Introduction to Adenosine Receptors as Therapeutic Targets

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Abstract Adenosine acts as a cytoprotective modulator in response to stress to an organ or tissue. Although short-lived in the circulation, it can activate four subtypes of G protein-coupled adenosine receptors (ARs): A_1, A_{2A}, A_{2B}, and A_3. The alkylxanthines caffeine and theophylline are the prototypical antagonists of ARs, and their stimulant actions occur primarily through this mechanism. For each of the four AR subtypes, selective agonists and antagonists have been introduced and used to develop new therapeutic drug concepts. ARs are notable among the GPCR family in the number and variety of agonist therapeutic candidates that have been proposed. The selective and potent synthetic AR agonists, which are typically much longer lasting in the body than adenosine, have potential therapeutic applications based on their anti-inflammatory (A_{2A} and A_3), cardioprotective (preconditioning by A_1

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and A_3 and postconditioning by A_{2B}), cerebroprotective (A_1 and A_3), and antinociceptive (A_1) properties. Potent and selective AR antagonists display therapeutic potential as kidney protective (A_1), antifibrotic (A_{2A}), neuroprotective (A_{2A}), and antiglaucoma (A_3) agents. AR agonists for cardiac imaging and positron-emitting AR antagonists are in development for diagnostic applications. Allosteric modulators of A_1 and A_3 ARs have been described. In addition to the use of selective agonists/antagonists as pharmacological tools, mouse strains in which an AR has been genetically deleted have aided in developing novel drug concepts based on the modulation of ARs.

Keywords Adenosine receptors · G protein-coupled receptors · Purines · Nucleosides · Imaging · Allosteric modulation · Agonists · Antagonists

Abbreviations

ADHF	Acute decompensated heart failure
ADP	Adenosine diphosphate
AMP	Adenosine 5′-monophosphate
AMP579	[1*S*-[1α, 2β, 3β, 4α(*S**)]]-4-[7-[[1-[(3-Chlorothien-2-yl)methyl] propyl]amino]-3*H*-imidazo[4,5-*b*]pyrid-3-yl]-*N*-ethyl 2,3-dihydroxycyclopentanecarboxamide
AR	Adenosine receptor
ATP	Adenosine triphosphate
BAY 60–6583	2-[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl] pyridin-2-ylsulfanyl]acetamide
BAY 68–4986	6-Amino-2-(2-(4-chlorophenyl)thiazol-4-ylthio)-4-(4-(2-hydroxyethoxy)phenyl)-5-isocyanonicotinonitrile
BG9719	1,3-Dipropyl-8-(2-(5,6-epoxy)norbornyl)xanthine
BG9928	3-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-propionic acid
BIIB014	3-(4-Amino-3-methylbenzyl)-7-(2-furyl)-3*H*- [1,2,3]triazolo [4,5-*d*]pyrimidine-5-amine (V2006)
CD39	Apyrase
CD73	Ecto-5′-nucleotidase
CF101	N^6-(3-Iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (IB-MECA)
CF102	2-Chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (Cl-IB-MECA)
CP-608,039	(2*S*, 3*S*, 4*R*, 5*R*)-3-Amino-5-{6-[5-chloro-2-(3-methylisoxazol-5-ylmethoxy)benzylamino]purin-9-yl-l-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide
CP-532,903	(2*S*, 3*S*, 4*R*, 5*R*)-3-Amino-5-{6-[2,5-dichlorobenzylamino]purin-9-yl-l-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide

CPFPX	8-Cyclopentyl-1-propyl-3-(3-fluoropropyl)-xanthine
CVT-3146	1-[6-Amino-9-[(2*R*, 3*R*, 4*S*, 5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]purin-2-yl]-*N*-methylpyrazole-4-carboxamide
CVT-6883	3-Ethyl-1-propyl-8-[1-(3-trifluoromethylbenzyl)-1*H*-pyrazol-4-yl]-3,7-dihydropurine-2,6-dione
EL	Extracellular loop
ENT	Equilibrative nucleoside transporter
E-NTPDase	Ectonucleoside triphosphate diphosphohydrolase
ERK	Extracellular receptor signal-induced kinase
FK 453	(+)-(*R*)-(1-(*E*)-3-(2-Phenylpyrazolo(1,5-*a*)pyridin-3-yl)acryl)-2-piperidine ethanol
FR194921	2-(1-Methyl-4-piperidinyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone
GPCRs	G protein-coupled receptors
GR79236	N^6-[(1*S*, 2*S*)-2-Hydroxycyclopentyl]adenosine
GRKs	G-protein-coupled receptor kinases
IL	Intracellular loop
KW3902	8-(Noradamantan-3-yl)-1,3-dipropylxanthine
KW6002	8-[(*E*)-2-(3,4-Dimethoxyphenyl)vinyl]-1,3-diethyl-7-methylpurine-2,6-dione
L-97-1	3-[2-(4-Aminophenyl)-ethyl]-8-benzyl-7-{2-ethyl-(2-hydroxy-ethyl)-amino]-ethyl}-1-propyl-3,7-dihydro-purine-2,6-dione
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinases
MRE0094	2-[2-(4-Chlorophenyl)ethoxy]adenosine
MRE-0470	2-[{Cyclohexylmethylene}hydrazino]adenosine (WRC-0470, binodenoson)
MRS5147	(1′*R*, 2′*R*, 3′*S*, 4′*R*, 5′*S*)-4′-[2-Chloro-6-(3-bromobenzylamino)-purine]-2′, 3′-*O*-dihydroxybicyclo-[3.1.0]hexane
N-0861	(±)-N^6-Endonorbornan-2-yl-9-methyladenine
NNC-21-0136	2-Chloro-N^6-[(*R*)-[(2-benzothiazolyl)thio]-2-propyl]-adenosine
OT-7999	5-*N*-Butyl-8-(4-trifluoromethylphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine
PET	Positron emission tomography
PI3K	Phosphoinositide-3 kinase
T-62	(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)-(4-chlorophenyl)-methanone
SLV-320	4-[(2-Phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]-*trans*-cyclohexanol
SDZ WAG94	N^6-Cyclohexyl-2′-*O*-methyl-adenosine
TM	Transmembrane helix
VER6947	2-Amino-*N*-benzyl-6-(furan-2-yl)-9*H*-purine-9-carboxamide
VER7835	2-Amino-6-(furan-2-yl)-*N*-(thiophen-2-ylmethyl)-9*H*-purine-9-carboxamide

V2006	see BIIB014
WRC-0571	8-(*N*-Methylisopropyl)amino-N^6-(5′-endohydroxy-endonorbornan-2-yl-9-methyladenine
ZM241385	4-2-[7-Amino-2-(2-furyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-yl-amino]ethylphenol

