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1. Introduction

The purine nucleoside adenosine is consensually identified as a major local regulator of tissue function especially when energy supply fails to meet cellular energy demand. Due to its ability to equalize energy intake to metabolic demand in the 1980s it earned the reputation of a "retaliatory metabolite".[1] Adenosine is omnipresent, released from almost all cells, and generated in the extracellular space by breakdown of ATP through a series of ectoenzymes, including apyrase (CD39) and 5′-nucleotidase (CD73).[2] The latter dephosphorylates extracellular AMP to adenosine, regulating the limiting step for its formation. Extracellularly, adenosine concentration is kept in equilibrium by reuptake mechanisms operated through the action of specific transporters. Then inside the cell it is phosphorylated to AMP by adenosine kinase or degraded to inosine by adenosine deaminase (ADA). Intracellularly, adenosine formation is dependent upon the hydrolysis of AMP by an intracellular 5-nucleotidase or hydrolysis of *S*-adenosyl-homocysteine. It is estimated that the levels of adenosine in the interstitial fluid are in the range 30−300 nM.[3] Adenosine concentrations increase under metabolically unfavorable conditions. Tissue hypoxia, for example, leads to an enhanced breakdown of ATP and the increased generation of adenosine. In addition to this route, the release of adenosine might be potentiated by hypoxia-dependent inhibition of the salvage enzyme adenosine kinase which rephosphorylates the nucleoside to AMP.[4] As adenosine is unstable and its half-life is limited by deamination or cellular reuptake, hypoxia-induced increase typically affects only local adenosine receptor signaling. As adenosine is not released in a transmitter or hormone-like fashion, it is likely to belong to the group of autacoids.

Adenosine mediates its effects through activation of a family of four G-protein-coupled adenosine receptors (ARs), named A_1, A_{2A}, A_{2B}, and A_3. These receptors differ in their affinity for adenosine, in the type of G proteins that they recruit, and finally in the downstream signaling pathways that are activated in the target cells. A_1 and A_3 ARs display high and low affinity for adenosine, respectively, and are inhibitory toward regulation of adenylyl cyclase activity. By contrast, activation of high-affinity A_{2A} and low-affinity A_{2B} subtypes stimulates adenylyl cyclase leading to an increase of cyclic AMP (cAMP) levels. Early pharmacological evidence for the existence of ARs has been provided by specific antagonism by methylxanthines, caffeine, and theophylline of adenosine-induced effects in the heart and brain.[5] These receptors are widely distributed through the body, and their presence on basically every cell makes them an

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Pier Andrea Borea obtained his Degree in Chemistry (*Summa cum Laude*) from the University of Ferrara in 1967. He has been Assistant Professor at the University of Ferrara since 1969. He moved as a Visiting Professor to the Preclinical Research Department, Sandoz, Basel (CH), in 1982–1983. He was Associate Professor of Pharmacology at the Faculty of Sciences, University of Ferrara, in 1984. He moved as a Visiting Professor to the Medical Research Council Molecular Neurobiology Unit, Cambridge (UK), in 1985–1986. Since 1994 he has been Full Professor of Pharmacology at the Faculty of Medicine and Surgery, University of Ferrara, and at present he is Director of the Department of Clinical and Experimental Medicine at the University of Ferrara. He has contributed 250 publications to international journals. His main field of interest is represented by the study, at the molecular level, of drug–receptor interactions. In the last years his major interest is represented by the P1 adenosine receptor system. He was Chairman of the 6th International Symposium on Adenosine and Adenine Nucleotides, Ferrara, Italy, May 19–24, 1998, and Chairman of the 8th International Symposium on Adenosine and Adenine Nucleotides, Ferrara, Italy, May 24–28, 2006. He is President of the Italian Chapter of the International "Purine Club".

interesting target for the pharmacological intervention in many pathophysiological situations linked to an increase of adenosine levels.

The first recorded report describing evidence for an AR originates from 1976. Now, 30 years later, advances in understanding the role of adenosine and its receptors in physiology and pathophysiology as well as new developments in medicinal chemistry of these receptors have enabled researchers to identify potential therapeutic areas for drug development. With the combination of pharmacological data, using selective ligands and genetically modified mice, important progress has been made toward an understanding of the role of ARs in a variety of diseases, such as inflammatory conditions, sepsis, heart attack, ischemia-reperfusion injury, vascular injury, spinal cord injury, chronic obstructive pulmonary disease (COPD), asthma, diabetes, obesity, inflammatory bowel disease, retinopathy, and Parkinson's Disease (PD). Nonselective AR antagonists are used to maintain wakefulness (caffeine) and, less commonly at present, treat bronchospasm (theophylline, aminophylline, enprofylline). Currently a number of new selective AR agonists and antagonists are in testing for a variety of new indications. Therefore, the purpose of this review is to analyze the structure−activity relationships of the ligands synthesized as antagonists for the ARs. We have included

Table 1. Affinity of AR Antagonists at the A_1, A_{2A}, A_{2B}, and A_3 ARs

	K_i[a] values for ARs (nM)			
A_1 antagonists	A_1	A_{2A}	A_{2B}	A_3
1, theophylline[3]	6770	1710	9200[h]	86 400
2, caffeine	55[b,i]	48[b,i]	10 400[j]	ND[g]
4, DPCPX[3,211,221]	3.9[f]	129	51	1100
5, KW-3902[13]	1.3[b,k]	380[b]	ND[g]	ND[g]
6, BG-9719[14]	0.45	1100	756[c,l]	19 900[c,l]
8, BG-9928	29[c,l]	4720[c,l]	690[c,l]	42 100[c,l]
9, L-97-1[18]	580	>100 000[e]	>100 000[e]	ND[g]
10, tricyclic imidazoline[20]	22	4400	580	>10 000
11, FK-453	18[m]	1300[m]	ND[g]	2800[m]
12, FK-838[24]	120[b,e]	5900[b,e]	ND[g]	ND[g]
13, FR-166124[24]	15[b,e]	6200[b,e]	ND[g]	ND[g]
14, N-0861	375[d,n]	3750[d,n]	ND[g]	ND[g]
15, WRC-0571	1.7[o]	105[o]	ND[g]	7940[o]

[a] Binding experiments at recombinant hA_1, A_{2A}, A_{2B}, and A_3 ARs unless noted. [b] Binding experiments at rat brain (A_1) and striatum (A_{2A}) ARs. [c] Binding experiments at recombinant dog ARs. [d] Binding experiments at guinea pig brain (A_1) and striatum (A_{2A}) ARs. [e] IC_{50} values. [f] Values are from saturation experiments. [g] ND = not determined. [h] Linden, J.; Thai, T.; Figler, H.; Jin, X.; Robeva, A. S. *Mol. Pharmacol.* **1999**, *56*, 705. [i] Daly, J. W.; Hide, I.; Bridson, P. K. *J. Med. Chem.* **1990**, *33*, 2818. [j] Kim, S. A.; Marshall, M. A.; Melman, N.; Kim, H. S.; Müller, C. A.; Linden, J.; Jacobson, K A. *J. Med. Chem.* **2002**, *45*, 2131. [k] Poulsen, S. A.; Quinn, R. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 357. [l] Auchampach, J.; Jin, X.; Moore, J.; Wan, T. C.; Kreckler, L. M.; Ge, Z. D.; Narayanan, J.; Whalley, E.; Kiesman, W.; Ticho, B.; Smits, G.; Gross, G.J. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 846. [m] Harada, H.; Asano, O.; Hoshino, Y. *J. Med. Chem.* **2001**, *44*, 170. [n] Belardinelli, L. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1167. [o] Martin, P. L.; Wysocki, R., Jr.; Barrett, R. J.; May, J. M.; Linden, J. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 490.

some synthetic schemes in order to show the chemistry involved in this field. In particular, we will investigate antagonists under active development that selectively target the four known AR subtypes.

2. A_1 Adenosine Receptor Antagonists

Considerable advances have been made recently in the pharmacological and molecular characterization of A_1 ARs, which had been proposed as targets for drug design and discovery. Xanthine and xanthine derivatives, including the natural compounds theophylline and caffeine, constitute the prototypical group of antagonists at all ARs, and modifications of the xanthine structure, in particular at the 8-position, give a choice of derivatives endowed in some cases of good subtype selectivity. Most of the selective A_1 AR antagonists are xanthine-based derivatives. Table 1 summarizes the examples of A_1 AR antagonists discussed in this review.

2.1. Chemistry

2.1.1. Xanthines

Alkylxanthines are the best-known class of compounds characterized as adenosine antagonists. There have been a large number of these derivatives prepared in an effort to increase potency and selectivity (Figure 1). Compounds of much higher potency than the prototypical caffeine have been identified, usually resulting from 1,3-dialkyl and 8-aryl or 8-cycloalkyl substitution.[3] However, this class of compounds has additional activities unrelated to adenosine receptor blockade, and there has been only limited success in preparing adenosine A_1-selective agents as therapeutic agents.

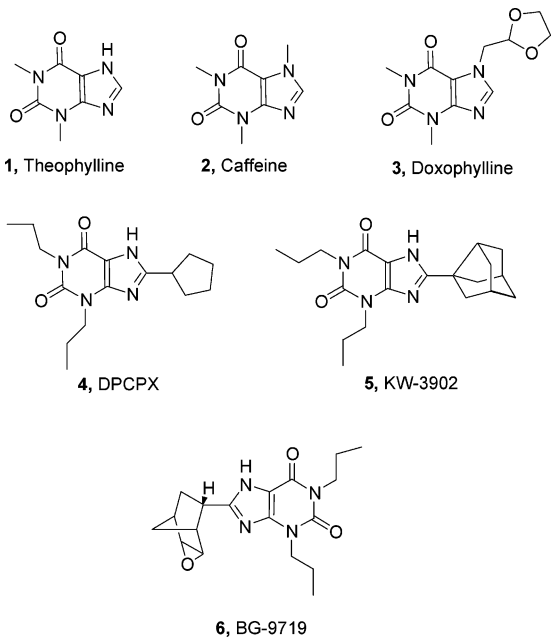


Figure 1. A_1 AR antagonists (xanthines).

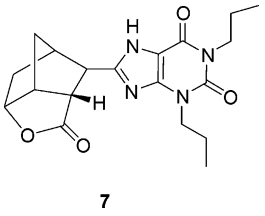


Figure 2. Norbornyl lactone analogue of BG-9719.

Theophylline (**1**, 1,3-dimethylxanthine) and caffeine (**2**, 1,3,7-trimethylxanthine) (Figure 1) are the classical nonselective xanthine antagonists of ARs that display micromolar affinity at AR subtypes.[3] Theophylline was first extracted from tea leaves around 1888. The drug was chemically identified and synthesized in 1896.[6,7] There are many examples of potent A_1 antagonists that contain bulky lipophilic substitution at the 8-position of 1,3-dipropylxanthines (Figure 1).[8−10] Doxofylline (**3**, 7-(1,3-dioxalan-2-ylmethyl)theophylline) is a xanthine bronchodilator which differs from theophylline in that it contains a dioxalane group in position 7. Doxofylline is prepared by reaction of theophylline-7-acetaldehyde with ethylene glycol by means of *p*-toluenesulfonic acid in refluxing benzene.[11]

A large number of modifications on the xanthine core at the 1-, 3-, and 8-positions led to the discovery of 8-cyclopentyl-1,3-dipropyl-xanthine (**4**, DPCPX, Figure 1), which was selective for rat A_1 AR compared with the A_{2A} AR and less selective at the human (h) hA_1 compared with hA_{2A} and hA_{2B} ARs.[3,12] Other substituted xanthines have been proposed as A_1 AR antagonists, in particular, 1,3-dipropyl-8-(3-noradamantyl)xanthine (**5**, KW-3902)[13] and 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)xanthine (**6**, BG-9719).[14] In this compound (also named Naxifylline), the xanthine ring and the epoxide are, respectively, situated endo and exo to the norbornane moiety, and the latter has an asymmetric center at C-2 (Figure 1). While a small stereochemical effect on the affinity was present between the enantiomers at guinea pig and hA_1 receptors, the *R*-isomer appeared to be less potent than the *S*-isomer in the rat. Very recently, a series of xanthines substituted with norbornyl-lactones structurally related to BG-9719 (**7**, Figure 2) was investigated. These

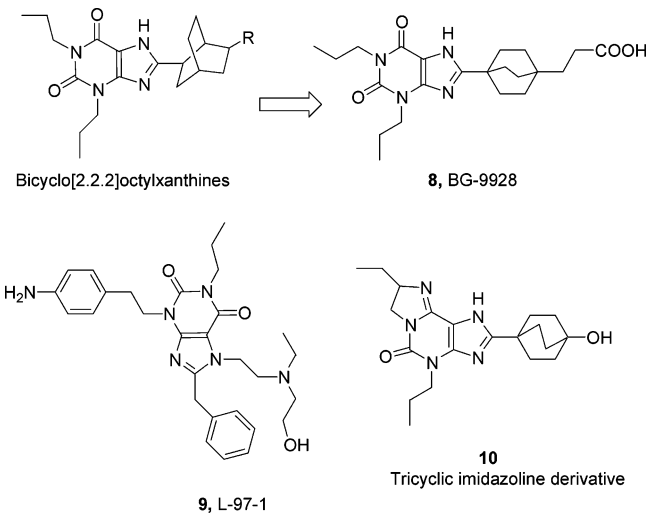


Figure 3. Water-soluble A_1 AR antagonists.

derivatives, in which the xanthine occupies the exo position on the norbornyl ring system, showed high A_1 binding affinity and selectivity over the closely related A_{2A} AR. The lactones possessed similar if not better in vivo activity to BG-9719 in the rat diuresis models.[15]

2.1.1.1. Water-Soluble Xanthine Derivatives as A_1 Adenosine Receptor Antagonists. The highest affinity xanthine-based molecules pictured in Figure 1 lack appreciably polar substituents. The utility of most of these compounds for intravenous administration in the treatment of acutely decompensated congestive heart failure patients in the clinic may be limited because of their low water solubility. In the search for a selective A_1 AR antagonist with greater aqueous solubility, a series of 1,3-substituted-8-cyclohexyl- and 8-bicyclo-[2.2.2]octylxanthines that contain linear substitution patterns was investigated by Kiesman et al.[15] (Figure 3). These authors have initially drawn their attention to the 8-cyclohexyl-*trans*-4-carboxylic acid xanthine derivatives published by Katsushima and co-workers[16] that showed low binding affinity at A_1 AR. They expanded this series of compounds and prepared the bicyclo-[2.2.2]octane derivatives by addition of a two-carbon bridge linking the 1- and 4-positions across the cyclohexane ring (Figure 3). Optimization of the bridgehead substituent led to propionic acid (**8**, BG-9928, Figure 3),[17] which retained high potency (dog A_1, K_i = 29 nM) and selectivity for the A_1 AR (163-fold vs A_{2A} AR; 24-fold vs A_{2B} AR; 1452 vs A_3 AR) with improved oral efficacy in a rat diuresis model as well as high oral bioavailability in rat, dog, and cynomolgus monkey.

