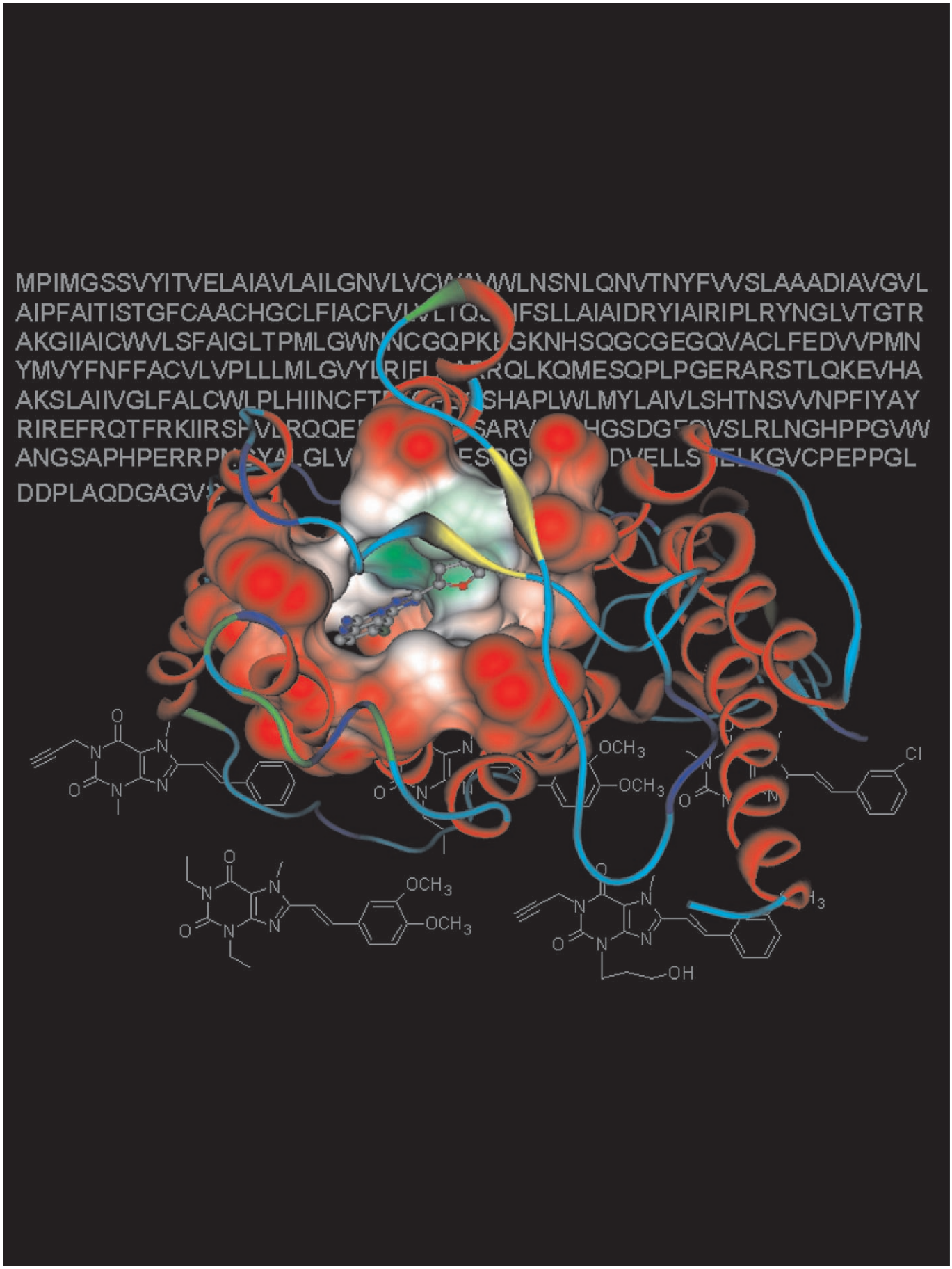

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Highlights on the Development of A_{2A} Adenosine Receptor Agonists and Antagonists