1 Introduction

Extracellular adenosine acts as a cytoprotective modulator, under both physiological and pathophysiological conditions, in response to stress to an organ or tissue (Fredholm et al. 2001; Haskó et al. 2008; Jacobson and Gao 2006). This protective response might take the form of increased blood supply (vasodilation or angiogenesis) (Ryzhov et al. 2008), ischemic preconditioning (in the heart, brain, or skeletal muscle) (Akaiwa et al. 2006; Cohen and Downey 2008; Liang and Jacobson 1998; Zheng et al. 2007), and/or suppression of inflammation (activation and infiltration of inflammatory cells, production of cytokines and free radicals) (Chen et al. 2006b; Martin et al. 2006; Ohta and Sitkovsky 2001). Adenosine acts on cell surface receptors that are coupled to intracellular signaling cascades. There are four subtypes of G-protein-coupled receptors (GPCRs); i.e., four distinct sequences of adenosine receptors (ARs) termed A_1, A_{2A}, A_{2B}, and A_3 (Fig. 1). The second messengers associated with the ARs are historically defined with respect to the adenylate cyclase system (Fredholm and Jacobson 2009). The A_1 and A_3 receptors inhibit the production of cyclic AMP through coupling to G_i. The A_{2A} and A_{2B} subtypes are coupled to G_s or G_o to stimulate adenylate cyclase. Furthermore, the A_{2B} subtype, which has the lowest affinity ($K_i > 1\,\mu M$) of all the subtypes for native adenosine, is also coupled to G_q (Ryzhov et al. 2006). Adenosine has the highest affinity at the A_1 and A_{2A} ARs (K_i values in binding of 10–30 nM at the high affinity sites), and the affinity of adenosine at the A_3AR is intermediate (ca. 1 μM at the rat A_3AR) (Jacobson et al. 1995).

Effector mechanisms other than the adenylate cyclase and phospholipase C are associated with the stimulation of ARs. For example, adenosine action can activate phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinases (MAPKs), and extracellular receptor signal-induced kinase (ERK) (Schulte and Fredholm 2003). The indirect regulation by adenosine of MAPKs can have effects on differentiation, proliferation, and apoptosis (Che et al. 2007; Fredholm et al. 2001; Jacobson and Gao 2006; Schulte and Fredholm 2003). Thus, the A_3AR activates Akt to inhibit apoptosis. These actions may be initiated through the β, γ subunits of the G proteins, which can also lead to the coupling of ARs to ion channels. The influx of calcium ions or the efflux of potassium ions can be induced by the activation of the A_1AR. The arrestin pathway, which has the dual role of signal transmission and downregulation of the receptor, is also activated by ARs (Klaasse et al. 2008; Penn et al. 2001). The A_{2A}AR forms a tight complex with G_s by a process described as “restricted collision coupling” (Zezula and Freissmuth 2008). The A_{2A}AR also

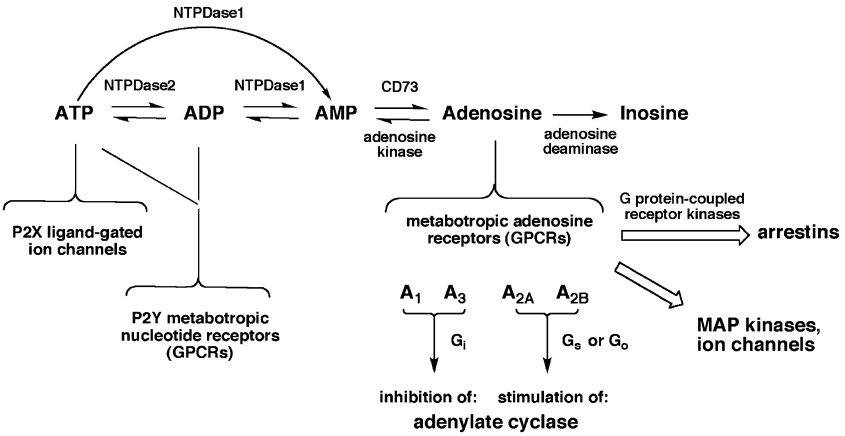

Fig. 1 Interconversion of extracellular adenine nucleotides and adenosine and their associated signaling pathways. These molecules may originate from intracellular sources. For example, adenosine may cross the plasma membrane through an equilibrative nucleoside transporter (ENT)1. The four subtypes of adenosine receptors (ARs) are grouped according to effects on adenylate cyclase. Inosine at micromolar concentrations also activates the A_3AR. Various extracellular nucleotides activate seven subtypes of P2X receptors and eight subtypes of P2Y, which are not specified here. The ARs and P2Y receptors are G-protein-coupled receptors (GPCRs), while the P2X receptors are ionotropic receptors. The ectonucleoside triphosphate diphosphohydrolases NTPDase1 and NTPDase2 are also known as CD39 (apyrase) and CD39L1, respectively. NTPDases3 and 8 (not shown) are also involved in breakdown of extracellular nucleotides

binds to additional "accessory" proteins, such as alpha-actinin, ARNO, USP4 and translin-associated protein-X (Zezula and Freissmuth 2008).

Adenosine suppresses various cytotoxic processes, such as cytokine-induced apoptosis. In the brain, both neuronal and glial cell functions are regulated by adenosine (Björklund et al. 2008; Fredholm et al. 2005). Adenosine acts as a local modulator of the action of various other neurotransmitters, including biogenic amines and excitatory amino acids. Adenosine attenuates the release of many stimulatory neurotransmitters and can counteract the excitotoxicity associated with excessive glutamate release in the brain. Adenosine can also modulate the interaction of neurotransmitters, such as dopamine, with their own receptors. In the periphery, adenosine has been shown to attenuate excessive inflammation, to promote wound healing, and to protect tissue against ischemic damage (Chen et al. 2006a; Haskó et al. 2008). In the cardiovascular system, adenosine promotes vasodilation, vascular integrity, and angiogenesis, and also counteracts the lethal effects of prolonged ischemia on cardiac myocytes and skeletal muscle (Cohen and Downey 2008; Zheng et al. 2007).

Therapeutic applications, both in the central nervous system and in the periphery, are being explored for selective AR agonists and antagonists. A large body of medicinal chemistry has been created around the four AR subtypes, such that selective agonists and antagonists are now available for each. These ligands have been used as pharmacological probes to introduce many new drug concepts. Mouse

strains in which an AR has been genetically deleted (each of the subtypes has now been deleted) have also been useful in developing novel drug concepts based on the modulation of ARs (Fredholm et al. 2005).

Adenosine itself is short-lived in the circulation, which has allowed its clinical use in the treatment of paroxysmal supraventricular tachycardia and in radionuclide myocardial perfusion imaging (Cerqueira 2006). The many selective and potent synthetic AR agonists, which are typically much longer lasting in the body than adenosine, have been slower to enter a clinical pathway than adenosine. Recently, the first such synthetic adenosine agonist, Lexiscan (regadenoson, CV Therapeutics, Palo Alto, CA, USA), an A_{2A}AR agonist, was approved for diagnostic use (Lieu et al. 2007).