BG-9928 is prepared by the cyclization of 5,6-diamino-1,3-dipropylpyrimidine-2,4(1*H*,3*H*)-dione I with bicyclo-[2,2,2]octane-1,4-dicarboxylic acid monoethyl ester II by means of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and triethylamine (TEA) in acetonitrile giving the dipropylxanthine derivative III, which is esterified with methanol and sulfuric acid, yielding the methyl ester IV. Reduction of the ester group of IV by means of lithium borohydride ($LiBH_4$) in refluxing tetrahydrofuran (THF) affords the hydroxymethyl derivative V, which is oxidized with Dess−Martin periodinane (DMP) in dichloromethane to provide the aldehyde VI. Condensation of aldehyde VI with phosphonate by means of potassium hexamethyldisilazane (KHMDS) in toluene leads to the acrylic acid methyl ester VII, which is hydrolyzed with lithium hydroxide (LiOH) in methanol/water to give the free acid VIII. Finally, the double bond of the acrylic moiety is hydrogenated with H_2 over palladium on carbon (Pd/C) in methanol to yield the target xanthine derivative 8 (Scheme 1).[18]

Another water-soluble molecule that has been described as a potential new oral drug for asthma is 3-[2-(4-aminophenyl)ethyl]-8-benzyl-7-{2-ethyl-(2-hydroxyethyl)amino]ethyl}-1-propyl-3,7-dihydropurine-2,6-dione] **9** labeled L-97-1 (Figure 3). This compound is an adenosine A_1 antagonist (K_i = 580 nM) with at least 100-fold selectivity over A_{2A} and A_{2B} ARs.[18]

2.1.2. Tricyclic Imidazoline Derivatives

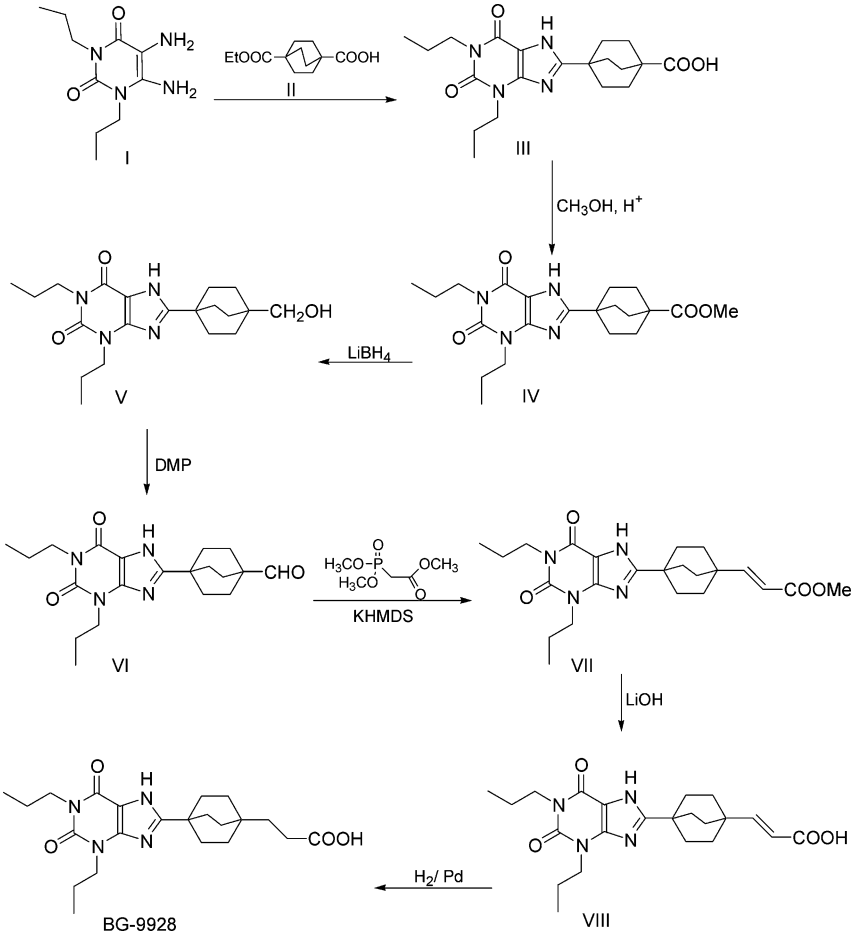
These compounds are essentially derivatives of xanthines in which the additional basic site significantly increased their water solubility relative to xanthines without substantial loss in A_1 binding affinity.[19] In connection with the discovery of BG-9928 and the tricyclic imidazoline derivatives reported in the literature, Vu et al.[20] reported the synthesis of compound **10**, (Figure 3) the *R*-isomer of 7,8-dihydro-8-ethyl-2-(4-bicyclo[2.2.2]octan-1-ol)-4-propyl-1*H*-imidazo-[2,1-*i*]purin-5(4*H*)-one, a potent A_1 AR antagonist with good selectivity over the other three ARs. Imidazoline **10** is a potent competitive A_1 AR antagonist, highly soluble in water (>100 mg/mL). In addition, it has an oral bioavailability of 84% and an oral half-life of 3.8 h in rats. When orally administered in a rat diuresis model, compound **10** promoted sodium excretion (ED_{50} = 0.01 mg/kg). This level of efficacy is comparable to that of BG-9928. Additional modifications of **10** also showed that the bridgehead hydroxyl group could be replaced with a propionic acid without a significant loss in binding affinity or in vivo activity.

2.1.3. Non-Xanthine Derivatives

Due to the nonspecific effects of xanthines, there has been considerable effort directed at identifying non-xanthine adenosine antagonists. A variety of heterocycles, including pyrazolopyridines, thiazolopyrimidines, imidazopyridines, and benzimidazoles, have been found to be AR antagonists. The desire for non-xanthine derivatives with increased potency and selectivity has driven continued interest in this area.[21]

2.1.3.1. Pyrazolo[1,5-*a*]pyridines. Another class of analogs structurally related to the xanthine core consists of derivatives the pyrazolo[1,5-*a*]pyridines nucleus. One compound in this series, FK-453, **11** (Figure 4), synthesized by Akahane et al.,[22] showed potent and selective adenosine A_1 antagonist activity and was selected for further evaluation. Various modifications have been performed on this nucleus such as constraining of acryloyl amide into a pyridazinone nucleus (compound **12**, FK-838, Figure 4) that produced a significant increase of potency and selectivity.[23] FK-838 is an A_1-selective adenosine antagonist, as demonstrated in radioligand binding (IC_{50} A_1 = 120 nM; IC_{50} A_2 = 5900 nM) and functional assays, whose diuretic and natriuretic effects appear to be due to both its renal hemodynamic effects and a direct inhibition of proximal tubular Na^+ reabsorption. The design process leading to the discovery of FR-166124 (**13**, 2-[2-[6-oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-1,6-dihydropyridazin-1-yl]-1-cyclohexenyl]acetic acid, Figure 4) involved introduction of various cyclic acid groups to the N^2 of the pyridazinone ring, in place of the butyric acid group of FK-838, as substituents to induce rigidity and mimic the postulated conformation of FK-453. A cyclohexenyl acetic

Scheme 1. Synthesis of BG-9928



acid group was found to be especially effective. Compound **13** is reported as a more potent derivative with higher A_{2A}/A_1 selectivity and very high water solubility as the sodium salt (>200 mg/mL).[24−26]

FK-838 is prepared as shown in Scheme 2. Friedel−Crafts reaction of I using acetic anhydride in the presence of a catalytic amount of concentrated sulfuric acid afforded II, which was then treated with glyoxylic acid monohydrate. The intermediate adduct III was cyclized with hydrazine monohydrate to give pyridazinone IV. Alkylation of IV with 4-bromobutyric acid ethyl ester was performed in the presence of sodium hydride to afford V, which was finally hydrolyzed with sodium hydroxide (NaOH) to give FK838.[23]

2.1.3.2. Adenine Derivatives. The initial report of adenine derivatives showing adenosine antagonist activity only covered 1-methyl- and 9-methyladenine.[27] The observation was made that adenine itself and 9-methyladenine showed specific competitive antagonism at low concentrations but exhibited nonspecific inhibitory activity at higher concentra-

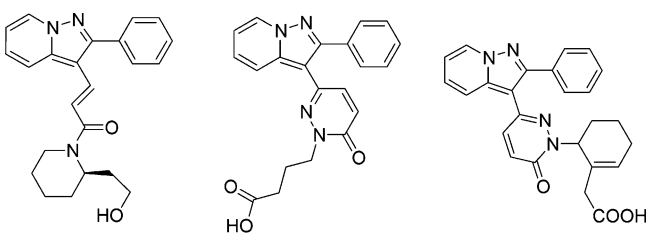
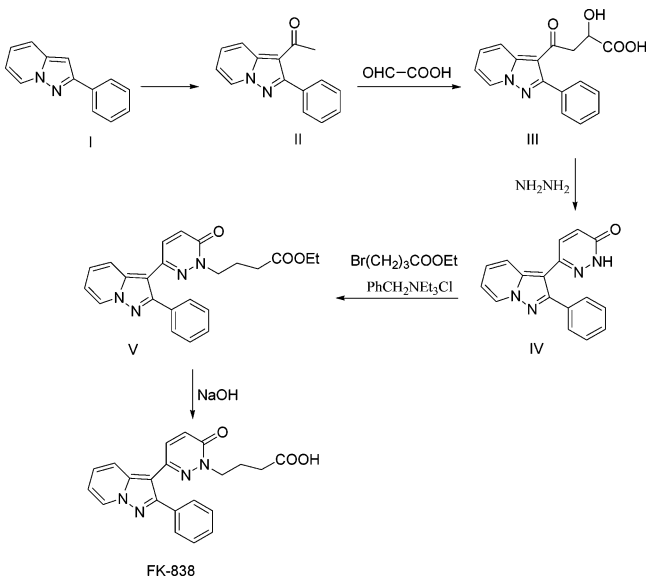


Figure 4. A_1 AR antagonists (pyrazolopyridines).

Scheme 2. Synthesis of FK-838



tions. Since N^6-substitution of adenosine had been used to confer A_1 AR agonist selectivity, it was clear that a selective antagonist could potentially be obtained by replacing the ribose sugar with a methyl group to generate N^6-substituted 9-methyladenines.[28−30]

Further structure−activity work has identified (±)-N^6-(*endo*-2-norbornyl)-9-methyladenine N-0861 (**14**, Figure 5) as a lead compound, which has been undergoing development as a cardiovascular agent for treatment of adenosine-related

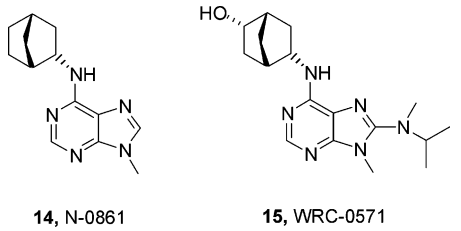
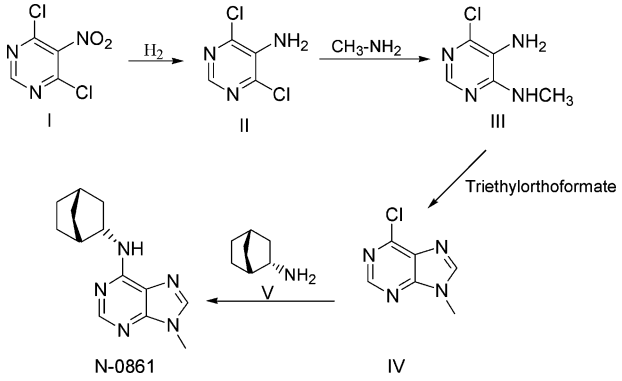


Figure 5. A_1 AR antagonists (adenines).

Scheme 3. Synthesis of N-0861



bradyarrhythmias. N-0861 receptor binding is specific for ARs, having negligible affinity (K_i > 10 000 nM) for alpha 1, alpha 2, and beta adrenoceptors, D_1 and D_2 dopamine receptors, and 5-HT_2 serotonin receptors.[31] Radioligand binding studies have shown that N-0861 binds with high affinity to A_1 ARs in bovine caudate membranes, where it is 600-fold selective for the A_1 vs the A_2 subtype of ARs.[31] N-0861 binds with lower affinity to A_1 ARs in rat and guinea pig cerebral cortex and to A_1 ARs in atrial tissue from guinea pig and human heart.[32]

Substitution in the 8-position of adenine with an isopropyl-methyl-amine moiety gave the best results as observed from compound **15** (WRC-0571, Figure 5), which is a highly potent and selective A_1 AR antagonist with superior potency and aqueous solubility relative to N-0861. In radioligand binding studies, it displayed high affinity for cloned hA_1 (K_i = 1.7 nM) and much lower affinity for cloned hA_{2A} and hA_3 ARs (K_i = 105 and 7940 nM, respectively). In functional studies, it potently inhibited the A_1-mediated negative inotropic response to 5′-(*N*-ethyl-carboxamido)adenosine (NECA) in isolated guinea pig atria (pK_B = 8.41), whereas it was much less active against NECA-induced, A_{2B}-mediated, relaxation in guinea pig aorta (pK_B < 5).[33]

The overall synthetic approach to N-0861 is described in Scheme 3: reduction of 4,6-dichloro-5-nitropyrimidine I followed by reaction with methylamine gives 5-amino-6-chloro-4-methylaminopyrimidine III. Cyclization with triethyl orthoformate under acidic conditions results in 9-methyl-6-chloropurine IV. Reaction of (±)-*endo*-2-aminonorbornane V with 9-methyl-6-chloropurine IV provides N-0861.[34]

2.2. Pharmacology

The A_1 AR has been cloned from several animal species including humans and is characterized by a close similarity at least for mammals.[35]As for signal transduction the A_1 AR is coupled to members of the Gi/Go family of G proteins inducing inhibition of adenylyl cyclase activity.[36] In addition, it might activate phospholipase-Cβ which is known to increase inositol 1,4,5-triphosphate (IP_3) and intracellular Ca^{2+}.[37] A_1 AR is coupled to pertussis toxine-sensitive potassium channels as well as K_{ATP} channels, essentially in cardiac tissue and neurons. Moreover, it could inhibit Q-, P-, and N-type Ca^{2+} channels[3] and modulate extracellular signal-regulated protein kinases (ERKs).[38]

The A_1 AR is widely distributed in the central nervous system (CNS) with high levels in brain, cortex, cerebellum, hippocampus, and dorsal horn of the spinal cord. It modulates the activity of the nervous system at the cellular level and is present in both pre- and postsynaptic terminals. At the presynaptical level it mediates inhibition of neurotransmitter release, while at the postsynaptical level it induces neurons hyperpolarization. Therefore, activation of A_1 ARs is responsible for sedative, anticonvulsant, anxiolytic, and locomotor depressant effects induced by adenosine. The endogenous levels of adenosine are sufficient to tonically activate inhibitory A_1 ARs, and caffeine, perhaps the most commonly used drug in the world,[39] mediates its excitatory effects through the antagonism of this inhibition. Recently the existence of heteromers constituted by A_1 and A_{2A} ARs has been demonstrated. A_1-A_{2A} heteromers constitute a unique target for caffeine, and chronic caffeine treatment leads to modification in the A_1-A_{2A} heteromers that could explain the tolerance to the psychostimulant effects of caffeine.[40]

A_1 ARs are responsible for many effects induced by adenosine in SNC[41] as well as in peripheral tissues.[42,43] Adenosine is a signaling nucleoside that has been implicated in the regulation of asthma and COPD.[43] Levels of adenosine are increased in the lungs of asthmatics, in which elevations correlate with the degree of inflammatory insult.[44] The early evidence that the A_1 AR is involved in asthma derived from studies on allergic rabbit models, where the adenosine-induced acute bronchoconstrictor response was reduced by pretreatments with A_1 AR antagonists.[45,46] Emerging scientific and clinical data indicate that through activation of this subtype adenosine mediates bronchoconstriction, inflammation, increased endothelial cell permeability, and mucin production.[47−49] The possible implication of A_1 AR in human asthma has been also suggested by early clinical trials with EPI 2010, a respirable antisense oligonucleotide (RASON), developed by EpiGenesis Pharmaceuticals. EPI-2010 is apparently metabolized locally by endogenous nucleases, confining its activity to the airways, and phase I clinical trials have shown EPI-2010 to be well tolerated with indications of efficacy.[50] Paradoxically, findings in ADA-deficient mice suggest the occurrence of anti-inflammatory actions of adenosine in the lung, through A_1 ARs chronic activation in macrophages.[51] If these effects could be extrapolated from mice to humans, then the potential clinical benefits of blocking A_1 ARs on airway smooth muscle may be offset by increased inflammation, and this is relevant in light of the fact that A_1 AR antagonists are under investigation as a potential treatment for asthma.[52]

At the cardiovascular level A_1 ARs mediate negative chronotropic, dromotropic, and inotropic effects. A_1 subtypes located on sinoatrial and atrioventricular nodes cause bradycardia and heart block, respectively, while the negative inotropic effects include a decrease in atrial contractility and action potential duration. Stimulation of A_1 ARs in the heart exerts cardioprotective effects by inhibiting norepinephrine release from sympathetic nerve endings.[53] Adenosine also protects tissues through its effect in ischemic preconditioning (IPC), a brief period of ischemia and reperfusion, that can

protect myocardium against infarction from a subsequent prolonged ischemic insult. Activation of A_1 ARs, protein kinase C, and mitochondrial K_{ATP} channels are responsible for this response.[54−56] IPC has been most widely investigated in the heart but also occurs in other tissues.[57,58]

In the kidney, A_1 ARs mediate vasoconstriction, decrease in glomerular filtration rate, inhibit renin secretion, and inhibit neurotransmitter release. A_1 AR antagonists represent a novel class of agents for potential use in the treatment of hypertension and edema.[59] A_1 AR antagonists produced diuresis and natriuresis of greater magnitude than thiazide diuretics[60] but without significant potassium wasting or reductions of renal blood flow and glomerular filtration rate.[61] Clinical trials in a limited number of subjects demonstrated that A_1 AR antagonists produced natriuretic and hypotensive effects in essential hypertensive patients[62] and attenuated the furosemide-induced decline of renal hemodynamic function in heart failure patients.[63]

The use of adenosine A_1 AR antagonists has been claimed for the treatment of neurological and psychiatric disorders, cardiac arrhythmia, restoration of cardiac function, congestive heart failure, renal failure, renal dysfunction, nephritis, hypertension, edema, asthma, and respiratory disorders.