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Although significant progress has been made in the past few decades demonstrating that adenosine modulates a variety of physiological and pathophysiological processes through the interaction with four subtypes of a family of cell-surface G-protein-coupled receptors, clinical evaluation of some adenosine receptor ligands has been discontinued. Major problems include side effects due to the wide distribution of adenosine receptors, low brain penetration (which is important for the targeting of CNS diseases), short half-life of compounds, or a lack of effects, in some cases perhaps due to receptor desensitization or to low receptor density in the targeted tissue. Currently, three A_{2A} adenosine receptor agonists have begun phase III studies. Two of them are therapeutically evaluated as pharmacologic stress agents and the third proved to be effective in the treatment of acute spinal cord injury (SCI), while avoiding the adverse effects of steroid agents. On the other hand, the great interest in the field of A_{2A} adenosine receptor antagonists is related to their application in neurodegenerative disorders, in particular, Parkinson's disease, and some of them are currently in various stages of evaluation. This review presents an update of medicinal chemistry and molecular recognition of A_{2A} adenosine receptor agonists and antagonists, and stresses the strong need for more selective ligands at the A_{2A} human subtype.

1. Introduction

Adenosine (Ado, **1**, Figure 1), a naturally occurring nucleoside, is present in all tissues of mammalian organisms where it modulates a series of physiological processes through the interaction with four G-protein-coupled receptor subtypes, named A_1, A_{2A}, A_{2B}, and A_3. Ado receptors (ARs) have widespread tissue distribution and are often co-expressed in the same cell type.[1a,b]

This nucleoside was reported to have potent hypotensive and bradycardic activity by Drury and Szent-Gyorgyi in 1929[2] but the clinical usefulness of adenosine has been recognized only late in the 1980s in the United States.[3,4]

The first evidence that there may be more than one type of A_2 receptor mediating increased formation of cAMP in brain tissue was the finding that in membrane preparations from striatum, but not in membranes from other parts of the brain, adenosine derivatives could potently activate adenylyl cyclase.[5,6] Based on the ability of adenosine to stimulate adenylyl cyclase in brain slices, Daly and co-workers divided A_2 receptors into two subforms:[7] A_{2A}, with high affinity for adenosine (0.1–1.0 μM), and A_{2B}, with considerably lower affinity ($\geq$ 10 μM). This subclassification was strongly supported by an extensive characterization of the binding properties of [^{3}H]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]NECA), a nonselective adenosine agonist, and its displacement by several nonlabeled adenosine agonists and antagonists in striatal membranes.[8]

In 1989, Libert and co-workers cloned several orphan G-protein-coupled receptors from the dog thyroid, one of which was subsequently identified as an A_{2A} receptor.[9] The amino acid sequence of the dog A_{2A} receptor is 410 amino acids long, and contains a long intracellular carboxy-terminal part. A_{2A} receptors have thereafter been cloned from several species including rat,[10,11] human,[12] mouse,[13] and guinea pig.[14]

Until now, much of the information on the distribution of the A_{2A} receptors has been obtained by using pharmacological and immunohistochemical tools. In the CNS, adenosine A_{2A} receptors are found to be concentrated in the dopamine-rich regions irrespective of whether ligand binding or mRNA is used for localization. However, there is also considerable data suggesting the presence of functionally important A_{2A} receptors in glutamatergic and GABAergic pathways intrinsic to the hippocampus and in the cortex. The distribution of A_{2A} receptors is not restricted to the medium-sized spiny neurons in the basal ganglia. As shown by PCR analysis or by in situ and northern blot analysis, the A_{2A} receptor gene is also expressed in numerous other tissues, namely blood vessels, endothelial cells, several lymphoid cells, smooth muscle cells, and several neurons

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Gloria Cristalli received her degree in medicinal chemistry in 1974 from the University of Camerino (MC, Italy), where she held the position of postdoctoral fellow (1975–1978), charged professor (1979–1984), and associate professor (1985–1994) of medicinal chemistry. Between 1977 and 1978, she worked at Cambridge University with Professor G. V. R. Born. Between 1986 and 1987 she was visiting professor in the Thrombosis Research Center of Philadelphia Temple University (USA). In 1994 she became full professor at the University of Modena, and in 1997 she returned to the University of Camerino, where she is currently working as full professor of medicinal chemistry. The scientific interests of her group are focused on the synthesis of nucleosides, nucleotides, and heterocyclic compounds acting as purinergic receptor agonists and antagonists, platelet aggregation, enzyme inhibitors, and antiviral and antitumor drugs.