Synthetic adenosine agonists have potential therapeutic applications based on their anti-inflammatory (A_{2A} and A_3) (Haskó et al. 2008; Ohta and Sitkovsky 2001), cardioprotective (preconditioning of the ischemic heart muscle by activation of the A_1 and A_3 ARs and its postconditioning by A_{2B}AR activation) (Cohen and Downey 2008), cerebroprotective (A_1 and A_3) (Chen et al. 2006a; Knutsen et al. 1999; von Lubitz et al. 1994), and antinociceptive (A_1) (Johansson et al. 2001) properties. Potent and selective AR antagonists display therapeutic potential as kidney protective (A_1) (Gottlieb et al. 2002), antifibrotic (A_{2A}) (Che et al. 2007), neuroprotective (A_{2A}) (Yu et al. 2004), antiasthmatic (A_{2B}) (Holgate 2005), and antiglaucoma (A_3) (Yang et al. 2005) agents. A_3AR agonists have been proposed for the treatment of a wide range of autoimmune inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel diseases, psoriasis, etc. (Guzman et al. 2006; Kolachala et al. 2008; Madi et al. 2007), and also for cardiac and brain ischemia. A_1AR agonists are useful in preclinical models of cardiac arrythmia and ischemia and in pain. Adenosine agonists are also of interest for the treatment of sleep disorders (Porkka-Heiskanen et al. 1997). Activation of the A_{2B}AR protects against vascular injury (Yang et al. 2008).

The alkylxanthines caffeine and theophylline are the prototypical antagonists of ARs, and their stimulant actions are produced primarily through blocking the depressant actions of adenosine through the A_1 and A_{2A} ARs (Fredholm and Jacobson 2009). Prior to the work of Rall, Daly, and other pioneers in the field, the stimulant actions of the alkylxanthines were thought to occur as a result of inhibition of phosphodiesterases. It is true that caffeine inhibits phosphodiesterases and has other actions, such as stimulation of calcium release, but these non-AR-mediated actions require higher concentrations of caffeine than are typically ingested in the human diet (Fredholm and Jacobson 2009).

The nonselective AR antagonist theophylline has been in use as an antiasthmatic drug (Holgate 2005), although its use is now limited as a result of side effects on the central nervous system and the renal system. Adenosine antagonists of various selectivities remain of interest as potential drugs for treating asthma (Wilson 2008). A large number of synthetic AR antagonists that are much more potent and selective than the prototypical alkylxanthines have been introduced, although none have yet been approved for clinical use. For example, AR antagonists have been proposed for neurodegenerative diseases (such as Parkinson's disease and Alzheimer's disease) (Schwarzschild et al. 2006), although a well-advanced A_{2A}AR antagonist

KW6002 (Istradefylline) (8-[(*E*)-2-(3,4-dimethoxyphenyl)vinyl]-1,3-diethyl-7-methylpurine-2,6-dione, Kyowa Hakko Kirin Co. Ltd, Tokyo, Japan) was recently denied FDA approval for the treatment of Parkinson's disease (LeWitt et al. 2008).

2 Sources and Fate of Extracellular Adenosine

Adenosine is not a classical neurotransmitter because it is not principally produced and released vesicularly in response to neuronal firing. Most tissues in the body and cells in culture release adenosine to the extracellular medium, from where it can feed back and act as an autocoid on the ARs present locally. The basal levels of extracellular adenosine have been estimated as roughly 100 nM in the heart and 20 nM in the brain, which would only partially activate the ARs present (Fredholm et al. 2005). In the case of severe ischemic stress, the levels can rapidly rise to the micromolar range, which would cause a more intense and generalized activation of the four subtypes of ARs. Nevertheless, it is thought that the exogenous administration of highly potent and selective AR agonists in such cases of severe ischemic challenge might still provide additional benefit beyond that offered by the endogenous adenosine generated (Jacobson and Gao 2006; Yan et al. 2003).

Extracellular adenosine may arise from intracellular adenosine or from the breakdown of the adenine nucleotides, such as adenosine triphosphate (ATP), outside the cell (Fig. 1). Adenosine, which is present in a higher concentration inside than outside the cell, does not freely diffuse across the cell membrane. There are nucleoside transporters, such as the equilibrative nucleoside transporter (ENT), ENT1, which bring it to the extracellular space. Extracellular nucleotides activate their own receptors, known as P2Y metabotropic and P2X inotropic receptors (Burnstock 2008). Extracellular nucleotides may also originate from cytosolic sources, including by vesicular release exocytosis, passage through channels, and cell lysis. Ectonucleotidases break down the adenine nucleotides in stages to produce free extracellular adenosine at the terminal step (Zimmermann 2000). For example, the extracellular enzyme ectonucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1) converts ATP and adenosine diphosphate (ADP) to adenosine monophosphate (AMP). A related ectonucleotidase, E-NTPDase2, primarily hydrolyzes 5′-triphosphates to 5′-diphosphates. The final and critical step, with respect to AR activation, of conversion of AMP to adenosine is carried out by ecto-5′-nucleotidase, also known as CD73. Overexpression of CD73 has been proposed to protect organs under stress by the formation of cytoprotective adenosine (Beldi et al. 2008). The adenosine produced extracellularly is also subject to metabolic breakdown by adenosine deaminase to produce inosine or (re)phosphorylation by adenosine kinase to produce AMP. Therefore, when an organ is under stress there is a highly complex and time-dependent interplay of the activation of many receptors in the same vicinity. In addition to the direct activation of ARs by selective agonists or their blockade by selective antagonists, inhibition of the metabolic or transport pathways surrounding adenosine is also being explored for therapeutic purposes (McGaraughty et al. 2005).

3 Adenosine Receptor Structure

The ARs, as GPCRs, share the structural motif of a single polypeptide chain forming seven transmembrane helices (TMs), with the N-terminus being extracellular and the C-terminus being cytosolic (Costanzi et al. 2007). These helices, consisting of 25–30 amino acid residues each, are connected by six loops, i.e., three intracellular (IL) and three extracellular (EL) loops. The extracellular regions contain sites for posttranslational modifications, such as glycosylation. The A_1 and A_3 ARs also contain sites for palmitoylation in the C-terminal domain. The A_{2A}AR has a long C-terminal segment of more than 120 amino acid residues, which is not required for coupling to G_s, but can serve as a binding site for "accessory" proteins (Zezula and Freissmuth 2008). The sequence identity between the human A_1 and A_3 ARs is 49%, and the human A_{2A} and A_{2B} ARs are 59% identical. Particular conserved residues point to specific functions. For example, there are two characteristic His residues in TMs 6 and 7 of the A_1, A_{2A}, and A_{2B} ARs. In the A_3AR, the His residue in TM6 is lacking but another His residue has appeared at a new location in TM3. All of these His residues have been indicated by mutagenesis to be important in the recognition and/or activation function of the receptor (Costanzi et al. 2007; Kim et al. 2003).