2.3. Clinical Development and Patents

Over the past few years, a number of new agents from the purinergic field have reached the clinical arena. These agents have demonstrated activity in a wide variety of preclinical models, and many have been found to have novel mechanisms of action. Some of these new agents are now in Phase I, II, and III trials, and in this section we will review the new A_1 AR antagonists in clinical development for asthma and heart failure. Bronchial asthma is a chronic debilitating disease which in its severe forms can be life threatening. At present, four classes of drugs have been available to combat the symptoms of this disease: beta-sympathomimetic agents, bronchodilators, antiallergic agents, and corticosteroids.

Theophylline, launched in 1939, is representative of bronchodilators and used in the treatment of asthma. Theophylline can mediate bronchodilatory and anti-inflammatory effects depending on the serum doses achieved. At higher doses (10−20 μg/L), bronchodilation is believed to occur through phosphodiesterase (PDE) inhibition. However, at lower concentrations (5−10 μg/L), which are responsible for anti-inflammatory effects, PDE antagonism is not relevant.[52] Therefore, different mechanisms have been proposed for the anti-inflammatory effects of low-dose theophylline.[64,65] One of these include antagonism of ARs which occurs at concentrations 20−100-fold lower than that required for PDE inhibition. Theophylline is a nonselective A_1, A_{2A}, A_{2B}, and A_3 antagonist with the potential to counteract the irritant and bronchoconstrictor effects induced by adenosine. The use of theophylline to treat asthma has undergone several cycles of enthusiasm and unpopularity over the past 50 years. The dissemination of clinical practice guidelines that indicate theophylline as a "not preferred" choice, the availability of newer agents, and concerns regarding the risk−benefit ratio of the drug have resulted in less frequent use of theophylline in developed countries. Nevertheless, its low cost and ease of administration offer advantages over other effective long-term maintenance medications such as inhaled corticosteroids. Further validation that theophylline is beneficial in asthma and COPD derives from recent clinical trials.[66−68] Therefore, good chances for theophylline exist in the future in clinical practice. Indeed, starting from 2004 different pharmaceutical companies, including AstraZeneca, Almirall Prodesfarma, Recordati, Mitsubishi Pharma, NikkenChemicals, and Schering-Plough, registered theophylline for treatment of asthma. In Japan, Nikken Chemical has launched Apnecut (theophylline) oral solution 10 mg for apnea of prematurity. It is the first oral theophylline preparation in Japan for primary apnea in premature and low-birth-weight newborns.

Doxofylline has been launched in 1987 as bronchodilator. Its mechanism of action is related to the inhibition of PDE activities, but in contrast to theophylline it appears to have decreased affinities toward A_1 and A_2 ARs, which may account for its better safety profile.[11] The bronchodilating activities of doxofylline have been demonstrated in clinical trials involving patients with either bronchial asthma or COPD. In contrast to other bronchodilators, experimental and clinical studies have shown that the drug is devoid of direct stimulatory effects. This may be of importance because the arrhythmogenic actions of bronchodilators may have a negative impact on the survival of patients with respiratory diseases. From the results of a multicenter, double-blind, randomized trial carried out in 11 Italian Pneumologic Clinics in 139 patients suffering from reversible chronic airways obstruction, doxofylline seemed to be a good alternative to theophylline in view of its better safety profile.[69] Additionally, the lack of cardiostimulant effects makes doxofylline highly suitable for the treatment of chronic obstructive lung disease particularly in combination with beta 2-adrenergic agonists.[70]

KW-3902, an A_1 AR antagonist developed at Kyowa Hakko, is currently in phase III development by NovaCardia for the intravenous treatment of heart failure in patients undergoing diuresis. In 2003, Kyowa Hakko entered into an agreement granting NovaCardia an exclusive license to develop and market the compound outside Asia and Japan. KW-3902 has also been studied for the treatment of renal disorders and renal failure. Development of the drug candidate for the treatment of hypertension was discontinued. NovaCardia's KW-3902 met its primary endpoints in a series of phase II trials designed principally to evaluate the effect of KW-3902 on fluid elimination and renal function in heart failure patients with renal impairment undergoing diuresis. Clinically significant improvements were observed in fluid elimination in patient treatment groups that were administered KW-3902 intravenously (iv) in combination with loop or other diuretics. There were also notable improvements in renal function in selected patient groups that received KW-3902 as part of their therapy.[71] NovaCardia has developed both intravenous and oral formulations of KW-3902, and in October 2006 they initiated phase III studies. The objectives of this study are to evaluate the effect of iv KW-3902 in addition to iv furosemide on heart failure signs and symptoms, renal function, and safety in subjects hospitalized with acute heart failure syndrome, volume overload, and renal impairment and estimate and compare within-trial medical resource utilization and direct medical costs between patients treated with iv KW-3902 vs placebo (PROTECT 2, registration number [clinicaltrial.gov] NCT00354458). Preliminary results of a multicenter, randomized, double-blind, dose-finding, placebo-controlled clinical study ($N = 146$) suggested that the combination of KW-3902 and furosemide was effective in maintaining renal function in patients with

acutely decompensated heart failure and renal impairment. KW-3902 has been patented for heart failure and hypertension,[72] renal disorders, and renal failure.[72−74]

N-0861 is an A_1 antagonist developed at Aderis. Studies in several species (rat, dog, pig) show that N-0861 selectively antagonizes adenosine A_1 ARs in vivo. N-0861, administered iv to anesthetized and vagotomized beta-blocked rats, antagonized A_1-mediated bradycardia induced by intravenous adenosine.[75] N-0861 is active in animal models of cardiovascular disease and is well tolerated in animals and man. In patients undergoing a clinically electrophysiology study to evaluate atrioventricular nodal conduction N-0861 was shown to antagonize adenosine-induced prolongation of atrioventricular nodal conduction.[76] N-0861 is currently in phase III studies for heart failure therapy. N-0861 and N^6-substituted 9-methyladenines have been patented as a new class of AR antagonists[77] and prevention and treatment of ischemia-reperfusion and endotoxin-related injury.[78]

FK-453 belongs to a series of pyrazolopyridines synthesized at Astellas Pharma that were shown to have antihypertensive and diuretic effects. The renal effects of FK-453 were examined in dogs. The diuretic and natriuretic properties of the title compound were similar to those of thiazide diuretics; however, FK-453 also had potent and selective renal vasodilating activity in dogs.[79] FK-453 was generally well tolerated at doses of 100, 400, and 800 mg administered to healthy male subjects. The most common side effects were headache and skin rash.[80] It is actually under phase II trials for acute renal failure.

FK-838 is a diuretic and antihypertensive A_1 antagonist developed at Astellas Pharma and under phase II studies for hypertension and as a diuretic agent. Its diuretic and natriuretic effects appear to be due to both its renal hemodynamic effects and direct inhibition of proximal tubular Na^+ reabsorption. In rats, it dose-dependently increased urine volume and sodium excretion similarly to hydrochlorothiazide with much less kaliuretic activity compared to the latter. In combination with furosemide, FK-838 enhanced the diuretic and natriuretic actions of furosemide to the same extent as hydrochlorothiazide and did not increase potassium loss in normal rats.[81] FK-838 has been patented for the treatment of anemia.[82]

BG-9719 is an adenosine A_1 antagonist undergoing phase II evaluation by CV Therapeutics and Biogen Idec for the treatment of congestive heart failure (CHF). By blocking the action of the A_1 AR, BG-9719 may reduce the amount of fluid waste that the kidneys retain without the associated decline in kidney function caused by other therapeutics. Originally developed at the University of Florida as CVT-124, BG-9719 was subsequently licensed to CV Therapeutics. Pursuant to a 1997 agreement, Biogen received exclusive worldwide rights from CV Therapeutics for the development, manufacture, and commercialization of A_1 AR antagonists for the treatment of acute and chronic CHF. Biogen Idec has also studied the potential of BG-9719 as a treatment for autoimmune disease. Administered intravenously to saline-loaded rats, it induced diuresis via antagonism of renal A_1 ARs.[14,83] BG-9719 does not exacerbate cardiac injury and does not interfere with IPC induced by multiple ischemia/reperfusion cycles in an in vivo dog model of infarction.[31] In a randomized, double-blind, crossover study evaluating 3 doses of BG-9719 in 63 patients with CHF on standard therapy, including ACE inhibitors, BG-9719 increased both urine output and glomerular filtration rate (GFR). In these same patients, furosemide increased urine output at the expense of decreased GFR. When BG-9719 was given in addition to furosemide, urine volume additionally increased and there was no deterioration in GFR. A_1 adenosine antagonism might preserve renal function while simultaneously promoting natriuresis during treatment for heart failure.[63] BG-9719 has been patented for renal and cardiovascular disorders.[84−86]

SLV-320 (structure unavailable) is an adenosine A_1 antagonist in phase II trials at Solvay for the treatment of CHF and renal failure. A study to evaluate cardiac hemodynamics and the safety of SLV-320 in subjects with CHF is running. The study is a randomized, placebo-controlled, multicenter, single-dose study to evaluate cardiac hemodynamics and safety of iv SLV-320 in 110 subjects with CHF requiring diuretic treatment. Each subject will receive one dose of SLV-320 or placebo or furosemide (registration number [clinicaltrial.gov] NCT00160134).

L-97-1 is described by Endacea researchers and collaborators at East Carolina University as a novel, water-soluble, small-molecule antagonist with high affinity and selectivity for the A_1 AR; it represents a potential new oral therapy for asthma and is in preclinical phase. In rabbits with allergic asthma, administration of the compound (10 mg/kg) 1 h before house dust mite (HDM) allergen significantly increased lung dynamic compliance up to 6 h, blocking both early- and late-phase allergic responses.[18] The effects of a lower dose (1 mg/kg) of L-97-1 were compared to montelukast, a cysteinyl leukotriene-1 receptor antagonist on airway reactivity and inflammation. L-97-1 blocks both the early allergic response and the late allergic response following HDM through the direct blocking of A_1 ARs on airway smooth muscle. As opposed to montelukast, L-97-1 may inhibit the T helper (Th2) lymphocyte response and cytokine release, e.g., Interleukin (IL)-4, IL-5, IL-13, associated with asthmatic airway responses following allergen challenge. By blocking both bronchoconstriction and airway inflammation it has been suggested that L-97-1 may be an effective oral antiasthma treatment.[87] L-97-1 has been patented for asthma and coronary artery disease,[88] dementia, Alzheimer's disease, depression, heart failure, and hypertension.[89]

3. A_{2A} Adenosine Receptor Antagonists

The discovery and development of potent and selective A_{2A} AR antagonists became, in the last 10 years, an attractive field of research to the discovery of new drugs for the treatment of neurodegenerative disorders, such as PD. Different compounds have been deeply investigated as A_{2A} AR antagonists, which could be classified in two great families: nitrogen polyheterocyclic systems and styrylxanthine derivatives. Table 2 summarizes the examples of A_{2A} AR antagonists reported in this section.

3.1. Chemistry

3.1.1. Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines

9-Chloro-2-furan-2-yl-[1,2,4]triazolo[1,5-*c*]quinazolin-5-ylamine named CGS-15943 (**16**, Figure 6) represented the first potent but poorly selective antagonist for the A_{2A} AR subtype.[90] Bioisosteric replacement of the phenyl ring of CGS-15943 with an N^7-substituted pyrazole led to the first example of an adenosine antagonist displaying the pyrazolo-triazolo-pyrimidine core named 8FBPTP (**17**, 8-(4-fluoroben-

Table 2. Affinity of AR Antagonists at the A_1, A_{2A}, A_{2B}, and A_3 ARs

	K_i[a] values for ARs (nM)			
A_{2A} antagonists	A_1	A_{2A}	A_{2B}	A_3
16, CGS-15943	3.5[d]	0.15[e]	71[f]	50.8[g]
17, 8FBPTP[91]	3.3[b]	1.2[b]	ND[c]	ND[c]
18, SCH-58261[95]	549	1.1	>10 000	>10 000
19, SCH-63390[95]	350	1.2	>10 000	>10 000
20, SCH-442416[94]	1111	0.048	>10 000	>10 000
22[93]	253	1.5	ND[c]	>10 000
23[95]	4927	4.63	>10 000	>10 000
24[95]	139	140	>10 000	>10 000
25[95]	2160	0.22	>10 000	>10 000
26, SCH-BT2[95]	369	3.8	>10 000	>10 000
27[99]	ND	0.94	ND[c]	ND[c]
28, SCH-420814[100]	ND	1.1	ND[c]	ND[c]
29, KF-17837	>10 000[h]	71[h]	ND[c]	2500[h]
30, CSC	28 000[b,i]	54[b,i]	ND[c]	>10 000[i]
31, BS-DMPX[104]	1200[b]	8.2[b]	ND[c]	ND[c]
32, KW-6002	2830[j]	36[j]	1800[j]	>3000[j]
33, ST-1535[108]	72	6.6	352	>1000

[a] Binding experiments at recombinant hA_1, A_{2A}, A_{2B}, and A_3 ARs unless noted. [b] Binding experiments at rat brain (A_1) and striatum (A_{2A}) ARs. [c] ND = not determined. [d] Ongini, E.; Dionisotti, S.; Gessi, S.; Irenius, E.; Fredholm, B. B. *Naunyn Schmiedebergs Arch. Pharmacol.* **1999**, *359*, 7. [e] Varani, K.; Gessi, S.; Dionisotti, S.; Ongini, E.; Borea, P. A. *Br. J. Pharmacol.* **1998**, *123*, 1723. [f] de Zwart, M.; Vollinga, R. C.; Beukers, M. W.; Sleegers, D. F.; von Frijtag Drabbe Kuenzel, J. K.; de Groote, M.; Ijzerman, A. P. *Drug Dev. Res.* **1999**, *48*, 95. [g] Klotz, K.-N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fredholm, B. B.; Lohse, M. J. *Naunyn-Schmied Arch. Pharmacol.* **1998**, *357*, 1. [h] Harada, H.; Asano, O.; Hoshino, Y. *J. Med. Chem.* **2001**, *44*, 170. [i] Jacobson, K. A.; Kim, H. O.; Siddiqi, S. M.; Olah, M. E.; Stiles, G. L.; von Lubitz, D. K. J. E. *Drugs Fut.* **1995**, *20*, 689. [j] Weiss, S. M.; Benwell, K.; Cliffe, I. A.; Gillespie, R. J.; Knight, A. R.; Lerpiniere, J.; Misra, A.; Pratt, R. M.; Revell, D.; Upton, R.; Dourish, C. T. *Neurology* **2003**, *61*, S101.

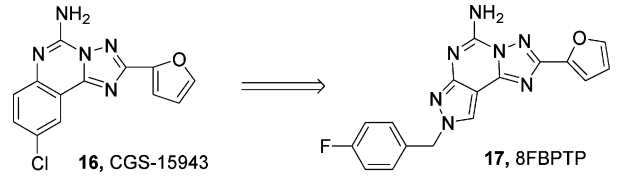


Figure 6. Structural relationships between CGS-15943 and 8FBPTP (the first A_{2A} AR antagonist).

zyl)-2-(2-furyl)-8*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine, Figure 6).[91] Some structural features of this compound highlighted the essential requirements for the A_{2A} affinity, i.e., the furyl moiety and the free amino group at the 5-position. Starting from these observations Baraldi et al.[92,93] focused their interest on the pattern of substitution on the pyrazolo preserving the other structural elements. Several alkyl, aryl, and phenylalkyl substituents have been introduced at both the N^7 and the N^8 positions. The biological data derived from the molecules obtained indicated that the best radicals were phenylalkyl chains, and among these it was possible to discern the length of the spacer introduced between the phenyl ring and the pyrazolo nitrogen that was optimized in two or three carbon atoms. Two selected compounds of this family named SCH-58261 (**18**, 5-amino-7-(β-phenylethyl)2-(2-furyl)-pyrazolo[4,3-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidine) and SCH- 63390 (**19**, 5-amino-7-(3-phenylpropyl)2-(2-furyl)-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine)[92,93] proved to be potent and selective A_{2A} AR antagonists both in rat and human models (Figure 7). It was also noted that the N^7 derivatives were more selective for the A_{2A} AR than the corresponding N^8 derivatives.

