently, Kyowa Hakko Kirin has reported the synthesis and SAR studies of the non-xanthine benzofuran derivatives **52**–**54** (Figure 11) as adenosine A_{2A} receptor antagonists.[167,168] These antagonists have a fused 5–6 heterocyclic structure similar to the Biotie/Roche's benzothiazoles **36** and tozadenant (Figure 10). The morpholine-substituted amide **52** (hA_{2A} % inh = 39 at 10 nM, hA_1 % inh = 37 at 1 μM) was efficacious in the mouse CGS21680-induced catalepsy model (78% at 10 mg/kg, po). The 4-morpholine-benzofuran analogue **53** also displayed good in vitro potency (hA_{2A} % inh = 30 at 10 nM) and an excellent selectivity profile (hA_1 % inh = 4, A_{2B} % inh = 21, both at 1 μM). Compound **53** showed efficacy in the mouse CGS21680-induced catalepsy model (76% at 10 mg/kg, po). Methyl carbamate **54** showed a similar in vitro potency (hA_{2A} % inh = 28 at 10 nM) to compound **53** and also remarkably reversed rat catalepsy (86% at 10 mg/kg, po). In this series, 4-morpholine derivatives such as compound **53** have superior aqueous solubility and pharmacokinetic profiles compared with their 4-phenyl analogues.

Biogen Idec. The Biogen Idec research group has reported on a variety of fused bicyclic cores derived from the known non-xanthine A_{2A} receptor antagonist ZM241385 (Figure 2). As mentioned in the historic antagonist section of this review, ZM241385 has a low oral bioavailability and limited brain penetration. With the aim of avoiding these liabilities, researchers at Biogen Idec have explored the SAR of the side chain. Adding the arylpiperazine tail discovered at Merck/Schering-Plough resulted in compound **55** (Figure 12), a bicyclic analogue of **32** with a core 1,2,4-triazolo[1,5-*c*]triazine instead of 1,2,4-triazolo[1,5-*c*]pyrimidine.[169] Compound **55**, which showed potent rat A_{2A} receptor binding affinity (rA_{2A} K_i = 4 nM) and a good selectivity over the rat A_1 receptor (205-fold), was orally active in the mouse catalepsy model at 3 mg/kg. Compound **56** (rA_{2A} K_i = 4 nM, 63-fold over rA_1) is a

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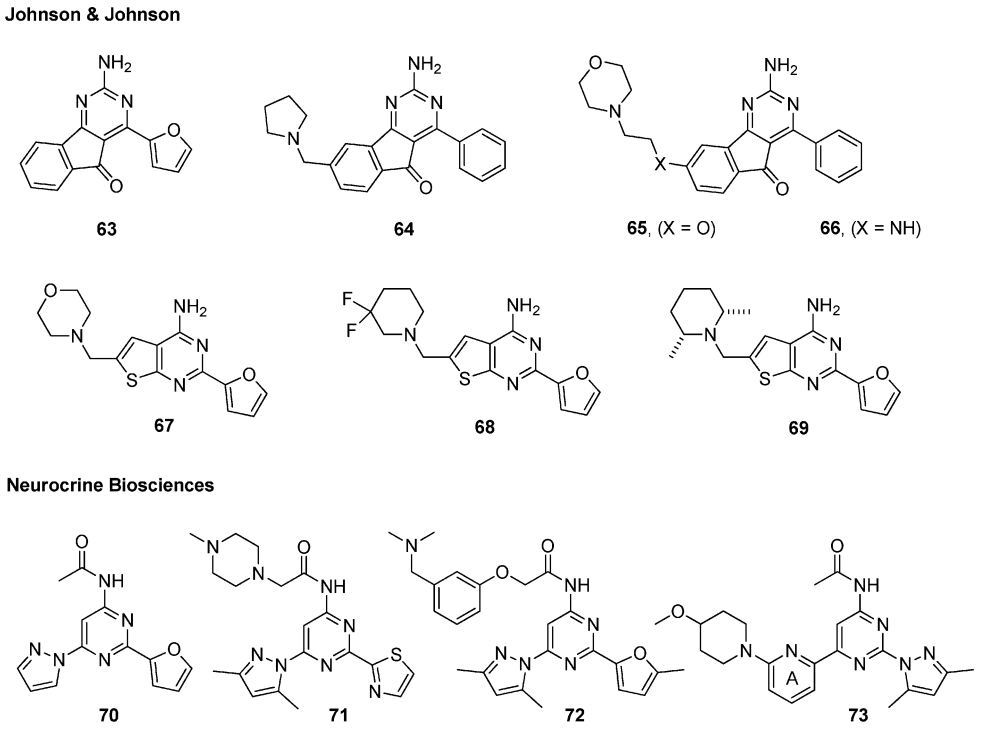