Recently, the human A_{2A}AR joined the shortlist of GPCRs for which an X-ray crystallographic structure has been determined (Jaakola et al. 2008). The reported structure (Fig. 2) contained a bound high-affinity antagonist ligand, ZM241385 (4-2-[7-amino-2-(2-furyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-yl-amino]ethylphenol), which is moderately selective for the A_{2A}AR. Prior to this dramatic step in bringing ARs into the age of structural biology, homology modeling of the ARs, based on a rhodopsin template, was the principal means of AR structural prediction and was useful in interpreting mutagenesis data. The modeling has defined two subregions within the putative agonist binding site (Costanzi et al. 2007; Kim et al. 2003). This putative binding site is located within the barrel or cleft created by five of the seven TMs (excluding TM1 and TM2), approximately one-third of the distance across the membrane from the exofacial side. The ribose moiety of adenosine binds in a hydrophilic region defined by TMs 3 and 7, and the adenine moiety binds in a largely hydrophobic region surrounded by TMs 5 and 6. Thus, the region of adenosine in the binding site is approximately the same as the position of the retinal in rhodopsin. Even the importance of the Lys residue in TM7 of rhodopsin that forms the covalent association (Schiff base) with retinal is conserved by analogy in the ARs, i.e., with a His residue that occurs at the same position (7.43) in all of the ARs. The His residue is predicted by molecular modeling to associate with the ribose moiety of adenosine. Features of the putative binding site of adenosine have been reviewed recently (Costanzi et al. 2007). Different labs have not been in agreement on the precise placement of the adenosine moiety when docked in the receptor. However, the major modeling publications in this area have zeroed in on the same limited region of the receptor structure for coordination of adenosine. One can consider the modeling approach to provide insights that are subject to refinement over time, as more is learned from mutagenesis studies and the modeling

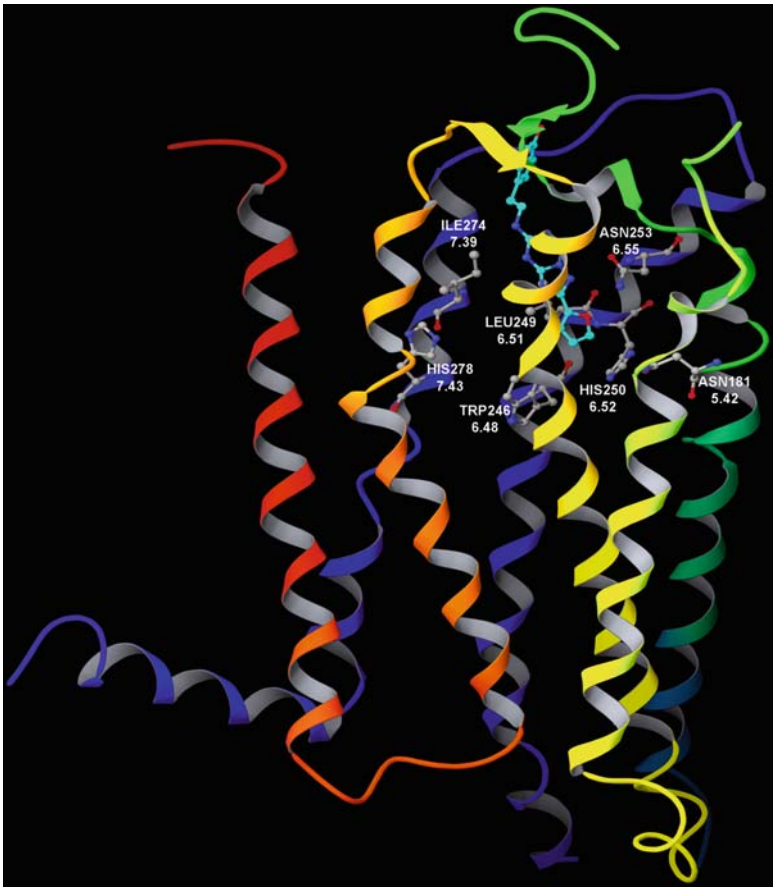

Fig. 2 X-ray crystallographic structure of the human A_{2A} adenosine receptor (AR), showing the bound antagonist ZM241385 (Jaakola et al. 2008). The structure of the A_{2A}AR is colored by region: N-terminus and transmembrane helical (TM) domain 1 in *orange*, TM2 in *ochre*, TM3 in *yellow*, TM4 in *green*, TM5 in *cyan*, TM6 in *blue*, TM7 and C-terminus in *purple*. The *p*-hydroxyphenylethyl moiety of the antagonist ligand points toward the exofacial side of the receptor

templates and computational methods are refined (Ivanov et al. 2009). Many amino acid residues predicted by molecular modeling to be involved in the coordination of antagonists by the A_{2A}AR were indeed in proximity to the bound ZM241385 in the X-ray structure, although the molecule was somewhat rotated from the orientation predicted in various docking models. These residues include Asn253 in TM6, which hydrogen bonds to the exocyclic NH of agonists and various antagonists in the AR models. The same residue was found to form a hydrogen bond with the exocyclic NH of ZM241385.

Dimerization has been proposed to occur between ARs, leading to homo- or heterodimers (Franco et al. 2006). Dimerization between ARs and other receptors has also been proposed; for example, A_1AR/D_1 dopamine receptor dimers

and $A_{2A}AR/D_2$ dopamine receptor dimers (Franco et al. 2006). Heterodimers of the A_1AR with either $P2Y_1$ or $P2Y_2$ nucleotide receptors or with metabotropic glutamate receptors have been detected (Prinster et al. 2005). The pharmacological properties of these heterodimers may differ dramatically from the properties of each monomer alone. For example, the $A_1AR/P2Y_1$ dimers have been characterized pharmacologically and were found to be inhibited by known nucleotide antagonists but not activated by known nucleotide agonists of the $P2Y_1$ receptor (Nakata et al. 2005). Dimers of A_{2A} adenosine/D_2 dopamine receptors are present in striatum and display a modified pharmacology relative to each of the individual subtypes. These receptor dimers are drug development targets for Parkinson's disease (Schwarzschild et al. 2006).

4 Regulation of Adenosine Receptors

Similar to the function and regulation of other GPCRs, both activation and desensitization of the ARs occur after agonist binding. Interaction of the activated ARs with the G proteins leads to second messenger generation and classical physiological responses. Interaction of the activated ARs with G protein-coupled receptor kinases (GRKs) leads to their phosphorylation. Downregulation of ARs should be considered in both the basic pharmacological studies and with respect to the possible therapeutic application of agonists. AR responses desensitize rapidly, and this phenomenon is associated with receptor downregulation, internalization and degradation. The internalization and desensitization of ARs has been reviewed recently (Klaasse et al. 2008). Mutagenesis has been applied to analyze the molecular basis for the differences in the kinetics of the desensitization response displayed by various AR subtypes. The most rapid downregulation among the AR subtypes is generally seen with the A_3AR, due to phosphorylation by GRKs. The $A_{2A}AR$ is only slowly desensitized and internalized as a result of agonist activation.

5 Adenosine Receptor Agonists and Antagonists in Preclinical and Clinical Trials

Potent and selective AR agonists and antagonists have been synthesized for all four AR subtypes, with selective $A_{2B}AR$ agonists being the most recently reported (Baraldi et al. 2009). Some of these ligands are selective for a single AR subtype, and others have mixed selectivity for several subtypes. Thus, numerous pharmacological tools for studying the ARs are available, and some of these compounds have advanced to clinical studies (Baraldi et al. 2008; Elzein and Zablocki 2008; Giorgi and Nieri 2008; Moro et al. 2006).