Barbara Cacciari received her degree in medicinal chemistry in 1990 at the University of Bologna, Italy and her PhD in medicinal chemistry in 1994 at the University of Ferrara (Italy) under the direction of Professor P. G. Baraldi. From 1992 to 1993 she worked in Professor Boger's group at The Scripps Research Institute in La Jolla, California. In 1995, she got her position as researcher in medicinal chemistry at the University of Ferrara, where she is currently working. Her main research interests are in the field of antitumoral agents related to anthramycin, distamycin and CC-1065, and also in the field of purinergic receptors, in particular, the development of agonists and antagonists of P1 and P2 receptors.

Diego Dal Ben graduated in medicinal chemistry in 2001 from the University of Padova, Italy and received his PhD in medicinal chemistry at the same university in 2005, after postdoctoral research at the school of pharmacy (University of London, UK) under the supervision of Professor Stephen Neidle and at the GlaxoSmithKline Research Center in Verona, Italy. Since June 2005, he has held the position of researcher (assistant professor) of medicinal chemistry at the Dipartimento di Scienze Chimiche, University of Camerino, Italy.

Catia Lambertucci graduated in medicinal chemistry in 1994 at the University of Camerino (MC, Italy). From 1994 to 1999 she worked on the chemistry of natural products, in particular, analogues of paclitaxel with fellowships from Tecnofarmaci S.C.p.A. Since January 2000 she has been working on the synthesis of ligands for purinergic receptors, and since December 2004, she has been a PhD student of medicinal chemistry at the Dipartimento di Scienze Chimiche, University of Camerino under the supervision of Professor Gloria Cristalli. During this period she spent some time at the Rega Institute of the University

Stefano Moro is associate professor of medicinal chemistry at the University of Padova, Italy. He received a BSc degree in medicinal chemistry and a PhD degree in physical organic chemistry from the University of Padova. He was a postdoctoral fellow at the National Institutes of Health in Bethesda (USA) and visiting professor at the ETH, Zürich (Switzerland). He is the chief of the Molecular Modeling Section at the University of Padova, Italy. His main interests concern the application of computational approaches to medicinal chemistry. He loves G-protein-coupled receptors and, in particular, adenosine receptors.

Giampiero Spalluto received his degree in chemistry and pharmaceutical technology in 1987 from the University of Ferrara, Italy. He obtained his PhD in organic chemistry from the University of Parma in 1992. Between 1995 and 1998 he worked at the University of Ferrara as assistant professor of medicinal chemistry. Since November 1998 he has held the position of associate professor of medicinal chemistry at the University of Trieste. His scientific interests have focused on the enantioselective synthesis of natural compounds and medicinal chemistry of ligands for adenosine receptor subtypes and antitumor agents.

Rosaria Volpini graduated in medicinal chemistry in 1990 from the University of Camerino (MC, Italy), and received her PhD in medicinal chemistry in 1994 after postdoctoral research at the University of Birmingham (UK) under the supervision of Professor R. T. Walker. She held the position of a postdoctoral fellow (1995–1998), researcher (assistant professor, 1999–2004), and associate professor (2005) of medicinal chemistry at the Dipartimento di Scienze Chimiche, University of Camerino, where she is currently working.