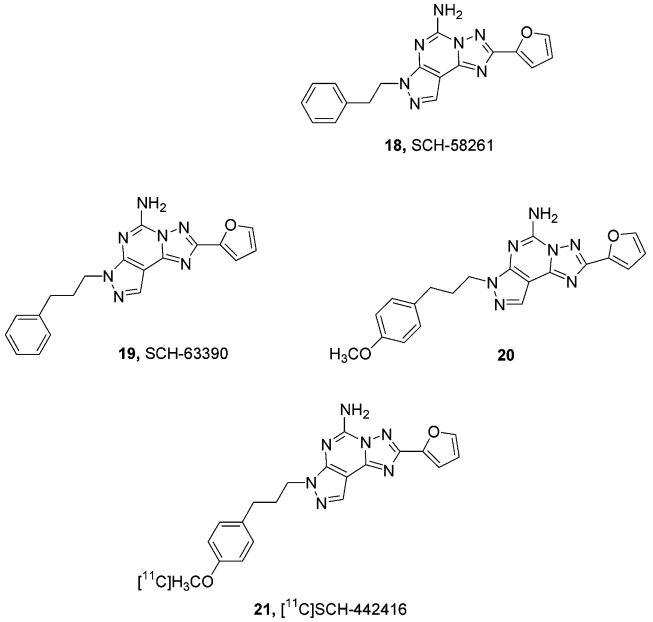
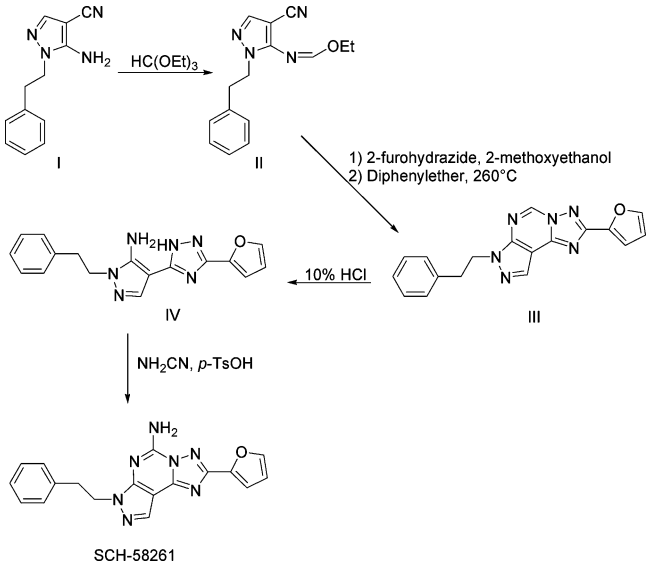


Figure 7. A_{2A} AR anatgonists (Pyrazolo-triazolo-pyrimidines).

Scheme 4. Synthesis of SCH-58261



SCH-58261 was prepared by transformation of the substituted pyrazole I in the corresponding imidate II through refluxing in triethylorthoformate (Scheme 4). The imidate II was then reacted with 2-furic acid hydrazide at reflux to provide the pyrazolo[4,3-*e*]pyrimidine intermediate. The latter compound was converted through a thermally induced cyclization in diphenyl ether to the tricyclic derivative III, which was treated with dilute hydrochloric acid induced pyrimidine ring opening to furnish the amine IV. This derivative was converted into the final compound by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140 °C.[93]

From the family of SCH compounds, 5-amino-7-(3-(4-methoxyphenyl)propyl)-2-(2 furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH-442416, **20**, Figure 7) was selected for the development of a new positron emission tomography (PET) ligand, whose chemical structure allows an easy introduction of a methyl group by direct *O*-alkylation of the phenolic function with $[^{11}C]CH_3I$ under alkaline conditions.[94] The aim of this study was to use [^{11}C]SCH-

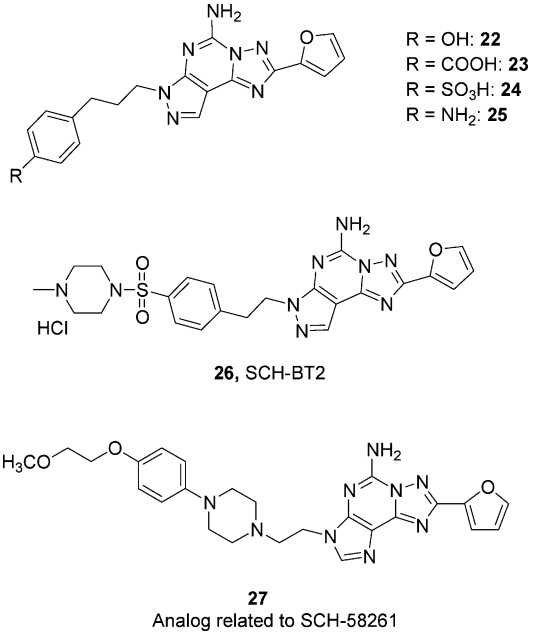


Figure 8. Water-soluble A_{2A} AR antagonists.

442416, **21**, as a new ligand for the in vivo imaging of A_{2A} ARs using PET. The in vitro binding in the brain and periphery, the good signal-to-noise ratio observed between 5 and 15 min after injection, and the low occurrence of radioactive metabolites all suggested that [^{11}C]SCH-442416 was applicable as the first non-xanthine ligand suitable for the in vivo imaging of A_{2A} ARs using PET. In addition, the data obtained from the binding experiments showed a higher affinity of the title compound for hA_{2A} vs rat ARs (0.048 vs 0.5 nM).[94]

3.1.2. Water-Soluble A_{2A} Adenosine Receptor Antagonists

The major restriction of the tricyclic adenosine antagonists was the low solubility in aqueous media that limited the pharmacological screening. Starting from this limit Baraldi et al.[93,95] reported a second generation of pyrazolo-triazolo-pyrimidines bearing oxygenated substituents on the phenyl-alkyl chains at the 7-position (compounds **22**−**25**). The most interesting compounds are depicted in Figure 8. Compound **22** displayed the best value of A_{2A} AR affinity indicating that the 4-hydroxy group positively influenced the receptor interaction but was not enough for reaching a good profile of water solubility. A water-soluble analogue of SCH-58261, named SCH-BT2 (**26**), was prepared by introduction of a 4-methyl-piperazine-1-sulfonyl moiety at the para position of the phenyl ring. SCH-BT2 altered neither motor behavior nor produced postural asymmetry by itself. However, when infused concomitantly with levodopa (L-DOPA) (capable of inducing modest controlateral rotational behavior), SCH-BT2 significantly potentiated the number of contraversive rotations.[96−98]

Very recently, a novel series of 3-substituted 8-furyl-[1,2,4]-triazolo[1,5-*i*]purin-5-amine analogs related to SCH-58261 was reported as A_{2A} AR antagonists.[99] Most of the N^3-substituted aryl piperazine and piperidine analogs demonstrated in vivo A_{2A} receptor binding affinity and A_1 receptor selectivity profiles superior to those of SCH-58261. In these series compound **27** displayed both superior in vitro and promising in vivo profiles (Figure 8).

Neustadt et al.[100] recently reported the arylpiperazine derivatives of pyrazolo[4,3-*e*]triazolo[1,5-*c*]pyrimidines with

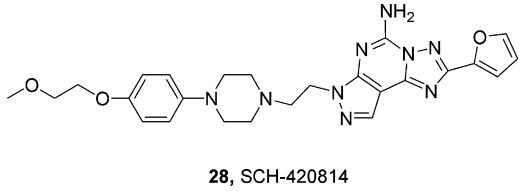
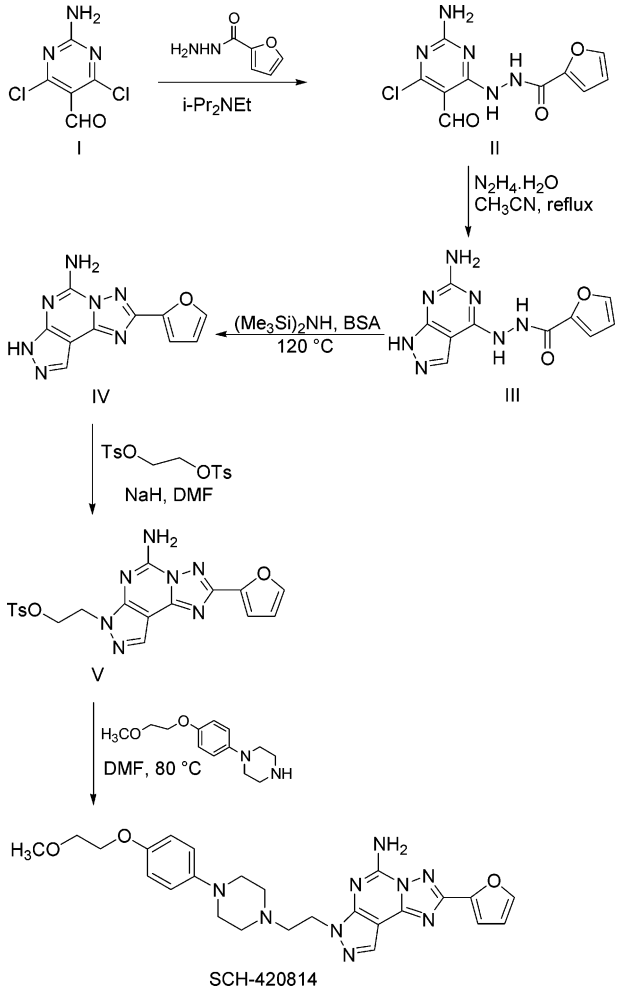


Figure 9. SCH-420814.

Scheme 5. Synthesis of SCH-420814



antagonist activity on the A_{2A} AR. Among these derivatives, SCH-420814 (**28**, Figure 9) demonstrated potent antagonist activity at the A_{2A} AR. Structure−activity relationship studies revealed additional compounds incorporating an aryl-piperazine side chain that also showed potent oral activity in the haloperidol-induced catalepsy model in rats. For the preparation of compound **28** they applied the synthetic route reported in Scheme 5.[100,101] The commercially available pyrimidine I was condensed with 2-furoic hydrazide producing the furoyl pyrimidinylhydrazine II. After cyclization of chloro aldehyde II with hydrazine hydrate in refluxing acetonitrile the resultant pyrazolopyrimidine hydrazide III was rearranged to the triazolo-fused compound IV upon heating with hexamethyldisilazane and *N*,*O*-bis(trimethylsilyl)acetamide. Then, alkylation of IV with ethylene glycol ditosylate in the presence of sodium hydride (NaH) furnished the 7-(2-tosyloxyethyl) pyrazolotriazolopyrimidine V. Subsequent amination with *N*-arylpiperazine in hot DMF furnished the title compound **28**.

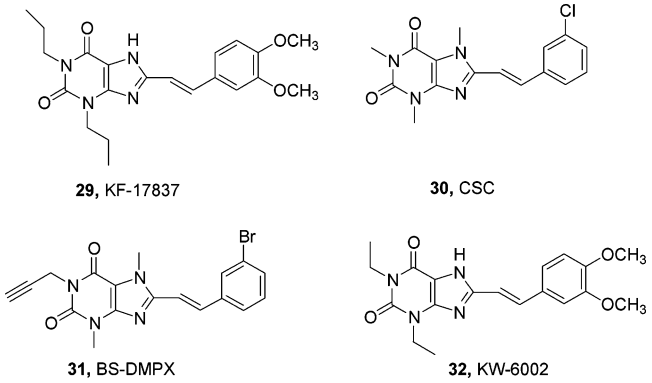
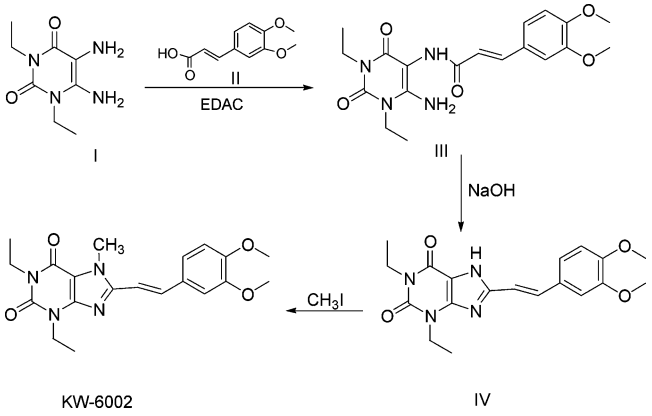


Figure 10. A_{2A} AR antagonists (styrylxanthines).

Scheme 6. Synthesis of KW-6002



3.1.3. Styrylxanthines

1,3-Dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine (**29**, KF17837, Figure 10) was the first A_{2A} AR antagonist in this chemical class of compounds.[102] The 3-chlorostyrylcaffeine **30** (CSC, Figure 10) was identified as being less potent than KF17837 but with an increased selectivity vs A_1 AR subtype.[103] Introduction of a propargyl at the 1-position in combination with the 8-styryl group in compound **31** (BS-DMPX, Figure 10) increased affinity to the A_{2A} AR with retention of selectivity.[104]1,3-Diethyl-7-methyl-8-(3,4-dimethoxystyryl)-xanthine **32** (KW-6002, also named istradefylline, Figure 10) is an 8-styrylxanthine with high affinity for the rat striatal A_{2A} AR. Due to its high affinity and selectivity, a radiolabeled derivative, [^{11}C]-KW-6002 labeled at the aromatic *O*-methyl position, was developed to be used in pharmacological testing to trace the A_{2A} ARs in vivo.[105,106]

KW-6002 was prepared by condensation of 6-amino-1,3-diethyluracil I with 3-(3,4-dimethoxyphenyl)-2(*E*)-propenoic acid II and 3-[3-(diethylamino)propyl]-1-ethylcarbodiimide (EDAC) in dioxane/water to provide the corresponding amide III. Cyclization of III by means of NaOH in the same solvent furnished the xanthine derivative IV, which was finally methylated with methyl iodide and potassium carbonate (K_2CO_3) in dimethylformamide (DMF) (Scheme 6).[107]

3.1.4. 9H-Purine derivatives

Minetti et al., on the basis of the molecular modeling of a number of potent AR antagonists, designed and synthesized a number of 2-alkyl-substituted purine derivatives as A_{2A} AR antagonists.[108] From them ST-1535 (2-*n*-butyl-9-methyl-8-[1,2,3]triazol-2-yl-9*H*-purin-6-ylamine **33**, Figure 11), was the most interesting.

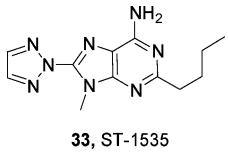


Figure 11. 9*H*-Purine derivative.