A general caveat in the design of selective agonists and antagonists is the frequent observation of a variation of affinity for a given compound at the same subtype in

different species. There are many examples of marked species dependence of ligand affinity at the ARs (Jacobson and Gao 2006; Yang et al. 2005). Therefore, caution must be used in generalizing the selectivity of a given compound from one species to another. In general, one must be cognizant of potential species differences for both AR agonists and antagonists.

5.1 Adenosine Receptor Agonists

Nearly all AR agonists reported are adenosine derivatives. A noteworthy exception is the class of pyridine-3,5-dicarbonitrile derivatives that fully activate ARs and that display varied selectivity at the AR subtypes (Beukers et al. 2004). One such compound is the A_{2B}AR-selective agonist BAY 60–6583 (2-[6-amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]pyridin-2-ylsulfanyl]acetamide) (Cohen and Downey 2008; Eckle et al. 2007). Another AR agonist of nonnucleoside structure is BAY 68–4986 (Capadenoson), which is a selective A_1AR agonist in clinical trials for the oral treatment of stable angina pectoris (Mittendorf and Wuppertal 2008). The structure–activity relationships (SARs) of adenosine derivatives as agonists of the ARs have been thoroughly probed (Jacobson and Gao 2006; Yan et al. 2003), and representative agonists are shown in Fig. 3. In general, substitution at the N6 position with certain alkyl, cycloalkyl, and arylalkyl groups increases selectivity for the A_1AR. Substitution with an N^6-benzyl group or substituted benzyl group increases selectivity for the A_3AR. Substitution at the 2 position, especially with ethers, secondary amines, and alkynes, often results in high selectivity for the A_{2A}AR.

All of the A_1AR agonists shown in Fig. 3 contain a characteristic N6 modification. The singly substituted A_1AR agonists NNC-21-0136 (2-chloro-N^6-[(*R*)-[(2-benzothiazolyl)thio]-2-propyl]-adenosine) and GR79236 (N^6-[(1*S*, 2*S*)-2-hydroxycyclopentyl]adenosine) (Merkel et al. 1995) and the doubly substituted selodenoson have been clinical candidates. NNC-21-0136 was the result of a program to develop CNS-selective AR agonists for use in treating stroke and other neurodegenerative conditions (Knutsen et al. 1999). A_1AR agonists are of interest for use in treating cardiac arrhythmias [for which adenosine itself, under the name Adenocard (Astellas Pharma, Inc., Tokyo, Japan), is in widespread use]. The A_1AR agonist SDZ WAG94 (2′-*O*-methyl-N^6-cyclohexyladenosine) was under consideration for treatment of diabetes (Ishikawa et al. 1998). The AR agonist of mixed selectivity AMP579 ([1*S*-[1α, 2β, 3β, 4α(S*)]]-4-[7-[[1-[(3-chlorothien-2-yl)methyl]propyl]amino]-3*H*-imidazo[4,5-b]pyrid-3-yl] *N*-ethyl-2,3-dihydroxycyclopentanecarboxamide) has cardioprotective properties (Cohen and Downey 2008). The 2-substituted A_{2A}AR agonists ATL-146e (4-{3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9*H*-purin-2-yl]-prop-2-ynyl}-cyclohexanecarboxylic acid methyl ester), binodenoson (2-[{cyclohexylmethylene}hydrazino]adenosine, MRE-0470 or WRC-0470), and MRE0094 (2-[2-(4-chlorophenyl)ethoxy]adenosine) have been cardiovascular clinical candidates (Awad et al. 2006; Desai et al. 2005; Udelson et al. 2004). Several of the A_{2A}AR agonists shown in Fig. 3 contain the 5′-uronamide

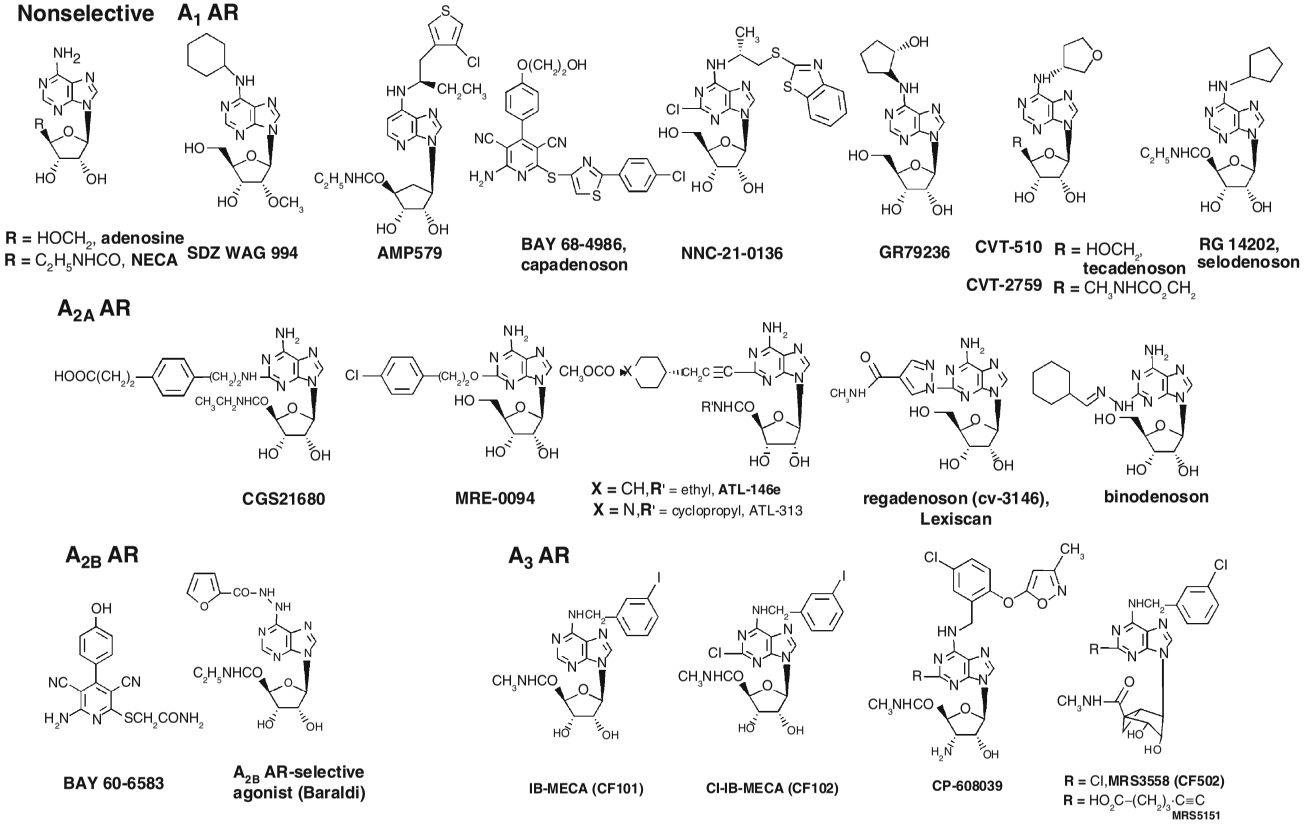

Fig. 3 Structures of selected adenosine receptor (AR) agonists. K_i values in binding are available in references (Baraldi et al. 2008; Jacobson and Gao 2006; Yan et al. 2003)