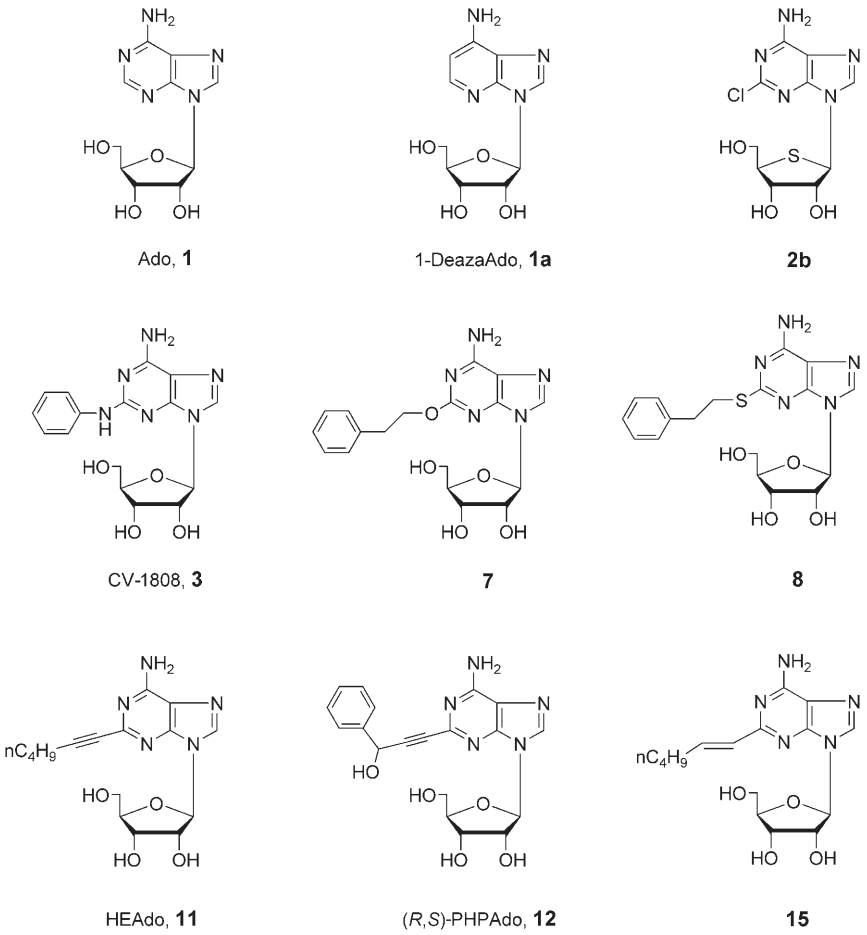


Figure 1. Adenosine derivatives.

of both the sympathetic and parasympathetic nervous systems. A wider outlook on the localization and physiological roles of A_{2A} receptors in different tissues and species is reported in recent pharmacological reviews.[1b,5b]

2. Structure–Activity Relationships of A_{2A} Adenosine Receptor Agonists

Modifications of the adenosine structure were first carried out following the observation that the activity of exogenous adenosine on the mammalian cardiovascular system is of short duration because of the rapid uptake of Ado into red blood cells and tissues,[15] its conversion to inosine by adenosine deaminase, and its phosphorylation by adenosine kinase.[16,17]

The first structure–activity studies pointed out that chemical modification of adenosine at the N6, 2-, or 5′-position increases the duration of coronary vasodilating activity, but it reduces potency with the exception of 2-chloroadenosine (2-ClAdo, **2**).[18]

Starting from these observations, a series of adenosine derivatives substituted at the 2-position with alkoxy, alkyl, amino, and mercapto groups were reported by Marumoto et al.[19]

2-Phenylaminoadenosine (CV-1808, **3**, Figure 1) proved to be the most potent compound in the series, also endowed with longer duration of effect than that of 2-chloroadenosine.