3.2. Pharmacology

The gene for the A_{2A} AR has been cloned from several species including dog,[109] rat,[110,111] human,[112] guinea pig,[113] and mouse[114] and demonstrated a high degree of homology among human, mouse, and rat.[35] The A_{2A} AR stimulates adenylyl cyclase activity through the coupling with Gs proteins leading to activation of cAMP-dependent protein kinase A. This in turn phosphorylates and activates various receptors, ion channels, phosphodiesterases, and phosphoproteins like CREB and DARPP-32.[115−117] Activation of protein kinase C has been also reported in PC12 cells.[118] In brain striatum the A_{2A} subtype stimulates Golf, another member of the Gs subfamily of G proteins.[119] In addition A_{2A} AR can interact with different types of Ca^{2+} channels to either increase intracellular Ca^{2+} or decrease Ca^{2+} influx[120,121] and is involved like the other adenosine subtypes in the modulation of ERKs activity.[38]

Due to a long carboxy terminal domain the A_{2A} AR shows a greater molecular weight (45 kDa) in comparison to the other subtypes (36−37 kDa). The A_{2A} AR C terminus has been defined as a crowded place where different accessory proteins may interact such as D_2-dopamine receptors,[122] α-actinin,[123] ADP-ribosylation factor nucleotide site opener (ARNO),[124] ubiquitin-specific protease (USP4),[125] and translin-associated protein X (TRAX).[126] The lack or presence of such different partners may explain conflicting results deriving by A_{2A} ARs activation, e.g., neuroprotection versus neurotoxicity.[127]

Within the brain A_{2A} ARs are richly expressed in the striatum, nucleus accumbens, and olfactory tubercle. A coexpression of A_{2A} with D_2 dopamine receptors has been reported in the GABAergic striatopallidal neurons where adenosine and dopamine agonists exert antagonistic effects in the regulation of locomotor activity. Activation of A_{2A} ARs in striatopallidal neurons decreases the affinity of D_2 receptors for dopamine, antagonizing the effects of D_2 receptors. The negative interaction between A_{2A} and D_2 receptors is at the basis of the use of A_{2A} antagonists as a novel therapeutic approach in the treatment of PD.[128] In addition, A_{2A} ARs may have an important role in the neurodegenerative process. Accordingly, a neuroprotective effect was demonstrated after caffeine intake or A_{2A} AR inactivation against dopaminergic neurodegeneration in a neurotoxin model of PD.[129] Concomitantly, two large prospective epidemiological studies have strongly associated caffeine consumption to a reduced risk of developing PD.[130,131] Last, the recent discovery that the A_{2A} can form functional heteromeric receptor complexes with other G-protein-coupled receptors such as D_2 and the mGlu5 receptors has also suggested new opportunities for the potential of A_{2A} antagonists in PD.[122] In the future development of bivalent ligands, able to activate D_2 and block A_{2A} ARs or antagonize both A_{2A} and mGlu5 subtypes, would be a promising strategy for the treatment of this neurodegenerative disease.[132−134]

In addition to the protection against striatal and nigral neuron loss by A_{2A} antagonists, there are data also supporting their protective role outside the basal ganglia.[135] Local

injection of an A_{2A} antagonist prevents glutamate-dependent death of neurons in hippocampal cortex[136] and also reduced cortical damage in a variety of ischemic stroke models. In A_{2A} knockout (KO) mice transient focal ischemia caused less neuronal damage in comparison to their wild-type (WT) littermates.[137] Therefore, it seems that tonic activation of A_{2A} ARs may be responsible for dangerous signal during injury, in contrast to the neuroprotective effects induced by endogenous A_1 activation. Recently, selective inactivation or reconstitution of A_{2A} ARs in bone-marrow cells revealed their contribution to the development of ischemic brain injury.[138] The involvement of A_{2A} ARs in neuroprotection is likely to be complex as stimulation of this subtype also diminishes brain damage after excitotoxic and traumatic injury.[139,140] A_{2A}-mediated protection has been reported against ischemia in the myocardia, kidney, and liver and in ischemia-reperfusion injury in the spinal cord.[141−144]

High expression of A_{2A} ARs has been found in platelets, leukocytes, vascular smooth muscle, and endothelial cells with important implications in the regulation of inflammatory responses. It is now well established that stimulation of the A_{2A} AR in immune cells induces anti-inflammatory effects, mostly due to its ability to increase cAMP levels, which has strong immunosuppressive effects.[145] Stimulation of A_{2A} ARs inhibits neutrophil adherence to the endothelium, degranulation of activated neutrophils and monocytes, plus superoxide anion generation. A_{2A} ARs have been recently defined as sensors and terminators of proinflammatory activities. The strongest evidence for the key role of A_{2A} in inflammation derived by the elegant study of Ohta et al.[146] using mice deficient in A_{2A} ARs. In this model the lack of A_{2A} subtype leads to increased tissue inflammation and damage, thus suggesting a negative and nonredundant regulatory role for the A_{2A} AR. This model permits one to appreciate that adenosinergic regulation of immune cells is fundamental in normal physiological control of inflammation in vivo in spite of the fact that other Gs-protein-coupled receptors and cAMP elevating ligands are present such as cathecolamines, prostaglandins, dopamine, and histamine.[145] Interestingly, the A_{2A} AR has been demonstrated to be involved in promotion of wound healing and angiogenesis in healing wounds.[147,148] Moreover, it plays an active role in the pathogenesis of dermal fibrosis, suggesting a role for antagonists as novel therapeutic approach in the treatment and prevention of dermal fibrosis in diseases such as scleroderma.[149]

3.3. Clinical Development and Patents

PD is a progressive, incurable disorder with no definite preventive treatment, although drugs are available to alleviate the symptoms and/or slow down the progress of the disease. Current therapy is based on dopamine replacement therapy, the most common drug treatments being dopaminomimetic agents, including L-DOPA, a dopamine precursor, as well as direct or indirect dopamine receptor agonists. L-DOPA is the mainstay in the treatment of PD, but because of tolerance problems and a wide range of adverse reactions, including involuntary movements and vomiting, a strong demand for new therapies exists. Among the various strategies, A_{2A} AR blockers are considered a potential approach to treatment of the disease.[128,150]

KW-6002, an adenosine A_{2A} antagonist, is currently undergoing phase III clinical trials at Kyowa Hakko for the oral treatment of PD. As monotherapy or combination therapy with L-DOPA or dopamine agonists, it has been shown to improve the symptoms of the disease in a parkinsonian monkey model without increasing the incidence or severity of dopaminergic-related side effects or inducing or worsening dyskinesia. The company had been developing the drug for the treatment of depression, but phase II studies were discontinued. In mice and rats, KW-6002, like other A_{2A} AR antagonists, dose-dependently prevented reserpine- and haloperidol-induced catalepsy, suggesting that it modulates dopaminergic neurotransmission.[151,152] On the other hand, in D_2 receptor knockout mice, which are a model of motor impairment that resembles PD, blockade of A_{2A} ARs with KW-6002 rescued the behavioral parameters and reestablished altered enkephalin and substance P expression, suggesting a nondopaminergic mechanism for the antiparkinsonian activity of KW-6002.[153] KW-6002 improved motor disability in experimental nonhuman primate parkinsonian models. Coadministration of KW-6002 and L-DOPA/benserazide potentiated the motor effects of levodopa (30%) without increasing the dyskinetic response.[154,155] Recently low doses of KW- 6002 coadministered with low doses of L-DOPA attenuated the development of L-DOPA-induced dyskinesia as well as rotational responses to repeated L-DOPA in hemiparkinsonian mice. These results encourage consideration of future A_{2A} antagonist trials in PD that are aimed at reducing the development rather than the expression of dyskinesia.[156] Kyowa Hakko Kogyo has completed three phase III studies of KW-6002 in development for the treatment of PD (registration number [clinicaltrial.gov] 6002-EU-007, 6002-US-013, or 6002-US-018). KW-6002 has a specific antagonistic effect on the A_{2A} AR in the brain. The studies were conducted in PD patients with wearing-off phenomenon on treatment with L-DOPA alone or L-DOPA administered concomitantly with other PD medications. Two studies were conducted in North America, and one study was conducted in 14 countries of the European Union and other regions. KW-6002 was administered for 12−16 weeks. The primary endpoint was the reduction in the percentage of awake time spent in the "off" state, which served as an indicator of the improvement in the wearing-off phenomenon. One of the North American studies revealed a statistically significant reduction in the percentage of awake time spent in the off state. The other North American study and the trial conducted in the European Union/other regions did not demonstrate a significant reduction in percentage of awake time per day spent in the off state compared with placebo patients but showed a significant improvement or a trend toward improvement in one of the secondary endpoints, the motor function score, assessed using the Unified Parkinson's Disease Rating Scale subscore III. Kyowa Hakko intended to submit a new drug application to the Food and Drug Administration in the latter half of 2006. The long-term safety of KW-6002 in patients who have completed 6002-EU-007, 6002-US-013, or 6002-US-018 studies has been assessed in an extension phase III study started in October 2004 (registration number [clinicaltrial.gov] 6002-INT-001). Other open-label phase III studies of the continued safety of KW-6002 for patients who completed the prior double-blind study 6002-INT-001 started in March 2005 (registration number [clinicaltrial.gov] 13711A) and in October 2005 (registration number [clinicaltrial.gov] 6002-US-025) and are currently recruiting patients. Phase II trials are also under way by the company for the treatment of restless legs syndrome (RLS). KW-6002 has been patented as a therapeutic agent for behavioral disorders,[157] anxiety,[158] and higher brain dysfunc-

tion,[159] in medicinal composition with dopaminergic agents, monoamine oxidase-B (MAO-B) inhibitors, or catechol-*O*-methyltransferase (COMT) inhibitors for PD, RLS, and attention deficit hyperactivity disorder,[160] in medicinal composition with antidepressant agent such as the serotonin and/or norepinephrine reuptake inhibitors for depression,[161] and for disease accompanied by chronic muscle/skeleton pain[162] and drug dependence.[163]

SCH-420814 is a selective, orally active A_{2A} AR antagonist discovered by scientists at Schering-Plough and currently under phase II investigation for PD.[100] It reversed haloperidol-induced catalepsy in rats and potentiated L-DOPA-induced turning behavior in neurotoxin 6-hydroxydopamine (6-OHDA)-lesioned rats. Also, it was effective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD and several rodent models of depression. Pharmacokinetic profiling revealed oral availability of 57%, 41%, and 4% in rats, dogs, and cynomolgus monkeys, respectively. SCH-420814 and SCH-412348 were tested in vivo in rats treated with the A_{2A} agonist CGS-21680, which reduces locomotion. At doses ranging from 0.1 to 1 mg/kg, both compounds dose-dependently reversed the effects of the A_{2A} agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680). They also potentiated L-DOPA-induced turning behavior in 6-OHDA-lesioned rats at the same dose ranges. These results suggest these agents may have potential in PD as well as in other conditions associated with reduced dopaminergic activity.[100] Both SCH-420814 and SCH-412348 have been patented for PD[164] and other involuntary movement disorders.[165] Moreover, SCH 420814 has been patented as a method for treating anxiety disorders including panic disorder, agoraphobia, obsessive-compulsive disorders, social phobia, and post-traumatic stress disorder.[158]

ST-1535 is an A_{2A} AR antagonist in preclinical phase at Sigma-Tau. The compound displayed A_{2A} AR antagonist activity in vivo as it increased spontaneous motor activity in mice and was able to antagonize haloperidol-induced catalepsy at a dose of 10 mg/kg. It also exhibited antidepressant activity in the mouse forced swim test. Potentially useful for the treatment of PD and other motor disorders, it was selected for in vivo characterization in animal models.[166] ST-1535 (10, 20, and 40 mg/kg, per os (po)) when administered alone to MPTP-treated common marmosets produced a dose-related increase in locomotor activity and tended to reverse motor disability. Treatment with a threshold dose of L-DOPA (2.5 mg/kg, po) produced an increase in locomotor activity and again tended to reverse motor disability.[167,168] ST-1535, at oral doses of 5 and 10 mg/kg, antagonizes catalepsy induced by intracerebroventricular administration of CGS 21680 in mice. Oral ST-1535, at 1.25 and 2.5 mg/kg, potentiates L-DOPA effects in reducing haloperidol-induced catalepsy.[169] ST-1535 potentiates the effects of a threshold dose of L-DOPA in unilaterally 6-OHDA-lesioned rats.[168] Subchronic (18 days, twice a day) ST-1535 (20 mg/kg ip) + L-DOPA (3 mg/kg ip) did not induce sensitization to turning behavior or abnormal involuntary movements during the course of treatment, indicating a low dyskinetic potential of the drug; acute administration of ST-1535 (20 mg/kg ip) proved capable of reducing jaw tremors in a tacrine model of Parkinson's disease tremor, thus representing a potential new compound, with long-lasting activity, for the treatment of PD.[170]

Table 3. Affinity of AR Antagonists at the A_1, A_{2A}, A_{2B}, and A_3 ARs

	K_i[a] values for ARs (nM)			
A_{2B} antagonists	A_1	A_{2A}	A_{2B}	A_3
34, MRS-1754[174]	403	503	2.0	570
35, MRE-2028-F20[176]	>1000	>1000	38	>1000
36, MRE-2029-F20[176]	200	>1000	5.5	>1000
37, MRE-2030-F20[176]	>1000	>1000	12	>1000
38[180]	102	1500	22	1200
39, CVT-6883[206]	>10 000	>10 000	8.3	>10 000
40[180]	370	1100	1.0	480
41, OSIP-339391[182]	37	328	0.410[b]	450

[a] Binding experiments at recombinant hA_1, A_{2A}, A_{2B}, and A_3 ARs unless noted. [b] Value is obtained from kinetic experiments.

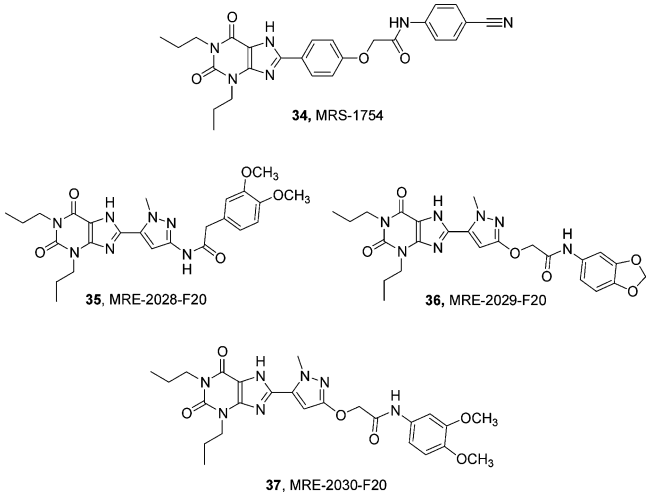


Figure 12. A_{2B} AR antagonists (xanthines).

ST-1535 has been patented for the treatment of PD and other motor disorders, Alzheimer's disease, Huntington's disease, Wilson's disease, and neurodegenerative conditions including cerebral ischemia.[171]

4. A_{2B} Adenosine Receptor Antagonists

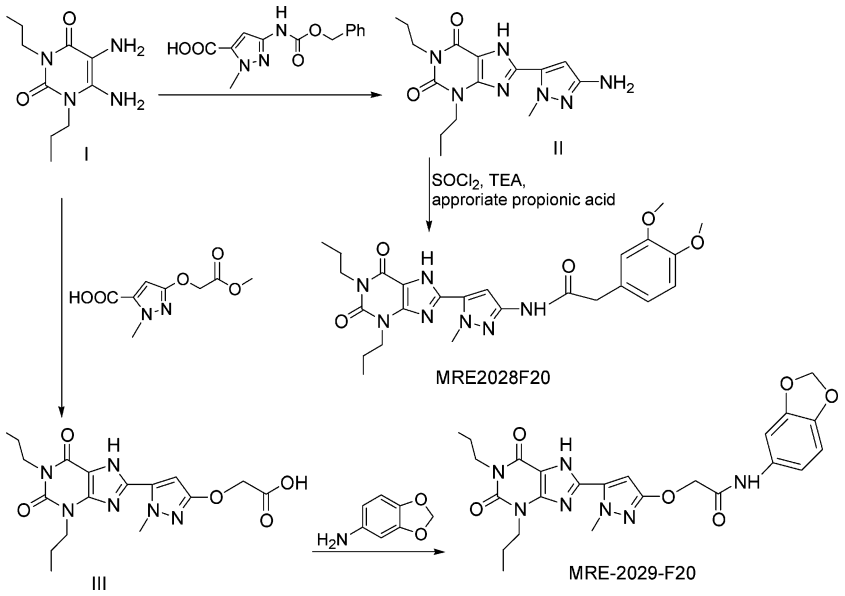
Pharmacological evidence for the therapeutic applications of A_{2B} AR antagonists has stimulated research for the development of potent and selective ligands for this subtype. Xanthine derivatives represent most of the high affinity receptor antagonists (Table 3).

4.1. Chemistry

4.1.1. Xanthines

Over the past several years the effort has been focused on studying the structure−activity relationships of xanthine derivatives to search for more selective and potent adenosine A_{2B} ligands. Potent and selective xanthine antagonists stem from multiple substitutions of the parent heterocycle. C^8-Substitution combined with N^1- and N^3- (and sometimes N^7) substitution have led to the development of potent and selective antagonists.[172,173] In the series of 8-phenyl xanthines, a large number of amide derivatives was shown to be selective for hA_{2B} vs hA_1, hA_{2A}, and hA_3, although less selective vs rat A_1 and A_{2A} ARS. As an example, the *p*-cyanoanilide derivative MRS-1754 (*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)phenoxy]-acetamide, **34**, Figure 12), was shown to be 204-, 255-, and 289-fold selective for hA_{2B} vs hA_1, hA_{2A}, and hA_3 ARs.[174]

Scheme 7. Synthesis of MRE-2028-F20 and MRE-2029-F20



Moreover, the tritium-labeled form of MRS-1754 has been prepared and utilized in radioligand binding assays.[175]

Baraldi et al.[176,177] published a series of 8-heterocycle-substituted xanthines as A_{2B} adenosine receptor antagonists. Several heterocycles, such as pyrazole, isoxazole, pyridine, and pyridazine at the 8-position of the xanthine nucleus, were studied. The synthesized compounds showed A_{2B} AR affinity in the nanomolar range and good levels of selectivity evaluated in radioligand binding assays at hA_1, hA_{2A}, hA_{2B}, and hA_3 ARs. These studies allowed identification of the derivatives 2-(3,4-dimethoxy-phenyl)-*N*-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-puri n-8-yl)-1-methyl-1*H*-pyrazol-3-yl]-acetamide (**35**, MRE-2028-F20), *N*-benzo[1,3]-dioxol-5-yl-2-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide (**36**, MRE-2029-F20), and *N*-(3,4-dimethoxy-phenyl)-2-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]acetamide (**37**, MRE-2030-F20, Figure 12), which showed high affinity to the A_{2B} AR subtype and very good selectivity vs the other AR subtypes. Substitution of the acetamide with a urea moiety afforded bioisosteric xanthines with good affinity and selectivity comparable to the acetamide derivatives. The derivatives with higher affinity to hA_{2B} AR proved to be antagonists, in the cAMP assay, capable of inhibiting the stimulatory effect of NECA (100 nM) with IC_{50} values in the nanomolar range and a trend significantly related to that observed in the binding assay. Consequently, Baraldi's group[178] synthesized the *N*-benzo-[1,3]dioxol-5-yl-2-[5-(1,3-diallyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide that led to preparation of the tritium-labeled form [^{3}H]MRE-2029-F20 (K_D value of 1.65 ± 0.10 nM in Chinese Hamster Ovary (CHO) cells expressing hA_{2B} receptors). This compound was found to be a selective, high-affinity radioligand useful for characterizing recombinant hA_{2B} receptors.