Figure 13. Selected A_{2A} antagonists from Johnson & Johnson and Neurocrine Biosciences.

representative example of a novel potent and selective series of A_{2A} antagonists that contain a (*R*)-2-(aminomethyl)pyrrolidine as the side chain.[135b] Compound **56** was orally active in the mouse catalepsy model at 10 mg/kg (po). Replacement of the triazolotriazine core with a triazolopyrimidine scaffold reduced A_{2A} receptor binding affinity and selectivity over the A_1 receptor, but interestingly in vivo efficacy was improved. Further attempts to optimize the side chain resulted in the discovery of novel fused bicyclic piperazine derivatives (e.g., compound **57**),[170] which can be viewed as constrained analogues of the 2-(aminomethyl)pyrrolidine linker as exemplified by compound **56** (Figure 12). In general, constrained analogues of **57** showed a reduced A_{2A} potency at the rat A_{2A} receptor. Triazolopyrimidine **57** (rA_{2A} K_i = 63 nM, 17-fold over rA_1) exhibited greater than 50% reduction of haloperidol-induced rat catalepsy within 30 min after oral administration, and this effect lasted for more than 120 min at a dose of 3 mg/kg (po). Compounds with the *R* stereochemistry in these novel pyrrolidine and fused piperazine-based side chains have greater in vitro potency and in vivo efficacy than the *S*. Novel alkynyl-substituted derivatives of the triazolopyrazine core were also reported. A representative analogue, compound **58** (rA_{2A} K_i = 12 nM, 3.4-fold over rA_1), was orally active at 3 mg/kg in both the mouse catalepsy and the 6-OHDA-lesioned rat model.[171]

Sigma-Tau. The Sigma-Tau research group has been exploring the possibility of substituting the furan ring with a triazole using a pyrimidoimidazo core derived from ZM241385. In this series, Sigma-Tau investigators identified ST1535 (**59**, Figure 12)[172] as a lead candidate for the treatment of PD. Antagonist **59** displayed potent A_{2A} receptor binding affinity (hA_{2A} K_i = 6.6 nM), 12-fold and 59-fold selectivity versus human A_1 and A_{2B} receptors, respectively, and was orally active at doses of 5 and 1.25 mg/kg in hypolocomotion and haloperidol-induced catalepsy models in rodents. It also potentiated L-DOPA activity in 6-OHDA-lesioned rats and MPTP-treated marmosets. Interestingly, it is reported that when dosed at 20 mg/kg in combination with L-DOPA (2.5 mg/kg), compound **59** significantly reversed motor disability compared with 20 mg/kg **59** alone.[173] Although **59** has been studied in phase I clinical trials, it is no longer listed on Sigma-Tau's pipeline. According to a recent patent application, ST3932 and ST4206 (**60** and **61**, Figure 12) are active oxidized metabolites of **59** with similar in vitro potencies (hA_{2A} K_i = 8 nM and K_i = 12 nM, respectively) and human A_1 selectivity (3- and 16-fold, respectively).[174] Antagonists **60** and **61** were orally active at 10, 20, and 40 mg/kg doses in haloperidol-induced catalepsy in mice. Additionally, these two compounds showed an increase in contralateral turning behavior induced by L-DOPA in rats (3 mg/kg dose).[174] This series of compounds possesses many good characteristics, but low selectivity over the A_1 receptor and possible stability issues with the triazole (a good leaving group) may be possible reasons for Sigma-Tau to decide to halt development.

Astellas Pharma. The monocyclic aminopyrazine derivative ASP5854 (**62**, Figure 12) was developed by the Astellas Pharma research group. It has a structure very similar to the structures of the Shire/Heptares A_{2A} antagonists discussed previously.[175] Compound **62** is a potent human A_{2A} (K_i = 1.8 nM) and A_1 receptor (K_i = 9 nM) dual antagonist that reversed haloperidol-induced rat catalepsy with a MED of 0.1 mg/kg. In addition, **62** significantly potentiated L-DOPA-induced rotational behavior in unilaterally 6-OHDA-lesioned rats with a MED of 0.03 mg/kg when dosed orally.[176] In non-human primates, the anticataleptic effect of **62** was achieved at more than 85% striatal A_{2A} receptor occupancy, and the compound reversed motor disability in MPTP-lesioned marmoset models at doses higher than 1 mg/kg dose, po.[177] In the rat passive avoidance test, **62** significantly reversed scopolamine-induced memory deficits at 0.3 mg/kg po and was efficacious in reversing the scopolamine-induced impairment of spontaneous alternation in the mouse Y-maze test at 0.1 mg/kg, po. In