Afterwards, structure–activity relationships for adenosine analogues provided evidence for two major subclasses of ARs, referred to as A_1 and A_2 subtypes. Such structure–activity relationships have been derived in many cases from biochemical studies, either in binding assays with ligands for A_1 and A_2 receptors of the brain, or in adenylate cyclase assays with A_1 AR inhibitory to cyclase of fat cell membranes and with A_2 AR stimulatory to adenylate cyclase in brain slices or membranes, in human fibroblast, in membranes of human platelets, or in rat pheochromocytoma PC12 cells.[20]

Structure–activity relationships in physiological systems have been examined for A_2 receptor-mediated inhibition of platelet aggregation,[21–31] relaxant effects in smooth muscle,[21–23,31–34] antihypertensive effects,[35–41] and enhancement of coronary blood flow.[42–55]

Although such studies were carried out only at A_1 and A_{2A} receptor subtypes, they had defined important features of the recognition sites for AR agonists. The 2′- and 3′-hydroxy groups of the ribose moiety appeared essential for full agonist activity,[56–59] whereas the 5′-hydroxy group could be modified with retention of activity, as for example, by replacing -CH_2OH with -CH_3, -CH_2Cl, -CH_2SCH_3, or -CONHR.[8,60] The adenine ring could be substituted at the 2-position leading to A_2 AR selectivity and monosubstitution on the N6-amino group was tolerated, particularly in the case of A_1 AR.[43,49] Substitution at both C2 and N6 generally does not have additive effects on the A_2/A_1 affinity ratio.[61]

Combination of substitutions at the 2-position of adenosine and replacing of -CH_2OH with -$CONH_2$ usually increased A_2 versus A_1 selectivity.[21–23,27,30–33,39,40,44,62–65] On the other hand, concurrent N6 and C5′ modifications leading to N6-substituted N-alkyladenosine-5′-uronamides, did not result in compounds more selective than the monosubstituted derivatives.[42,66]

The nitrogen atoms in the 3- and 7- positions are required for high affinity of adenosine analogues at all subtypes.[25,65] 1-Deazaadenosine derivatives showed somewhat reduced AR affinity, the N6-substituted compounds being A_1 AR selective, as the A_1 agonist activity is retained to a larger extent than the A_2 affinity.[59,67,68]

In the following years, four human ARs, belonging to the superfamily of G-protein-coupled receptors, have been cloned[69,70] and classified as A_1, A_{2A}, A_{2B}, and A_3.[71]

Moreover, cloning and expression of recombinant ARs have been carried out in several other species including chick, cow, dog, pig, rat, mouse, rabbit, and sheep, demonstrating that there are species differences between the same subtype.[72,73] Since then few reports have been published, in which compounds were characterized at the four human AR subtypes.[74–79]

2.1. 2-Substituted adenosine derivatives

In the last 35 years a relevant number of C2 substituted derivatives of adenosine were synthesized and tested for their affinity at A_1 and A_{2A} receptors. From the whole series, which includes 2-amino-,[80] 2-hydrazino-,[32,45,81] 2-alkoxy-,[82] 2-alkylthio-,[83–85] and 2-alkynyl-derivatives,[37,38,77,79] the compounds showing the highest A_{2A} affinity bore a phenylethyl (or cyclohexylethyl) group directly linked to the heteroatom or triple bond (see Table 1 compounds **4**–**9**).

The alkynyl derivatives 2-phenylethynyladenosine (PEAdo, **10**), 2-(hexyn-1-yl)adenosine (HEAdo, **11**, Figure 1), (*R*,*S*)-2-phenylhydroxypropynyladenosine ((*R*,*S*)-PHPAdo, **12**, Figure 1), and the corresponding diastereomers **13** and **14** were recently tested in binding studies on A_1, A_{2A}, and A_3 rat membrane receptors (Cristalli et al., unpublished data) and on the four human recombinant receptor subtypes, stably transfected into Chinese hamster ovarian (CHO) cells (the potency at the A_{2B} receptor was measured using adenylate cyclase activity assays).[77] All the compounds showed A_{2A} affinity in the low nanomolar range and HEAdo proved to be also slightly A_{2A}-selective in rat membrane receptors ($A_1/A_{2A} \approx 20$ and $A_3/A_{2A} \approx 5$). The phenylhydroxypropynyl derivatives are, in general, very potent but rather unselective at both rat and human AR subtypes (Table 1). Partial reduction of the HEAdo triple bond led to *E* and *Z* alkenyl isomers **15** (Figure 1) and **16**, and full reduction of it gave 2-hexyladenosine (**17**).[23] These compounds were tested for their affinity at A_1 and A_{2A} receptors and the *trans* isomer **15** showed good A_{2A} affinity and moderate selectivity ($A_1/A_{2A} \approx 24$). The alkyl derivative **17** was inactive at both the A_1 and A_{2A} subtypes (Table 1).[23]