modification, characteristic of NECA; others have the adenosine-like CH_2OH group. Such agonists are of interest for use as vasodilatory agents in cardiac imaging [adenosine itself, under the name Adenoscan (Astellas Pharma, Inc., Tokyo, Japan), is in use for this purpose] and in suppressing inflammation (Cerqueira 2006). CVT-3146 (1-[6-amino-9-[(2*R*, 3*R*, 4*S*, 5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]purin-2-yl]-*N*-methylpyrazole-4-carboxamide, Lexiscan, regadenoson) is already approved for diagnostic imaging (Lieu et al. 2007).

All of the A_3AR agonists shown in Fig. 3 contain the NECA-like 5′-uronamide modification and have nanomolar affinity at the receptor. CP-608,039 ((2*S*, 3*S*, 4*R*, 5*R*)-3-amino-5-{6-[5-chloro-2-(3-methylisoxazol-5-ylmethoxy)benzylamino]purin-9-yl-l-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide) and its N^6-(2,5-dichlorobenzyl) analog CP-532,903 ((2*S*, 3*S*, 4*R*, 5*R*)-3-amino-5-{6-[2, 5-dichlorobenzylamino]purin- 9-yl- l- 4-hydroxytetrahydrofuran-2-carboxylic acid methylamide) (Wan et al. 2008) (not shown) are selective A_3 agonists that were developed for cardioprotection. CF101 (N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine, IB-MECA) is being studied by Can-Fite Biopharma (Petah-Tikva, Israel) for the treatment of rheumatoid arthritis (Phase IIb), dry eye syndrome (Phase II) and psoriasis (Phase II) (http://clinicaltrials.gov). Can-Fite Biopharma is also developing the A_3AR agonist CF102 (2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine, Cl-IB-MECA) for the treatment of liver conditions, including liver cancer, hepatitis infections and liver tissue regeneration (Bar-Yehuda et al. 2008; Madi et al. 2004). The North conformation of the ribose ring was found to be the preferred conformation at the A_3AR, which accounts for the high potency and selectivity of the rigid analog MRS3558 ((1′S, 2′R, 3′S, 4′R, 5′S)-4′-{2- chloro - 6- [(3-chlorophenylmethyl)amino]purin-9-yl}-1-(methylaminocarbonyl)bicyclo[3.1.0]hexane-2,3-diol) at the human and rat A_3ARs (Ochaion et al. 2008). The bicyclic ring constrains the ribose-like moiety in the desired conformation. The recent generation agonist in the same chemical series MRS5151 ((1′S, 2′R, 3′S, 4′R, 5′S)-4′-[6-(3-chlorobenzylamino)-2-(5-hydroxycarbonyl-1-pentynyl) -9-yl]-2′, 3′-dihydroxybicyclo [3.1.0] hexane-1′-carboxylic acid *N*-methylamide) is designed to be A_3AR selective in at least three different species, including mouse (Melman et al. 2008a).

Recently, macromolecular conjugates (e.g., dendrimers) of chemically functionalized AR agonists were introduced as potent polyvalent activators of the receptors that are qualitatively different in pharmacological characteristics in comparison to the monomeric agonists (Kim et al. 2008; Klutz et al. 2008). The feasibility of using dendrimer conjugates to bind to AR dimers was studied using a molecular modeling approach (Ivanov and Jacobson 2008).

5.2 Adenosine Receptor Antagonists

The newer and most selective AR antagonists are more chemically diverse than the classical 1,3-dialkylxanthines, which have been used pharmacologically as

antagonists of the A_1 and A_2 ARs. A range of AR antagonists and their synthetic methods were recently reviewed (Baraldi et al. 2008; Moro et al. 2006).

Purine AR antagonists, including both xanthine and adenine derivatives, have provided a wide range of receptor subtype selectivity, depending on the substitution (Fig. 4). In general, modifications of the xanthine scaffold at the 8 position with aryl or cycloalkyl groups has led to high affinity and selectivity for the A_1AR. Highly selective xanthine antagonists of the A_1AR (e.g., the epoxide derivative BG 9719 (1,3-dipropyl-8-(2-(5,6-epoxy)norbornyl)xanthine) and the more water soluble BG9928 (3-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)

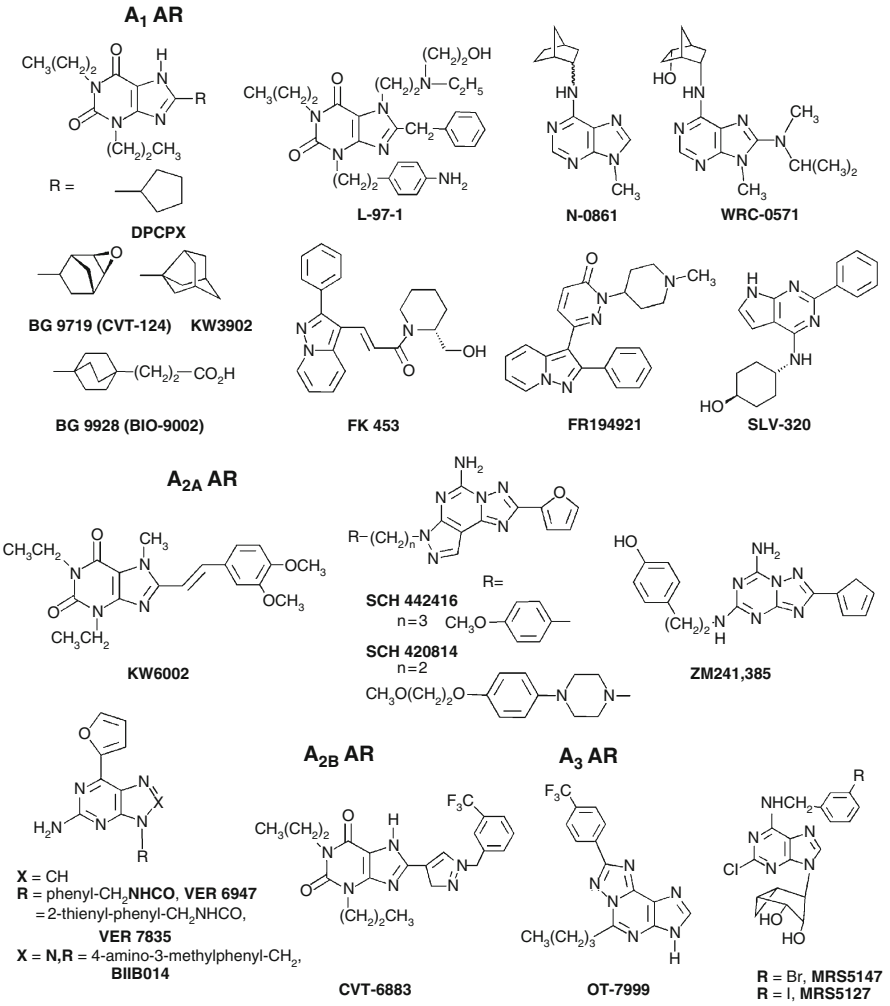

Fig. 4 Structures of selected adenosine receptor (AR) antagonists. K_i values in binding are available in references (Baraldi et al. 2008; Jacobson and Gao 2006)