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contrast, the specific adenosine A_{2A} receptor antagonist istradefylline was not efficacious in either of these tests. Importantly, these results demonstrate that the orally active dual adenosine A_{2A} and A_1 receptor antagonist **62** can repair motor impairments via A_{2A} receptor antagonism. It is noteworthy to mention that via A_1 receptor antagonism, **62** also showed an improved cognitive function in animal cognition models; however, this subject is outside the scope of this review.[178] Because Astellas has not reported any recent development of **62**, it is assumed that this program has been discontinued.

Johnson & Johnson. Researchers at Johnson & Johnson have developed a novel arylindenopyrimidine scaffold from initial screening hits.[179] The initial lead, compound **63** (Figure 13), had superior functional in vitro activity (no binding affinity has been reported for this class of compounds) in both human A_{2A} and A_1 receptors (hA_{2A} cAMP K_i = 0.1 nM, hA_1 cAMP K_i = 0.4 nM) and reversed haloperidol-induced catalepsy in mice with an ED_{50} of 5 mg/kg (po). However, compound **63** had poor aqueous solubility and was Ames positive.[179] In order to attempt to address these two liabilities, the furan ring of **63** was replaced by a phenyl ring, and a variety of amines were incorporated at the 8 and 9 positions of the arylindenopyrimidine scaffold. These efforts resulted in the discovery of compound **64**, a promising dual A_{2A} and A_1 receptor antagonist that showed potent functional in vitro activities (hA_{2A} cAMP K_i = 4.1 nM; hA_1 cAMP K_i = 17 nM), had a good pharmacokinetic profile, and achieved desirable brain levels. Compound **64** also exhibited good in vivo efficacy with ED_{50} values of 0.2 and 0.5 mg/kg (po) in the mouse and rat catalepsy models, respectively. In addition, compound **64** demonstrated excellent in vivo efficacy in the reserpine-induced akinesia mouse model at 1 mg/kg (po), the 6-OHDA-lesioned rat model at 1 mg/kg (po), and the reversing motor disability model in MPTP-treated marmosets at 10 mg/kg (po).[138] Nevertheless, further development of compound **64** was discontinued because of its genotoxicity in both the Ames test and the mouse lymphoma L5178Y assay. Metabolic identification studies showed that after oxidative metabolism, two reactive metabolites were produced, one with an endocyclic iminium ion in the pyrrolidine ring and another containing an arylaldehyde, both of which are known to cause genotoxicity. Therefore, these analogues were further modified to yield compounds **65** and **66**, ether and amino analogues, respectively, that do not contain a benzylic hot spot for oxidative metabolism. Both compounds **65** (hA_{2A} cAMP K_i = 6.5 nM; hA_1 cAMP K_i = 48.2 nM) and **66** (hA_{2A} cAMP K_i = 4.4 nM; hA_1 cAMP K_i = 32.7 nM) maintained functional in vitro potency and were very efficacious in the mouse catalepsy model with ED_{50} < 0.1 mg/kg, po. In particular, compound **65** was effective in reversing haloperidol-induced catalepsy with an ED_{50} value of 0.3 mg/kg, po, in mouse/rat models of reserpine-induced akinesia at 1 mg/kg.[180,181]