Synthesis of MRE-2028-F20 and MRE-2029-F20 is depicted in Scheme 7: the xanthine amide derivative 35 was prepared by a coupling reaction with the amine derivative II and the appropriate phenylacetic acid chloride derivatives obtained, in turn, by reaction of the corresponding phenylacetic acid with thionyl chloride and triethylamine in dichloromethane.

Xanthine derivative III was synthesized through condensation of 5,6-diaminouracil I with the appropriate carboxylic acid intermediate. Subsequently, condensation with dioxymethylen-aniline in the presence of EDAC and 1-hydroxy benzotriazole (HOBt) in DMF at room temperature yielded the target compound 36.[176]

In this field of research, very recently Zeng et al.[179] patented a series of 8-pyrazole xanthine derivatives as A_{2B} AR antagonists. The new 8-(1*H*-pyrazole-4-yl)xanthines displayed good affinity to A_{2B} AR, confirming the relevance of a pyrazole ring at the 8-position of xanthine nucleus. The chemical structures and relative binding data of the most potent compounds are reported in Figure 13 and Table 3. In

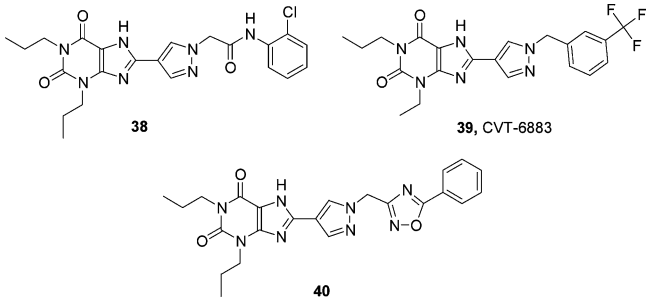


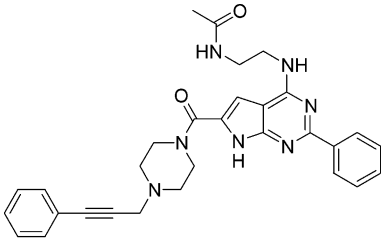
Figure 13. A_{2B} AR antagonists (8-pyrazol-4-yl-xanthines).

this report the authors presented compounds **38** and **39** (3-ethyl-1-propyl-8-[1-[3-(trifluoromethyl)benzyl]-1*H*-pyrazol-4-yl]-2,3,6,7-tetrahydro-1*H*-purine-2,6-dione, CVT-6883), which showed good affinity to the hA_{2B} AR.

The same authors synthesized a series of heterocyclic 5-membered rings by bioisosteric replacement of the amide bond with different heterocyclic 5-membered rings (1,2,4-oxadiazoles and isoxazoles).[180] Derivative **40** was the most active and selective analogue among these classes of compounds, displaying high affinity (K_i = 1 nM) and selectivity for the hA_{2B} AR vs A_1, A_{2A}, and A_3 AR subtypes (A_1/A_{2B}, A_{2A}/A_{2B}, and A_3/A_{2B} selectivity ratios of 370, 1100, and 480, respectively; Figure 13).

4.1.2. Pyrrolopyrimidines

Recently in a patent by OSI Pharmaceuticals Inc. a series of pyrrolopyrimidines is reported to be A_{2B} AR antagonists.[181]



41, OSIP-339391

Figure 14. Pyrrolopyrimidine derivative.

The most important compound of this family, coded OSIP-339391 (**41**, Figure 14), displays a 70-fold selectivity for A_{2B} ARs over the other h AR subtypes. The radiolabeled form [^{3}H]OSIP-339391 was prepared and characterized in kinetic, saturation, and competition binding experiments at recombinant hA_{2B} ARs. From the association and dissociation rate studies, the affinity was 0.41 nM, and that found in saturation binding experiments was 0.17 nM.[182]

The synthetic route for the preparation of OSIP-339391 is reported in Scheme 8: the reaction of 4-chloro-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine I with benzenesulfonyl chloride II and NaH in DMF gives 4-chloro-2-phenyl-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine III, which is condensed with CO_2 by means of lithium diisopropylamine (LDA) in THF to yield 4-chloro-2-phenyl-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid lithium salt III. Reaction of III with *N*-(2-aminoethyl)acetamide in hot dimethyl sulfoxide (DMSO) affords 4-(2-acetamidoethylamino)-2-phenyl-7-(phenylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid IV, which is desulfonated by means of NaOH in methanol to provide 4-(2-acetamidoethylamino)-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid V. Condensation of V with the acetylenic piperidine derivative by means of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and TEA in DMF gives the target compound OSIP-339391.[181]

4.2. Pharmacology

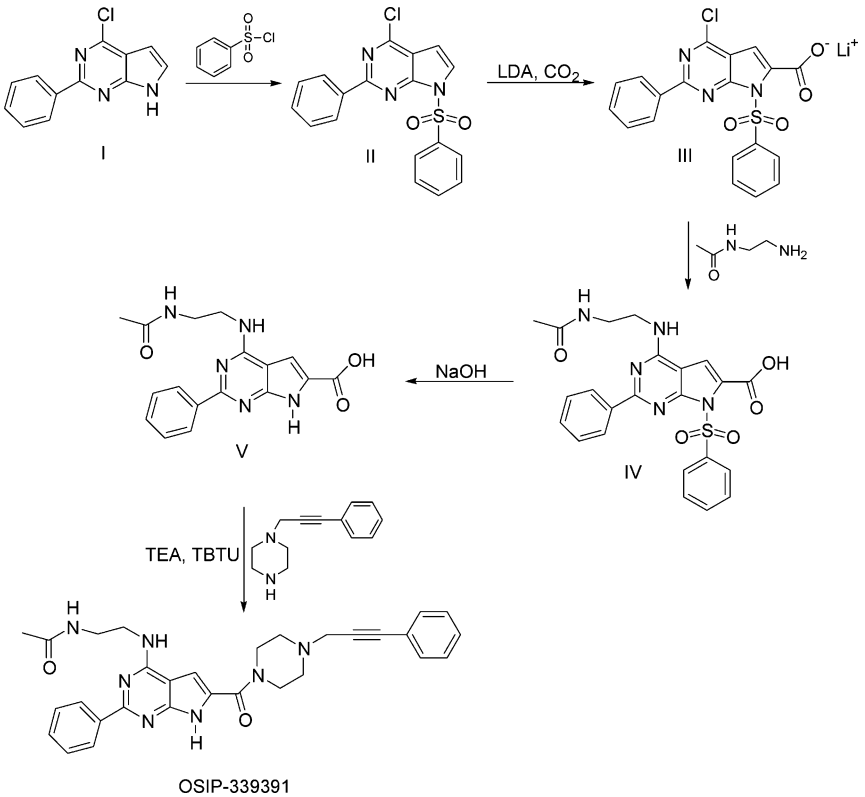
A_{2B} ARs were cloned from rat hypothalamus,[183] human hippocampus,[184] and mouse mast cells.[114] Following initial studies indicating selective induction of A_{2B} ARs by hypoxia, analysis of the cloned hA_{2B} promoter identified a functional hypoxia-responsive region, including a functional binding site for hypoxia-inducible factor (HIF) within the A_{2B} promoter.[185] These results demonstrated transcriptional coordination of A_{2B} by HIF-1α and amplified adenosine signaling during hypoxia, suggesting an important link between hypoxia and metabolic conditions related with inflammation and angiogenesis.

A_{2B} ARs have long been known to couple to adenylyl cyclase activation through Gs proteins. However, other intracellular signaling pathways have been demonstrated to be associated to A_{2B} ARs including Ca^{2+} mobilization through Gq proteins and mitogen-activated protein kinase (MAPK) activation.[186] A_{2B} AR-induced stimulation of phospholipase C results in mobilization of intracellular calcium in HMC-1 cells and promotion of IL-8 production.[187]

Initially tissue distribution of A_{2B} ARs was reported in peripheral organs like bowel, bladder, lung, and vas deferens. As for the brain, mRNA and protein were detected in hippocampal neurones[188,189] and glial cells but not in microglial cells.[190,191] Stimulation of A_{2B} ARs mediates the release of IL-6 from astrocytes.[191,192] Due to the neuroprotective effect of IL-6 against hypoxia and glutamate neurotoxicity, activation of A_{2B} subtype provides a damage-control mechanism during CNS injury.[193]

Functional studies have identified A_{2B} ARs in airway smooth muscle, fibroblasts, glial cells, the gastrointestinal tract, and the vasculature. Vascular A_{2B} may be associated

Scheme 8. Synthesis of OSIP-339391



to vasodilatation in both smooth muscle and endothelium. This subtype is important in modulation of vasodilatation in certain vessels such as rat mesenteric artery and guinea-pig pulmonary artery but not in others where the A_{2A} effect predominates. Recent findings also support the involvement of A_{2B} subtype in adenosine-mediated coronary vasodilation.[194,195] Activation of A_{2B} receptors may prevent cardiac remodeling after myocardial infarction.[196] A protective effect from infarction has been also attributed to A_{2B} ARs in ischemic postconditioning through a pathway involving protein kinase C and phosphatidylinositol-3-kinase.[197]

According to mRNA analysis revealing high amounts of A_{2B} message in the cecum and large intestine, it has been reported that A_{2B} ARs in intestinal epithelial cells trigger an increase in cAMP levels that is responsible for Cl^- secretion. This Cl^- secretion pathway results in movement of isotonic fluid into the lumen, a process that naturally serves to hydrate the mucosal surface but, in extreme, produces secretory diarrhea.[198−200]

A_{2B} ARs have been cloned from mouse bone-marrow-derived mast cells and reported to mediate degranulation and activation of canine mastocytoma and human mast cells, thus suggesting a possible role in allergic and inflammatory disorders.[35] Adenosine constricts airways of asthmatic patients through the release of histamine and leukotrienes from sensitized mast cells.[201,202] The receptor involved seems to be the A_{2B} in humans or the A_3 in rats. Recently, A_{2B} ARs have been reported to mediate several proinflammatory effects of adenosine in inflammatory cells of the lung. In addition to mast cells, functional A_{2B} ARs have been found in bronchial smooth muscle cells and lung fibroblasts. In these cells adenosine, through stimulation of A_{2B} subtype, increases the release of various inflammatory cytokines, supporting the evidence that A_{2B} ARs play a key role in the inflammatory response associated with asthma. The first evidence for the involvement of A_{2B} ARs in asthma derived from studies concerning selectivity of enprofylline, a methylxanthine structurally related to theophylline.[203] Further support for the role of A_{2B} in asthma comes from studies demonstrating their presence on different types of cells important for the cytokine release in asthmatic disease such as smooth muscle cells, lung fibroblasts, endothelial cells, bronchial epithelium, and mast cells. Expression of A_{2B} ARs was also found in mast cells and macrophages of patients affected by COPD.[204] Activation of A_{2B} in HMC1 mast cell line induced an increase of IL-8 release.[187] Furthermore, A_{2B} antagonists potently inhibited activation and degranulation of human mast cells induced by adenosine.[205] Recently, it has been reported that ADA-deficient mice treated with the selective A_{2B} antagonist CVT-6883 showed reduced elevations in proinflammatory cytokines and chemokines as well as mediators of fibrosis and airway destruction.[206] Interestingly, other authors investigated the role of A_{2B} ARs in inflammation in vivo.[207] This study was carried out on A_{2B} KO mice in which exon 1 of the A_{2B} was replaced by a reporter gene, allowing examination of endogenous A_{2B} ARs expression in various tissues and cell types in vivo. Results show that there is abundant reporter expression in the vasculature and macrophages. This new animal model emphasizes a role for the A_{2B} ARs in attenuating inflammation through regulation of proinflammatory cytokine production and adhesion properties of the vasculature. In contrast with the function of A_{2B} ARs in vasodilation, the A_{2B} KO mice have normal blood pressure.[207]

Due to the above-described biological effects, at present about 166 A_{2B} compounds are in biological testing, 18 are in preclinical stage, and 1 is in the phase I stage as antiasthmatic, antiallergic, antidiabetic, antidiarrheal, antiatherosclerotic, and oncolytic drugs for cardiovascular and ocular disorders.

4.3. Clinical Development and Patents

CVT-6883 is a selective, potent, and orally available A_{2B} AR antagonist. CV Therapeutics has initiated a clinical program for CVT-6883 intended to treat asthma with once a day dosing. A randomized, double-blind, placebo-controlled, single-dose phase I study of oral CVT-6883 in 24 healthy adults is assessing the safety and tolerability of a range of doses of CVT-6883. Blockade of the A_{2B} AR may limit or prevent mast cell degranulation, which can lead to bronchoconstriction and the inflammatory process associated with asthma and cardiopulmonary disease. In a recent study, mice deficient in ADA developed severe pulmonary inflammation and injury, which could be attenuated by CVT-6883 treatment. This compound was also able to decrease elevated proinflammatory cytokine lung levels in ADA-deficient mice. CVT-6883 also suppressed the increased expression of matrix metalloproteinases and their inhibitors as well as pulmonary fibrosis developed by mutant mice. ADA deficiency was also related with enhanced lung expression of adenosine and A_{2B} AR, the levels of which tended to be lower in the presence of CVT-6883. In a mouse model of bleomycin-induced pulmonary fibrosis, lung injury was successfully reduced by CVT-6883. CVT-6883 also attenuated the airway reactivity induced by NECA, AMP, or allergen in an allergic mouse model of asthma.[208]

Together, these results suggest that antagonizing A_{2B} AR may be beneficial in the management of chronic pulmonary diseases. Results from a randomized, double-blind, placebo-controlled, single-ascending dose phase I clinical study of CVT-6883 indicated that it was well tolerated with no serious adverse events reported.[206] CVT-6883 has been patented for asthma and COPD.[209] Moreover, A_{2B} AR antagonists have been claimed for use in promoting the healing of wounds caused by mechanical, chemical, or thermal activity, for example, for diabetic ulcer.[179]

MRE-2029-F20 is one of several high-affinity antagonists of King Pharmaceuticals in the preclinical phase. This compound has been reported to be a potent and selective A_{2B} antagonist able to counteract stimulation of cAMP levels from in vitro studies performed in HEK293 cells and in inflammatory cells relevant for asthma-like neutrophils, lymphocytes, and HMC1 cells.[210,211] Several A_{2B} antagonists have been patented for asthma and eye disorders.[177]

Other interesting A_{2B} antagonists in the preclinical phase include MRS 1754,[212] MRS 1706,[212] MRS 1668,[212] and PSB-1115[213] developed at the National Institutes of Health (NIH) and PSB 53[213] from the University of Bonn (Integrity source)

5. A_3 Adenosine Receptor Antagonists

A_3-selective AR antagonists have been postulated as novel anti-inflammatory and antiallergic agents; recent studies also indicated a possible employment of these derivatives as antitumor agents. In recent years many efforts have been made to search for potent and selective hA_3 AR antagonists (Table 4).

Table 4. Affinity of AR Antagonists at the A_1, A_{2A}, A_{2B}, and A_3 ARs

A_3 antagonists	K_i[a] values for adenosine receptors (nM)			
	A_1	A_{2A}	A_{2B}	A_3
42, PSB-10[216]	1700[b]	2700[b]	ND	0.43
43, KF-26777[217]	1800	470	620	0.20
44[218]	>1000	>1000	> 1000	0.80
45, OT-7999[219]	>10 000[d]	>10 000[d]	>10 000[d]	0.95
46, MRS 1097[223]	5930[c]	4770[c]	ND[e]	108
47, MRS 1191[223]	40 100[c]	<10%[c]	ND[e]	31.4
48, MRS 1334[224]	>100[c]	>100[c]	ND[e]	2.69
49, MRS 1523[225]	15 600[c]	2050[c]	ND[e]	18.9
51, MRE-3008-F20[231]	1200	141	2100	0.82
52, MRE-3005-F20[233]	250	60	200	0.04
55, VUF-5574[235]	>10 000[c]	>10 000[c]	ND[e]	4.0

[a] Binding experiments at recombinant hA_1, A_{2A}, A_{2B}, and A_3 ARs unless noted. [b] Binding experiments at human cortex (A_1) and striatum (A_{2A}) ARs. [c] Binding experiments at rat cortex (A_1) and striatum (A_{2A}) ARs. [d] IC_{50} values. [e] ND = not determined.