-bicyclo[2.2.2]oct-1-yl]-propionic acid, Biogen Idec, Cambridge, MA, USA), as well as KW3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine, Merck and Co., Inc., Whitehouse Station, NJ, USA) have been (BG 9719) (Gottlieb et al. 2002) or are currently (BG9928 and KW3902) (Cotter et al. 2008; Dittrich et al. 2007; Givertz et al. 2007; Greenberg et al. 2007) in clinical trials for treatment of acute decompensated heart failure (ADHF) with renal impairment. In dogs, both BG9719 and BG9928 have high affinity for both the A_1AR and A_{2B}AR (Auchampach et al. 2004) with A_{2B}/A_1 ratios of 21 and 24, respectively (Doggrell 2005). The selectivity of BG 9928 for the human A_1AR compared to the human A_{2B}AR is 12 (Kiesman et al. 2006). The 8-cyclopentyl derivative DPCPX (8-cyclopentyl-1,3-dipropylxanthine), also known as CPX, which is selective for the A_1AR in the rat with nanomolar affinity but less selective at the human AR subtypes, has been in clinical trials for cystic fibrosis through a non-AR-related mechanism (Arispe et al. 1998). The highly selective A_1AR antagonist L-97-1 (3-[2-(4-aminophenyl)-ethyl]-8-benzyl-7-{2-ethyl - (2-hydroxy-ethyl)-amino]-ethyl}-1-propyl-3,7-dihydro-purine-2,6-dione, Endacea Inc., Research Triangle Park, NC, USA) is water soluble and in late preclinical development for the treatment of asthma (Wilson 2008). As in the cases of DPCPX, BG 9719, N-0861 ((±)-N^6-endonorbornan-2-yl-9-methyladenine), and others, a persistent problem in the development of A_1AR antagonists is low aqueous solubility, e.g., high lipophilicity, corresponding low water solubility, and low bioavailability (Hess 2001); thus, A_1AR antagonists, e.g., BG 9928 and L-97-1, with good water solubility are preferable clinical candidates. Moreover, a persistent problem in the use of xanthine derivatives as AR antagonists is their interaction at the A_{2B}AR. Modification of xanthines at the 8 position with certain aryl groups has given rise to preclinical candidates that are selective for the A_{2B}AR (e.g., CVT-6883, 3-ethyl-1-propyl-8-[1-(3-trifluoromethylbenzyl)-1H-pyrazol-4-yl]-3,7-dihydropurine-2,6-dione, CV Therapeutics, Palo Alto, CA, USA) (Mustafa et al. 2007). Use of the adenine derivatives WRC-0571 (8-(*N*-methylisopropyl)amino-N^6-(5′-endohydroxy-endonorbornan-2-yl-9-methyladenine) as an inverse agonist at the A_1AR provides A_1AR selective antagonism without blocking the A_{2B}AR (Martin et al. 1996). Nonxanthine antagonists of the A_1AR have also been shown to have high receptor subtype selectivity, e.g., FK453 (Terai et al. 1995) and SLV 320 (4-[(2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]-trans-cyclohexanol, Solvay Pharmaceuticals SA, Brussels, Belgium) (Hocher et al. 2008). Moreover, various nonxanthine A_1AR antagonists have been or are currently being explored for clinical applications (Jacobson and Gao 2006). For example, SLV 320 is in clinical trials as an intravenous treatment for ADHF with renal impairment (http://clinicaltrials.gov).

Modification of xanthines at the 8 position with alkenes (specifically styryl groups) has led to selectivity for the A_{2A}AR. Such derivatives include the A_{2A}AR antagonist KW6002 (istradefylline), which has been in clinical trials. Some 8-styrylxanthine derivatives, such as CSC (8-(3-chlorostyryl)caffeine), have been discovered to inhibit monoamine oxidase-B, as well as the A_{2A}AR (Vlok et al. 2006). The triazolotriazine ZM241385 and the pyrazolotriazolopyrimidine

SCH 442416 (5-amino-7-(3-(4-methoxy)phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) are highly potent A_{2A}AR antagonists (Moresco et al. 2005; Palmer et al. 1996). ZM241385 also binds to the human A_{2B}AR with moderate affinity, and has been used as a radioligand at that subtype (Ji and Jacobson 1999). SCH 442416 displays $>$ 23, 000-fold selectivity for the human A_{2A}AR (K_i 0.048 nM) in comparison to human A_1AR and an $IC_{50} > 10\,\mu M$ at the A_{2B} and A_3 ARs. A_{2A}AR antagonists, such as the xanthine KW6002 and the nonxanthines SCH 442416, VER 6947 (2-amino-*N*-benzyl-6-(furan-2-yl)-9*H*-purine-9-carboxamide), and VER 7835 (2-amino-6-(furan-2-yl)-*N*-(thiophen-2-ylmethyl)-9*H*-purine-9-carboxamide), are of interest for use in treating Parkinson's disease (Gillespie et al. 2008; LeWitt et al. 2008; Schwarzschild et al. 2006). The A_{2A}AR antagonist BIIB014 (V2006) has begun Phase II clinical trials (Biogen Idec, Cambridge, MA, USA, in partnership with Vernalis, Cambridge, UK) for Parkinson's disease (Jordan 2008).

Cyclized derivatives of xanthines, such as PSB-11 (8-ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*imidazo[2.1-*i*]purin-5-one), are A_3AR-selective, and similar compounds have been explored by Kyowa Hakko. Selective A_3AR antagonists, such as the heterocyclic derivatives OT-7999 (5-*n*-butyl-8-(4-trifluoromethylphenyl)-3*H*-[1,2,4]triazolo-[5,1-*i*]purine), are being studied for the treatment of glaucoma (Okamura et al. 2004), and other such antagonists are under consideration for treatment of cancer, stroke, and inflammation (Gessi et al. 2008; Jacobson and Gao 2006). MRS5147 ((1′*R*, 2′*R*, 3′*S*, 4′*R*, 5′*S*)-4′-[2-chloro-6-(3-bromobenzylamino)-purine]-2′, 3′-*O*-dihydroxybicyclo-[3.1.0]hexane) and its 3-iodo analog MRS5127 are highly selective A_3AR antagonists in both human and rat, based on a conformationally constrained ribose-like ring that is truncated at the 5′ position (Melman et al. 2008b). No selective A_3AR antagonists have yet reached human trials. However, an antagonist of mixed A_{2B}/A_3AR selectivity in the class of 5-heterocycle-substituted aminothiazoles from Novartis (Horsham, UK), QAF 805 (Press et al. 2005), was in a Phase Ib clinical trial for the treatment of asthma. This antagonist failed to decrease sensitivity to the bronchoconstrictive effects of AMP in asthmatics (Pascoe et al. 2007).