Recently Johnson & Johnson has reported a series of aminothieno[2,3-*d*]pyrimidines, exemplified by compounds **67**–**69** (Figure 13), that were potent adenosine A_{2A} receptor antagonists with varying degrees of selectivity over A_1 receptors. A number of cyclic and acyclic amines were explored as side chains, and some interesting SAR results were obtained. The less basic the amino group incorporated, the higher are the in vitro and in vivo activities of the compound bearing this group. For example, compounds containing very basic amines such as pyrrolidines (calcd pK_a = 8.2) and piperidines (calcd pK_a = 8.5) showed good in vitro potency but did not reverse catalepsy in vivo; however, compounds with a reduced basic amine, such as morpholine (calcd pK_a = 6.5) in compound **67** (hA_{2A} cAMP K_i = 29 nM; hA_1 cAMP K_i = 1680 nM), were potent in vivo with an ED_{50} for compound **67** of 1.3 mg/kg, po, in the mouse catalepsy model. Compound **68** containing a difluorinated piperidine (calcd pK_a = 4.7) was significantly more potent in vitro (hA_{2A} cAMP K_i = 6.6 nM; hA_1 cAMP K_i = 290 nM) than **67** and also very active in vivo, with ED_{50} < 1 mg/kg, po, in the mouse catalepsy model. Attempts to avoid metabolic oxidation at the benzylic carbon led to compound **69**, where steric hindrance is provided by the methyl groups of a *cis*-2,6-dimethylpiperidine. Compound **69** is the most potent analogue of this series (hA_{2A} cAMP K_i = 5.3 nM; hA_1 cAMP K_i = 100 nM) and showed a robust in vivo efficacy in the mouse catalepsy model at 10 mg/kg, po.[182] It would be particularly interesting to develop one of these molecules to investigate the effects of a dual A_{2A}/A_1 antagonist in treating the motor and cognitive symptoms of PD.

Neurocrine Biosciences. Neurocrine Biosciences has reported that a series of trisubstituted pyrimidines act as adenosine A_{2A} receptor antagonists.[183] The initial 4-acylaminopyrimidine lead compound **70** (Figure 13, hA_{2A} K_i = 0.6 nM) suffered from a lack of selectivity over the human A_1 receptors (17-fold), and its furan moiety had the potential for metabolic instability. Simultaneous optimization of the acetamide moiety and the pyrazole and furan rings of **70** led to compound **71**. This analogue maintained a high binding affinity (hA_{2A} K_i = 9.0 nM) and had an improved selectivity versus the human A_1 receptor (222-fold selectivity); however, it showed a weak inhibition of CYP3A4 (IC_{50} = 13 μM).[184] Most of the piperazine derivatives such as compound **71** were found to be potent inhibitors of the hERG channel. In an effort to address this liability, replacement of the piperazine moiety was explored. As a result, compound **72**, which is a potent adenosine A_{2A} receptor antagonist (hA_{2A} K_i = 0.4 nM) with good selectivity over the human A_1 receptor (100-fold), showed a clean hERG profile (patch-clamp IC_{50} = 1200 nM) and good aqueous solubility (0.21 mg/mL, pH 5). Although the pharmacokinetic profile of compound **72** displayed rapid plasma clearance (330 mL min^{-1} kg^{-1}), the brain levels were high, even after 4 h (360 ng/g). In line with the in vivo data, compound **72** exhibited significant oral activity with a MED of 10 mg/kg in the rat haloperiodol-induced catalepsy model.[185] Further development of this series led to the discovery of compound **73** (Figure 13), an analogue that showed potent and selective A_{2A} antagonism (hA_{2A} K_i = 0.4 nM, 193-fold over hA_1). Despite its moderate solubility (30 μg/mL at pH 7.4), compound **73** was efficacious at an oral dose of 1 mg/kg in the haloperidol-induced rat catalepsy model and exhibited 88% inhibition of catalepsy at 10 mg/kg, a dose at which plasma exposure (150 ng/mL) was moderately low but brain exposure (560 ng/g) was relatively high. Oral administration of compound **73** also potentiated L-DOPA-induced rotational behavioral in unilaterally 6-OHDA-lesioned rats with a MED of 3 mg/kg (po).[186] In order to improve the solubility of this series, a number of different small cyclic amines were incorporated at the pyridyl A-ring of **73**; however, most of these analogues showed much lower levels of in vitro potency and A_1 selectivity.[187]

Miscellaneous Series of A_{2A} Receptor Antagonists. Palobiofarma is currently developing a non-furan adenosine A_{2A} receptor antagonist PBF509 (structure not disclosed) for