5.1. Chemistry

5.1.1. Xanthines

Natural antagonists for ARs, such as caffeine and theophylline, show in general low affinity for the A_3 AR subtype.[214] Different positions of the xanthine core have been modified with the aim of improving A_3 AR affinity. A series of tricyclic imidazo[2,1-*i*]purinones and ring-enlarged analogues derived from xanthine derivatives has been prepared as AR antagonists. In comparison with xanthines, the tricyclic compounds exhibit increased water solubility due to a basic nitrogen atom, which can be protonated under physiological conditions.[215] Among this series PSB-10, 8(*R*)-ethyl-4-methyl-2-(2,3,5-trichlorophenyl)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one (**42**, Figure 15), is a high-affinity

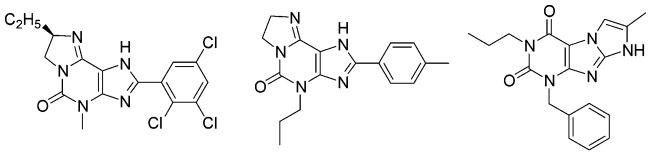
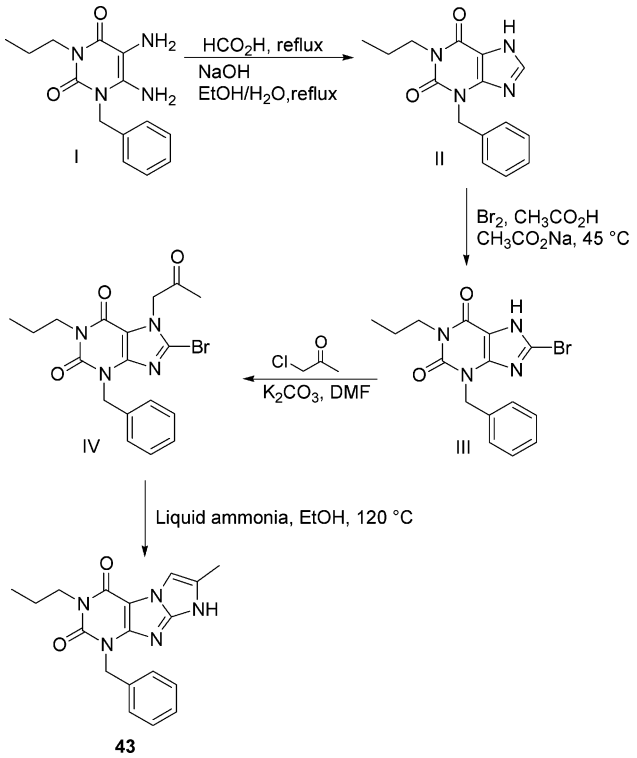


Figure 15. A_3 AR antagonists (xanthines).

ligand for A_3 ARs (hA_3, K_i = 0.43 nM) with high selectivity over hA_1 and hA_{2A} ARs (K_i = 1700 and 2700 nM, respectively). The compound showed inverse agonist activity in binding studies in CHO cells expressing recombinant hA_3 ARs (IC_{50} = 4 nM).[216] Another similar compound is 2-(4-bromophenyl)-7,8-dihydro-4-propyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one, also named KF-26777 (**43**, Figure 15), endowed with subnanomolar affinity to hA_3 ARs (K_i = 0.20 nM) and high selectivity over A_1, A_{2A}, and A_{2B} ARs (9000-, 23 500-, and 31 000-fold, respectively). It concentration-dependently inhibited 2-chloro-N^6-(3-iodobenzyl)-*N*-methyl-5′-carbamoyladenosine (Cl-IB-MECA)-induced [^{35}S]guanosine 5′-*O*-(3-thiotriphosphate) ([^{35}S]-GTPγS) binding to human embryonic kidney 293 cells (HEK293) (IC_{50} = 270 nM) and enhanced intracellular Ca^{2+} concentration in human promyelocytic cells (K_B = 0.42 nM). This agent was indicated for potential interest for treatment of brain ischemia and inflammatory diseases such as asthma.[217]

The discovery of 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-diones by cyclization between the 7- and 8-positions of the xanthine core lead to **44** (Figure 15), a highly

Scheme 9. Synthesis of Compound 43



potent and selective A_3 adenosine receptor antagonist.[218] This compound shows a subnanomolar affinity (hA_3, K_i = 0.8 nM) toward the desired receptor target with a noteworthy selectivity versus the other adenosine receptors subtypes.

Compound **44** was synthesized by the approach reported in Scheme 9. The 3-benzyl-1-propyl-3,7-dihydro-purine-2,6-dione II was obtained by reacting the diamino derivative I with formic acid. Bromination at the 8-position with Br_2 and sodium acetate in acetic acid at 60 °C led to the key 8-bromo-intermediate III. Alkylation at the N^7-position with 1-bromo-propan-2-one yielded 3-benzyl-8-bromo-7-(2-oxo-propyl)-1-propyl-3,7-dihydro-purine-2,6-dione IV. Treatment of IV with liquid ammonia in a sealed tube at 120 °C in ethanol involved substitution of the bromine at the 8-position followed by in situ cyclization of the amino group with the N^7 carbonyl function to give the desired 7-benzyl-2-methyl-5-propyl-1*H*,7*H*-1,3a,5,7,8-pentaaza-cyclopenta[*a*]indene-4,6-dione V.[218]

In this field of research the triazolopurine derivatives in which the xanthine structure is extended are also reported. One example is OT-7999 (**45**, Figure 16), which proved to be a potent and selective hA_3 AR ligand. In receptor binding assays, OT-7999 displayed high affinity for the A_3 AR (K_i = 0.95 nM) and >10 500-fold selectivity relative to other AR subtypes. Significant reductions in intraocular pressure were obtained in cynomolgus monkeys at 2−4 h following topical application to the eye of OT-7999 (500 mcg).[219,220]

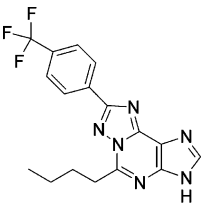
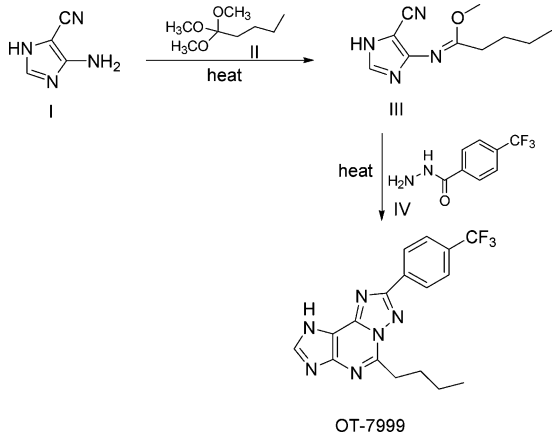


Figure 16. Triazolopurine derivative.

Scheme 10. Synthesis of OT-7999



Reaction of 4-amino-1*H*-imidazole-5-carbonitrile I with trimethyl orthopentanoate II in hot DMF gives methyl *N*-(5-cyano-1*H*-imidazol-4-yl)pentanoimidate III, which is then cyclized with benzoic hydrazide IV in hot DMF to yield the target triazolo-purine derivative OT-7999[219] (Scheme 10).

5.1.2. 1,4-Dihydropyridine and Pyridines

Starting from the experimental observations that 1,4-dihydropyridines bind A_1 adenosine receptors in the rat brain,[221,222] Jacobson et al. used the 1,4-dihydropyridine nucleus as a template for probing the SAR profile at the A_3 AR subtype.[223] SAR studies of adenosine receptor antagonists indicated that sterically bulky groups are well tolerated at the 4-, 5-, and 6-positions. The combination of substitutions led to the discovery of MRS 1097 (2-methyl-6-phenyl-4-styryl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester, **46**, Figure 17), MRS 1191, (2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 5-benzyl ester, **47**, Figure 17), and MRS 1334 (2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-ethyl ester 5-(4-nitro-benzyl) ester, **48**, Figure 17) as the first A_3 antagonists related to 1,4-dihyropyridines. In this study, they also synthesized pyridine derivatives[223,224] through oxidation of the corresponding 1,4-dihydropyridine.

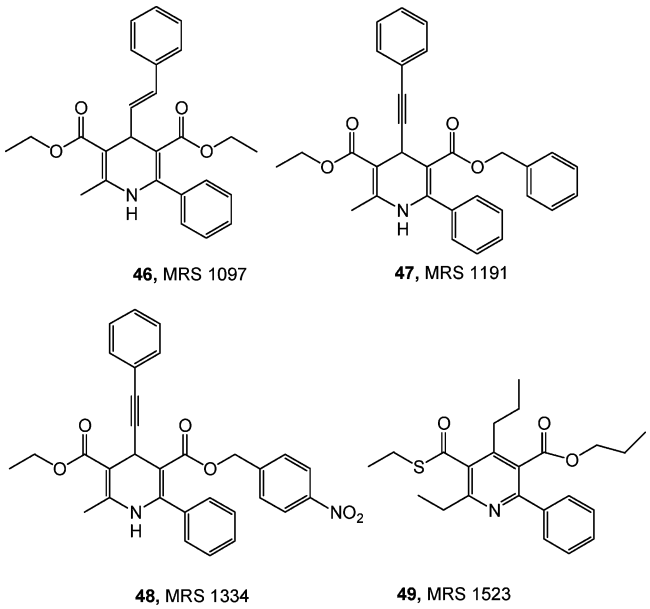


Figure 17. Dihydropyridine and Pyridine derivatives as A_3 AR antagonists.

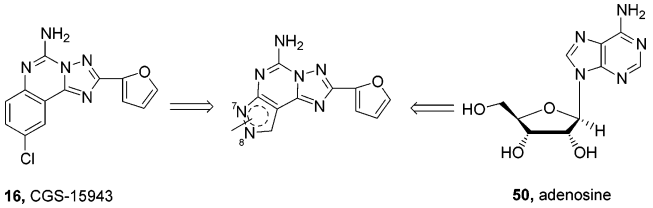


Figure 18. Structural correlation with CGS-15943 and the adenine nucleus present in the adenosine.

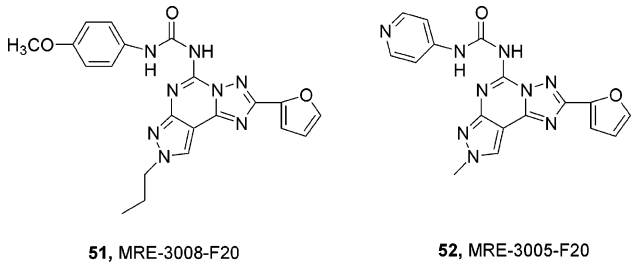


Figure 19. A_3 AR antagonists (pyrazolo-triazolo-pyrimidines).

In this class of compounds, small groups at the 4-position were found to be essential such as in MRS 1523 (6-ethyl-5-ethylsulfanylcarbonyl-2-phenyl-4-propyl-nicotinic acid propyl ester, **49**, Figure 17), which showed favorable affinity at the hA_3 AR subtype. Comparing the structural requirements for the two related classes of compounds indicated that bulky substituents at the 4-position and a 5-benzyl ester, which are affinity enhancing in dihydropyridines, are not well tolerated in the pyridine series for A_3 receptor binding. At other positions, structural parallels occur between corresponding dihydropyridine and pyridine analogues.[225]

5.1.3. Pyrazolo-triazolo-pyrimidines

The pyrazolo-triazolo-pyrimidine nucleus, due to its strong structural correlation with the nonselective antagonists **16**, CGS-15943, and the adenine nucleus present in the endogenous modulator adenosine **50** (Figure 18), has been strongly investigated in the past decade as a prototypical template for adenosine antagonists. The triazolo-quinazoline derivative CGS-15943 represented the starting point for searching for new potent and selective hA_3 adenosine receptor antagonists. MRS-1220, a 5-*N*-phenylacetyl derivative of CGS-15943, in receptor binding studies displayed K_i values of 305 ± 51, 52.0 ± 8.8, and 0.65 ± 0.25 nM for rat A_1, A_{2A}, and hA_3 receptors, respectively, being 470- and 80-fold selective for hA_3 ARs vs rat A_1 and A_{2A} ARs, respectively. MRS-1220 also antagonized the effects of an A_3 agonist in functional assays.[226,227]

An innovative series of tricyclic compounds (MRE series) reported by Baraldi's group represented new selective A_3 AR antagonists. In this class attention was focused on the N^8 patterns of substitution due to the quite complete inactivity of the N^7-substituted derivatives at the hA_3 subtype (e.g., SCH-58261).

MRE-3008-F20 (**51**, Figure 19), one of several high-affinity antagonists, is an A_3 AR ligand (K_i = 0.29 nM against 4-aminobenzyl-5′-*N*-methylcarboxamidoadenosine ([^{125}I]-AB-MECA) binding to human receptors expressed in HEK293 cells) with high selectivity over rat A_1 and A_{2A} ARs (K_i > 10 000 and 1993 nM, respectively) as well as hA_1 and hA_{2A} ARs (K_i = 1197 and 141 nM, respectively).[228] The compound showed antagonist activity in a functional

assay being capable of blocking the effect of IB-MECA on cAMP production in CHO cells ($IC_{50} = 4.5$ nM).[229−231] The tritium-labeled compound was able to bind hA_3 ARs expressed in CHO cells with a K_D value of 0.82 nM and a B_{max} value of 297 fmol/mg protein and represents the first high-affinity, selective radiolabeled antagonist for this subtype resulting in a useful tool for characterization of A_3 ARs in both normal and pathological conditions.[232] The isosteric replacement of the phenyl with a 4-pyridyl moiety provided higher hydrosolubility and led to the first water-soluble hA_3 antagonist (MRE-3005-F20, **52**, Figure 19) which is an ideal candidate for the pharmacological and clinical investigations of the hA_3 AR subtype.[233]

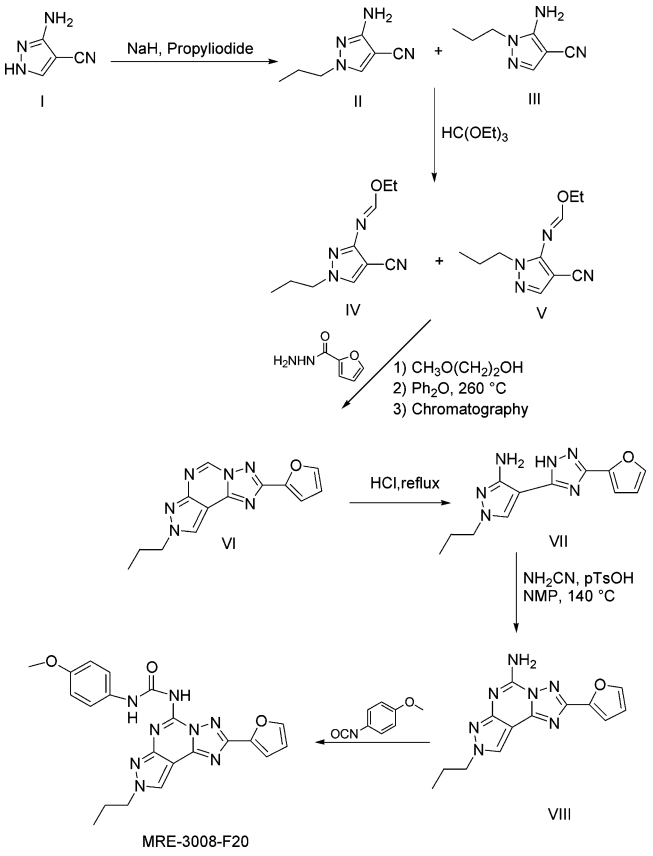
In molecular modeling studies reported by Moro et al. on pyrazolo-triazolo-pyrimidines, a combined target-based and ligand-based drug design has been carried out to define a novel pharmacophore model for the hA_3R antagonists. A high-throughput docking strategy has been applied on the pyrazolo-triazolo-pyrimidine series. All low-energy docked conformations have been superimposed and used to characterize the common features crucial to the recognition process. A novel target-based pharmacophore model has been proposed for human A_3 AR antagonists. A CoMFA (comparative molecular field analysis) approach has been used as an alternative scoring function for prediction of ligand receptor binding affinity. The new target-based pharmacophore model was coherent with the structure−activity relationships collected on the pyrazolo-triazolo-pyrimidine analogues.[234,235] Moreover, very recently Botta, Martinelli, and Baraldi et al. performed a pharmacophoric study using the software Catalyst, which yielded three different common feature hypotheses for antagonists of the hA_3R. The three pharmacophores referred to a recurring scheme consisting of three hydrophobic interactions lying at the vertexes of a triangle. They seemed particularly good in handling pyrazolo-triazolo-pyrimidine derivatives.[236]

These results confirm the importance of this tricycle as the most potent class of A_3 AR antagonists.