5.3 Radioligands for In Vivo Imaging

With the established relevance of ARs to human disease states, it has been deemed useful to develop high-affinity imaging ligands for these receptors, for eventual diagnostic use in the CNS and in the periphery. Ligands for in vivo positron emission tomographic (PET) imaging of A_1, A_{2A}, and A_3 ARs have been developed. For example, the xanthine [^{18}F]CPFPX (8-cyclopentyl-1-propyl-3-(3-fluoropropyl)-xanthine, similar in structure to DPCPX) and the nonxanthine [^{11}C]FR194921 (2-(1-methyl-4-piperidinyl)-6-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)-3(2*H*)-pyridazinone) have been developed as centrally-active PET tracers for imaging of the A_1AR in the brain (Bauer et al. 2005). The first PET

ligand for the $A_{2A}AR$ was [7-methyl-^{11}C]-(*E*)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]TMSX) (Ishiwata et al. 2000). This is a caffeine analog related to the series of KW6002, introduced by the Kyowa Hakko. 5-Amino-7-(3-(4-[^{11}C]methoxy)phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine ([^{11}C]SCH442416) has recently been explored as a PET agent in the noninvasive in vivo imaging of the human $A_{2A}AR$ (Moresco et al. 2005). [^{11}C]SCH442416 displays an extremely high affinity at the human $A_{2A}AR$ (K_i 0.048 nM). Recently, an A_3AR PET ligand, [F-18]FE@SUPPY (5-(2-fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate), based on a series of pyridine A_3AR antagonists, was introduced (Wadsak et al. 2008). Several nucleoside derivatives that bind with nanomolar affinity at the A_3AR and that contain ^{76}Br for PET imaging were recently reported, including the antagonist MRS5147 (Kiesewetter et al. 2008).

6 Allosteric Modulation of Adenosine Receptors

In addition to directly acting AR agonists and antagonists, allosteric modulators of A_1 and A_3 ARs have been introduced (Gao et al. 2005). Allosteric modulators have advantages over the directly acting (orthosteric) receptor ligands in that they would magnify the effect of the native adenosine released in response to stress at a specific site or tissue and, in theory, would not induce a biological effect in the absence of an agonist. Various allosteric enhancers of the activation of ARs by agonists are under consideration as clinical candidates. The benzoylthiophenes, represented by PD-81,723 (Fig. 5), were the first AR allosteric modulators to be identified. A structurally related benzoylthiophene derivative known as T-62 ((2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)-(4-chlorophenyl)-methanone), which acts as a selective positive enhancer of the A_1AR, like PD-81,723 (2-amino-4,5-dimethyl-3-thienyl-[3-trifluoromethylphenyl]methanone), had progressed toward clinical trials for neuropathic pain (Li et al. 2004). LUF6000 (*N*-(3,4-dichlorophenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine) is a selective positive enhancer of the human A_3AR (Gao et al. 2008).

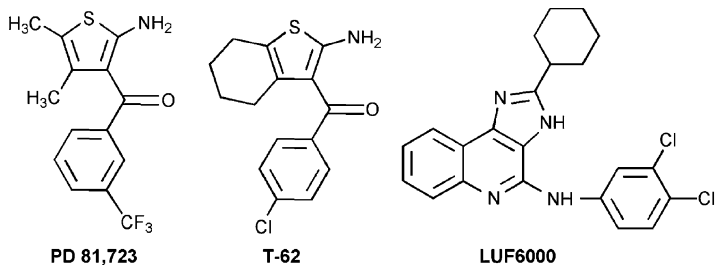

Fig. 5 Allosteric modulators of adenosine receptors (ARs)

7 Genetic Deletion of Adenosine Receptors

Deletion of each of the four AR subtypes has been carried out, and the resulting single-AR knockout (KO) mice are viable and not highly impaired in function (Fredholm et al. 2005; Yang et al. 2008). The pharmacological profile indicates that the analgesic effect of adenosine is mediated by the A_1AR, and analgesia is lost in mice in which the A_1AR has been genetically eliminated. Genetic KO of the A_1AR in mice removes the discriminative-stimulus effects but not the arousal effect of caffeine and increases anxiety and hyperalgesia. Study of A_{2A}AR KO mice reveals functional interaction between the spinal opioid receptors and peripheral ARs. A_1AR KO mice demonstrate a decreased thermal pain threshold, whereas A_{2A}AR null mice demonstrate an increased threshold to noxious heat stimulation, supporting an A_1AR-mediated inhibitory and an A_{2A}AR-mediated excitatory effect on pain transduction pathways. KO of the A_{2A}AR eliminates the arousal effect of caffeine. Genetic KO of the A_{2A}AR also suggests a link to increased anxiety and protected against damaging effects of ischemia and the striatal toxin 3-nitropropionic acid. Genetic KO of the A_3AR leads to increased neuronal damage in a model of carbon monoxide-induced brain injury. Neutrophils lacking A_3ARs show correct directionality but diminished speed of chemotaxis (Chen et al. 2006b). Although studies on A_{2B}AR KO mice have been reported (Yang et al. 2008), the importance of A_{2B}AR in the brain still awaits future investigation.

8 Conclusions

In conclusion, adenosine is released in response to organ stress or tissue damage and displays cytoprotective effects, in general, both in the brain and in the periphery. When excessive activity occurs in a given organ, adenosine acts as an endogenous quieting substance, to either reduce the energy demand or increase the energy supply to that organ. Nearly every cell type in the body expresses one or more of the AR subtypes, which indicates the central role of this feedback system in protecting organs and tissues and in tissue regeneration. Thus, a common theme to the therapeutic applications proposed for agonists is that adenosine acts as a cytoprotective modulator in response to stress to an organ or tissue.

Selective agonists and antagonists have been introduced and used to develop new therapeutic drug concepts. ARs are notable among the GPCR family in terms of the number and variety of agonist drug candidates that have been proposed. Thus, this has led to new experimental agents based on anti-inflammatory (A_{2A} and A_3), cardioprotective (preconditioning by A_1 and A_3 and postconditioning by A_{2B}), cerebroprotective (A_1 and A_3), and antinociceptive (A_1) effects. Potent and selective AR antagonists display therapeutic potential as kidney-protective (A_1), antifibrotic (A_{2A}), neuroprotective (A_{2A}), and antiglaucoma (A_3) agents. Adenosine agonists for cardiac imaging and positron-emitting adenosine antagonists are in development for diagnostic use. Allosteric modulation of A_1 and A_3 ARs has been demonstrated.

In addition to selective agonists/antagonists, mouse strains in which an AR has been genetically deleted have been useful in developing novel drug concepts based on modulation of ARs.

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