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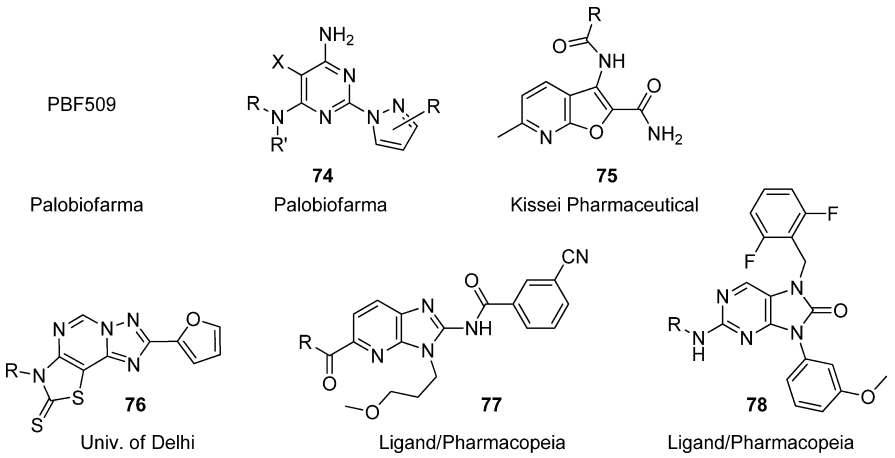

Figure 14. Other known A_{2A} antagonists.

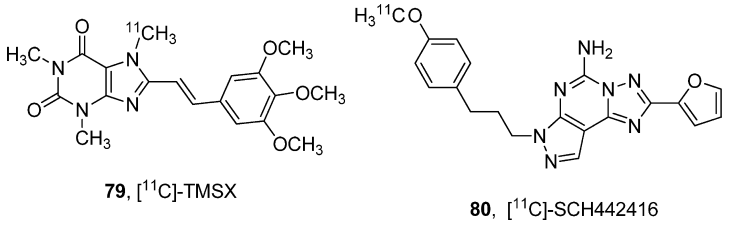

Figure 15. A_{2A} antagonists radioligands.

the treatment of PD. According to Palobiofarma's pipeline, PBF509 is currently in phase I clinical trials.[188] A recent patent application from this company claimed 4-aminopyrimidine derivatives such as **74** (Figure 14) as adenosine A_{2A} receptor antagonists. Interestingly, A_{2A} binding affinities were shown only for selected intermediates and no data for final targets were reported.[189]

In addition to the companies mentioned in this section, other research groups have also reported SAR studies of A_{2A} receptor antagonists as potential agents to treat PD. These include Kissei Pharmaceutical (**75**, benzofurans/pyridofurans, patent applications only),[190,191] University of Delhi (**76**, thiazolotriazolopyrimidines),[192] Ligand Pharmaceuticals/Pharmacopiea, Inc. (**77**, 2-aminoimidazopyridines),[193] and Ligand Pharmaceuticals (**78**, trisubstituted purinones).[194] Although in general these derivatives (shown in Figure 14) have a desirable in vitro A_{2A} binding affinity and selectivity profile, no in vivo results have been reported.

As we have seen in this section, the design of novel and orally active A_{2A} antagonists has been focused on two different strategies. First, to avoid the formation of reactive metabolites, many non-furan-containing aryl and heteroaryl analogues have been prepared. Second, to improve the low aqueous solubility found in most of these polycyclic ligands, introduction of polar substituents and the development of novel monocyclic and bicyclic cores have been extensively studied.

Other Therapeutic Areas. According to Thompson Pharma, CV Therapeutics (acquired by Gilead Sciences Inc.) is conducting preclinical studies with a series of A_{2A} antagonists for the potential treatment of chemical dependence.[195] In addition, Agenus Inc. and NewVac LLC are investigating analogues of istradefylline as selective A_{2A} adenosine receptor antagonists as adjuvants for use with oncovaccines and adoptive immunotherapy in potential personalized cancer vaccines.[196]

Despite the vast number of publications regarding the utility of historic A_{2A} antagonists for many different therapeutic applications, the current ongoing clinical trials involving A_{2A} antagonists show that the focus of big pharma is currently confined to Parkinson disease.

Antagonist Radioligands. A number of radioligands have been studied over the past few decades. Not only are they useful for mapping the A_{2A} receptors, but they are also of paramount importance for determining binding affinities and the relationships between dose, plasma levels, and receptor occupancy, critical information for the development of centrally acting drugs.

Various radioligands of the xanthine type of A_{2A} antagonists have been evaluated. In most cases these molecules have been labeled with ^{3}H or ^{11}C to study binding to A_{2A} receptors in human platelets and rat striatal membranes or for use as PET radioligands to map A_{2A} receptors in the heart and brain. Most of these efforts have been focused on finding a good PET ligand that specifically binds to the A_{2A} receptors in the brain. This is not an easy task, and the major challenges that have been observed are compound accumulation and nonspecific binding (the compound is in the brain but not at the target receptors). On the basis of the literature, the most suitable radioligand of the xanthine type is **79** ([^{11}C]TMSX, Figure 15), a compound developed by Mishina et al. as a PET ligand to map the A_{2A} receptors in the brain of PD patients.[197] However, because of the susceptibility of xanthine **79** to photoisomerization, more suitable radioligands of the non-xanthine type have been developed.