Synthesis of MRE-3008-F20 is depicted in Scheme 11: alkylation of 5-amino-4-cyanopyrazole I with *n*-propyl iodide led to a 4:1 inseparable mixture of regioisomers II and III. This mixture was transformed into the respective imidates IV and V through refluxing in triethyl orthoformate. Subsequent addition of 2-furoic hydrazide to the nitrile group of IV + V, followed by thermal cyclization in diphenyl ether at 260 °C, furnished the corresponding pyrazolo-triazolo-pyrimidines. The desired regioisomer VI was then isolated by flash chromatography. Hydrolysis of VI with aqueous HCl afforded the aminopyrazole VII, which was further converted to the tricyclic compound VIII by reaction with cyanamide and *p*-toluenesulfonic acid. Finally, coupling of VIII with *p*-methoxyphenyl isocyanate provided the title urea.[231]

5.1.3.1. Fluorosulfonyl- and Bis(*β*-chloroethyl)amino-phenylamino- pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines. Synthesis of irreversible A_3 antagonists was realized to provide useful tools for structure−activity studies. Electrophilic groups, specifically sulfonyl fluoride and nitrogen mustard (bis-(*β*-chloroethyl)amino) moieties, have been incorporated at the 4-position of the aryl urea group (Compunds **53** and **54**, Figure 20).[237] Compounds containing a fluorosulfonyl moiety proved to be irreversible antagonists at the hA_3 AR (at 100 nM, 79% of inhibition), while the corresponding nitrogen mustard derivatives were unable to covalently bind this receptor subtype. This difference in the receptor interaction between the 53 and 54 series has been explained on the basis of chemical reactivity of the two different groups: the $-SO_2F$ group is highly reactive versus all nucleophilic functions, while the nitrogen mustard reacts only with amino functions.

Scheme 11. Synthesis of MRE-3008-F20



5.1.4. Isoquinoline and Quinazoline Urea Analogues as Antagonists for the Human Adenosine A_3 Receptor

A structure−affinity analysis reported by IJzerman et al.[238] indicated that on the 2-position of the quinazoline ring or the equivalent 3-position of the isoquinoline ring a phenyl or heteroaryl substituent increased the A_3 AR affinity in comparison to unsubstituted or aliphatic derivatives. Combination of the optimal substituents in the two series led to the potent hA_3 AR antagonist *N*-(2-methoxyphenyl)-*N*′-(2-(3-pyridyl)quinazolin-4-yl)urea (VUF5574, **55**, Figure 21) with a K_i value of 4 nM and a selectivity of at least 2500-fold vs A_1 and A_{2A} ARs. In an in vitro functional assay the compound competitively antagonized the inhibition of cAMP production induced by the adenosine agonist NECA in CHO cells expressing hA_3 ARs with a pA_2 value of 8.1.[238]

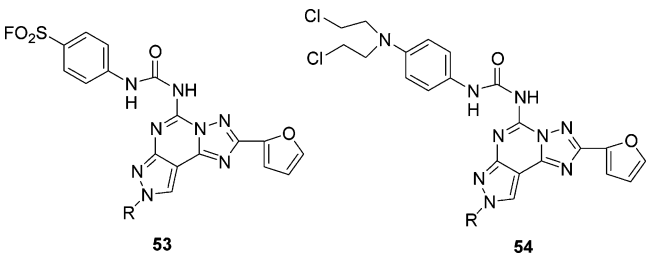
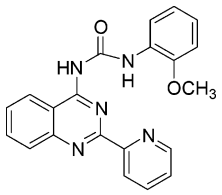


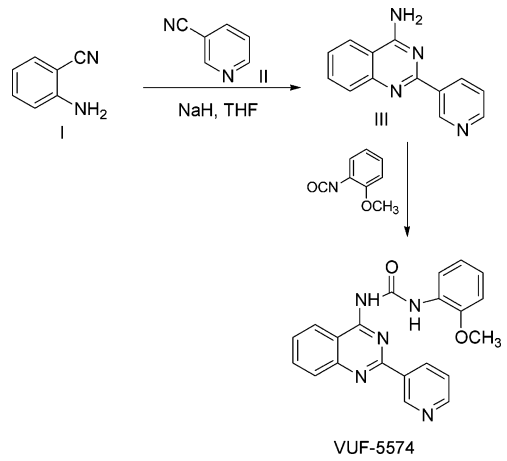
Figure 20. A_3 irreversible antagonists.



55, VUF-5574

Figure 21. Quinazoline urea derivative.

Scheme 12. Synthesis of VUF-5574



VUF5574 was prepared as reported in Scheme 12. The amide anion obtained from 2-aminobenzonitrile I and NaH in THF was added to 3-cyanopyridine II yielding 2-(3-pyridyl)-4-aminoquinazoline III. This was then condensed with 2-methoxyphenyl isocyanate to furnish the title urea.[238]

5.2. Pharmacology

The A_3 AR is the only adenosine subtype cloned before its pharmacologic identification.[239] It was originally isolated as an orphan receptor from rat testis, having 40% sequence homology with canine A_1 and A_{2A} subtypes.[240] Homologs of the rat striatal A_3 AR have been cloned from sheep and human. Interspecies differences in A_3 AR structure are large, showing the rat A_3 AR only 74% sequence homology with sheep and human.

A_3 ARs activation inhibits adenylyl cyclase activity by coupling with G_i proteins.[241] In the rat mast cell line RBL-2H3 and rat brain, A_3 ARs stimulation activate phospholipase C through Gq proteins.[242,243] The A_3 AR is widely distributed with its mRNA expressed in testis, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon, and eye of rat, sheep, and humans.[239,244−247]

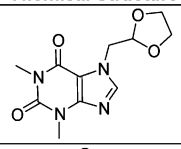
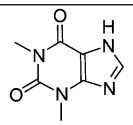
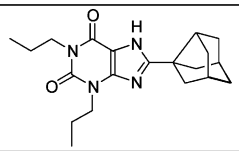
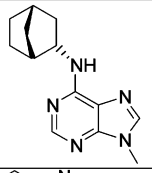
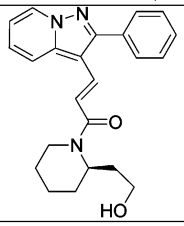
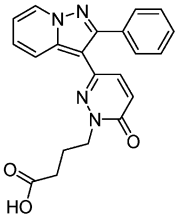
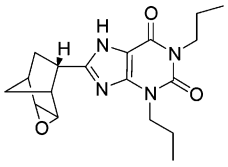
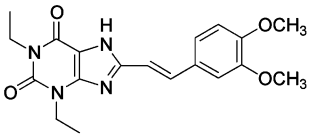
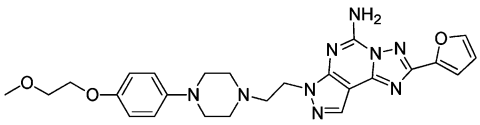
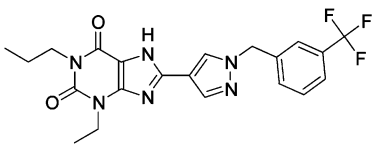
A dual role of A_3 ARs has been reported in the brain. In particular, it seems that chronic preischemic administration of the agonist IB-MECA induces a significant neuronal protection and reduction of the subsequent mortality, while acute administration of the drug results in a pronounced worsening of neuronal damage and postischemic mortality. Mice with functional deletions of the A_3 AR (A_3 $AR^{-/-}$) reveal a number of CNS functions where the A_3 ARs play a role, including nociception, locomotion, behavioral depression, and neuroprotection. Consistent with previous reports of the neuroprotective actions of A_3 AR agonists, A_3 $AR^{-/-}$ mice show an increase in neurodegeneration in response to repeated episodes of hypoxia suggesting the possible use of A_3 agonists in the treatment of ischemic, degenerative conditions of the CNS.[248]

To date, much evidence supports that activation of A_3 ARs is crucial for cardioprotection during and following ischemia-reperfusion, and it is likely that a consistent part of the cardioprotective effects exerted by adenosine, once largely attributed to the A_1 AR, may now in part be ascribed to A_3 AR activation.[249,250] The molecular mechanism of A_3 AR cardioprotection has been attributed to regulation of ATP-sensitive potassium channels. The cardioprotective effects of A_3 ARs were also detected in mice overexpressing low levels of A_3 ARs without detectable adverse effects, while higher levels of A_3 expression lead to the development of a dilated cardiomyopathy.[251] Similar data were observed in the case of A_1 ARs overexpression.[252]

In addition to reducing injury in myocardial and vascular tissues, other beneficial actions at the inflammatory level have been attributed to the A_3 subtype. For example, A_3 ARs are expressed in human neutrophils where they are involved together with A_{2A} in the reduction of superoxide anion generation[253] and have been implicated in suppression of tumor necrosis factor alpha (TNFα) release induced by endotoxin from human monocytes.[254] Moreover, A_3 activation seems to inhibit degranulation and superoxide anion production in human eosinophils.[255] Transcript levels for the A_3 subtype are elevated in the lungs of asthma and COPD patients, where expression is localized to eosinophilic infiltrates. Similar evidence was observed in the lungs of ADA-deficient mice that exhibited adenosine-mediated lung disease. Treatment of ADA-deficient mice with MRS 1523, a selective A_3 antagonist, prevented airway eosinophilia and mucus production. These results are in contrast to experiments performed in human eosinophils ex vivo, where chemotaxis was reduced by A_3 AR activation, suggesting that significant differences exist between the impact of A_3 signaling on eosinophil migration ex vivo and in the whole animal.[256] The functional role of the A_3 subtype in the pathogenesis of asthma remains controversial, and differences in the pharmacology of A_3 subtype from different species render it difficult to understand whether an A_3 AR agonist or antagonist is better for use in antiasthmatic therapies.

A very interesting area of application of A_3 ligands concerns cancer therapies. The possibility that A_3 AR plays a role in the development of cancer has aroused considerable interest in recent years.[257] A_3 subtype has been described in the regulation of the cell cycle, and both pro- and antiapoptotic effects have been reported depending on the level of receptor activation.[258−261] A_3 activation has been demonstrated to be involved in inhibition of tumor growth both in vitro and in vivo, leading to the development of A_3 agonists in clinical trials for colon carcinoma. The molecular mechanisms involved in the anticancer effects induced by A_3 agonists included regulation of the WNT pathway.[262] On the other hand, it has been reported that adenosine upregulates HIF-1α protein expression and vascular endothelial growth factor (VEGF) protein accumulation by activating A_3 AR subtype in tumoral cells, suggesting a role for A_3 subtype in the regulation of angiogenesis.[263] Overexpression of the A_3 subtype has been demonstrated in colon cancer tissues obtained from patients undergoing surgery in comparison to normal mucosa. Overexpression in tissues was also reflected at the level of peripheral blood cells, rendering this adenosine subtype a possible marker for cancer detection.[264] Similar data were also found in the case of arthritis, where A_3

Table 5. Selected Adenosine Receptor Antagonists in Clinical Development

Name	Chemical Structure	Phase	Pharmacological profile	Therapeutic group
Doxofylline		*Launched*	*A_1AR antagonist*	*Bronchodilator*
Theophylline		*Launched*	*A_1AR antagonist*	*Bronchodilator*
KW-3902		*Phase III*	*A_1AR antagonist*	*Agent for heart failure*
N-0861		*Phase III*	*A_1AR antagonist*	*Agent for heart failure*
FK-453		*Phase II*	*A_1AR antagonist*	*Agent for renal failure*
FK-838		*Phase II*	*A_1AR antagonist*	*Agent for hypertension, diuretic*
BG-9719		*Phase II*	*A_1AR antagonist*	*Agent for heart failure*
SLV-320		*Phase II*	*A_1AR antagonist*	*Agent for* heart *failure and renal failure*
KW-6002		*Phase III*	*A_{2A}AR antagonist*	*Antiparkinsonian*
SCH-420814		*Phase II*	*A_{2A}AR antagonist*	*Antiparkinsonian*
CVT-6883		*Phase I*	*A_{2B}AR antagonist*	*Antiasthmatic*

activation shows beneficial effects by suppression of TNFα production.[265,266] Adenosine receptors have been implicated in many ocular and systemic ischemic diseases (e.g., retinal ischemia). The A_3 KO mouse showed lower intracellular pressure, suggesting a role for A_3 antagonists in the therapy of glaucoma.[267,268]

5.3. Clinical Development and Patents

At the moment there are not A_3 antagonists in clinical phases. However, in light of the plethora of biological effects attributed to A_3 ARs, substantial efforts in medicinal chemistry have been addressed to develop antagonists for the A_3 subtype.[269] As a result a number of molecules are in biological testing as therapeutic agents for asthma and COPD, glaucoma, cancer, and stroke.

Use of A_3 antagonists has been patented for inhibition of tumor growth.[270] The pre- or coadministration of pharmaceutical compositions comprising high-affinity adenosine A_3 receptor antagonists, such as MRE-3008-F20, has been patented for synergistically accentuating the response to chemotherapy consisting of taxane (e.g., paclitaxel), vinca alkaloid (e.g., vincristine), camptothecin (e.g., irinotecan), or antibiotic (e.g., doxorubicin) treatment.[271] The claim further embodies the prevention of multidrug resistance (MDR) and targeted tumors include those expressing MDR-associated protein (MRP), A_3 ARs, or P-glycoprotein, as found in leukemia, melanoma, and carcinoma of the pancreas, ovary, and lung. Moreover, MRE-3008-F20 has been also patented for the treatment of cardiac hypoxia, allergic diseases, cerebral ischemia, and cancers with high concentrations of A_3 ARs.[272]

Other patents of A_3 antagonists also concern their use for cognitive disorders, multiple sclerosis, neurodegeneration, PD, stroke, traumatic brain injury,[273] asthma and COPD,[274−277] glaucoma,[278] and arthritis.[279]

6. Future Perspectives and Conclusions

In the 30 years that have passed since the initial identification of ARs, intense and extensive basic biological research has resulted in a fairly complete understanding of the importance of the adenosinergic system in human pathophysiology.[280] Development of receptor antagonists with good tissue specificity is the most challenging aspect for researchers working in the drug discovery area. In general, in the adenosinergic field, recent efforts in medicinal chemistry and pharmacology have yielded substantial numbers of compounds with selectivity at each AR subtype; this is also attested from the impressive number of published patents: 119, 129, 48, 29 (Integrity source) for A_1, A_{2A}, A_{2B}, and A_3 ARs, respectively. In spite of the problem related to the ubiquity of adenosine itself in the body and difficulty in devising adenosine antagonists that affect only one physiological function, nine molecules targeting ARs are now in clinical development (Table 5).

In particular, A_1 ARs have emerged as a promising strategy for the treatment of neurological disorders, asthma, and heart and renal failure. KW-3902 and N-0861 are at the highest phase of clinical studies for treatment of heart failure in patients undergoing diuresis, while BG-9719, FK 453, FK 838, and SLV-320 are in phase II trials.

A_{2A} antagonists are now the most realistic alternatives, and support to dopaminergic treatment of PD and KW-6002 is currently in late-stage clinical trials; SCH 420814 is under phase II studies for PD treatment.

As for A_{2B} antagonists, CVT-6883 is in phase I studies for management of chronic pulmonary diseases, and other molecules are in development as antiasthmatic drugs and also of potential value as antidiabetic, antidiarrheal, antiatherosclerotic, and cardiovascular disorders.

Finally, A_3 antagonists are being considered for application in asthma and COPD, glaucoma, cancer, and stroke. However, clinical efficacy still remains to be demonstrated, and it would be in the clinic that our understanding of A_3 ARs biology will be challenged.

Although many of the agents reviewed are in the early stages of development, the future for generation of AR antagonists in the treatment of human health can be considered promising, and we believe that several new drugs useful for the treatment of important pathologies will be available in the next few years.

7. Database

Part of the information reported in this review derives from the following database: http://Integrity.prous.com.

8. Supporting Information

List of AR antagonist patents. This material is available free of charge via the Internet at http://pubs.acs.org.

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