A number of studies using [^{125}I]ZM241385 and [^{3}H]-ZM241385 (both from **16**, Figure 2) have been published, but the applications of these radioligands have been limited, since these compounds also have a high binding affinity at the A_{2B} receptor. In 1996, Zocchi et al. were the first to document a specific, saturable, and reversible binding of [^{3}H]SCH58261 in

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rat striatal membranes (hK_d = 0.70 nM).[198] Calculation of the binding of different agonists and antagonists was based on displacement from the A_{2A} receptor of [^{3}H]SCH58261, which proved to be an excellent probe for studying the A_{2A} receptor subtype in mammalian brain. [^{3}H]SCH58261 has also been successfully used to develop binding assays in porcine coronary arteries, porcine striatum, and PC12 cells.[199] Furthermore, successful [^{3}H]SCH58261 labeling of adenosine A_{2A} receptors in human platelets and in human neutrophil membranes has also been accomplished.[200] In addition, [^{3}H]SCH58261 has been used to characterize the A_{2A} receptors in human lymphocyte membranes[201] and proven to be a useful tool in autoradiographic studies in rats.[202]

Fazio et al. have developed [^{11}C]SCH442416 (**80**, Figure 15) as a suitable PET tracer.[203] Its parent compound (SCH442416) possesses a methoxy group to allow a fast and easy chemical approach to the radiosynthesis of the labeled form and a high specific radioactivity of the final product by direct alkylation of the phenolic function with [^{11}C]CH_3I. Radioligand **80** has a high binding affinity at the adenosine A_{2A} receptor (hK_d = 0.048 nM) and is the first non-xanthine radioligand applicable for the in vivo PET imaging of adenosine A_{2A} receptors due its adequate regional distribution in the brain and the periphery, a good signal-to-noise ratio observed between 5 and 15 min after injection, and the low occurrence of radioactive metabolites. Papapetropoulos et al. successfully used **80** as a PET radiotracer to investigate the relationships between dose, steady-state plasma levels, and receptor occupancy of vipadenant (Figure 10) in healthy male volunteers.[204]

CONCLUSION

Following the discovery of adenosine, almost a century of intense research on adenosine receptors has led to the selection of the adenosine A_{2A} receptor as a research target for developing small molecules in the treatment of various medical conditions.

The vast store of knowledge and numerous research tools available make the A_{2A} receptor a fascinating target for the medicinal chemist to explore. Not only does knowledge about receptor distribution combined with the availability of selective ligands allow us to obtain critical information such as receptor occupancy, but insight into its structure, particularly that coming from X-ray crystal structures, also serves to inspire the design of novel potent and selective A_{2A} ligands.

On the basis of a large number of publications in the literature, A_{2A} ligands have therapeutic potential for a wide spectrum of medical conditions. One of these applications, the vasodilating effect of an A_{2A} agonist, has been validated, and adenosine and regadenoson are currently being marketed for myocardial perfusion imaging. Furthermore, A_{2A} agonists are being investigated as agents to treat a number of conditions such as asthma, COPD, neuropathic pain, and diabetic foot ulcers.

The potential therapeutic use of an A_{2A} antagonist still remains to be validated. As we have seen in this review, most of the efforts in this area are focused on treating the symptoms of Parkinson disease. Unfortunately the lack of efficacy in phase III studies of istradefylline, and recently of preladenant, constitutes a major setback and raises questions about the therapeutic validity of this mechanism in humans. In our opinion, the main question that needs to be answered is whether the lack of efficacy of istradefylline and preladenant is due to a particular intrinsic feature of these compounds or to the complexity involved in designing these types of clinical studies, such as appropriate dose selection and recruitment of the correct patient populations. It is also possible that the commonly assumed connection between A_{2A} receptors and the symptoms of PD is invalid because in vivo efficacy does not translate from animal models to humans, a situation unfortunately often observed in CNS programs. The current vigorous stream of research and development, including ongoing clinical trials with tozadenant in phase IIb and V81444 in phase IIa studies, may enable us to reach a conclusion about the possible connection between the A_{2A} mechanism and PD in the near future.

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The author declares no competing financial interest.

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