More recently, a broad screening of 2-alkoxyadenosine derivatives has been carried out by Gao et al. aimed mainly at defining the affinity and selectivity of these compounds at A_3 AR subtypes. However, these single substitutions at the 2-position, which were previously found to contribute to the affinity for the rat A_{2A} AR, were also demonstrated to be important for the affinity and selectivity at the human A_{2A} AR homologue.[86]

Other adenosine derivatives, with pyrazole or thiophene rings substituted at the 2-position (compounds **18**–**20**, Table 1) were found to be short acting functionally selective coronary vasodilators with good potency, but they possess low affinity for the A_{2A} AR ($K_i = 1122$ nM, 2895 nM, and 692 nM, respectively).[46]

2.2. Ribose ring and purine modified adenosine derivatives

The only ribose ring modification, which has been reported to improve A_{2A} AR affinity and reduce A_1 AR activity, was the isosteric substitution of the endocyclic oxygen by sulfur (compound **2b**, Figure 1). In fact, comparison of 2-chloroadenosine (**2**) and the thio-ribosyl analogue **2b** shows that the latter was 3.2-fold more potent than **2** at A_{2A} AR, whereas its A_1 AR affinity was diminished by 32-fold. On the other hand, compounds **2** and **2b** were of similar potency at A_3 AR.[58]

Recently the synthesis and biological activity as potential agonists for human A_{2A} receptors of adenosine derivatives containing an ethyl-substituted tetrazole moiety at the 4′-position of the ribose and an amino alcohol at the 2-position of the adenine core were reported.[87,88] The activity of these compounds has been evaluated in radioligand binding assays using the four cloned human ARs. The compounds have also been profiled in cAMP assays using human receptors expressed on transfected CHO cells, and in functional assays using rat aorta, guinea pig aorta, and guinea pig tracheal rings.[88]

Results of these sets of experiments show that substitution at the *para*-position of the phenyl ring at the 2-side chain by different groups greatly increases the binding affinity for the A_{2A} AR. At the same time, the tested substituted derivatives have reduced affinity for A_1 and A_3 receptors, thus remarkably improving the A_1/A_{2A} and A_3/A_{2A} selectivity. Among the tested adenosine derivatives, compound **21**, lacking the hydroxy group in the side chain was the most potent and selective in binding studies (see data in Table 1).

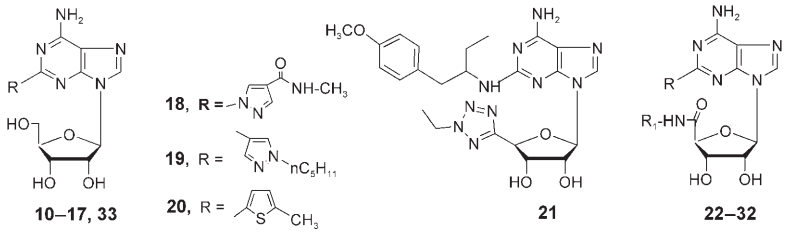
Among the purine modified nucleosides, 1-deazaadenosine derivatives showed, in general, reduced AR affinity, 1-deazaadenosine itself (**1a**, Figure 1) and its N6-substituted analogues being A_1 AR selective.[59,67,68] Conversely, 2-chloro-1-deazaadenosine (**2a**) showed an A_{2A} and A_3 affinity similar to that of the parent compound **2** (which is slightly A_1-selective), and a reduced A_1 AR activity, thus resulting selective for the A_{2A} AR (Table 1).[58,67]

2.3. 2-Substituted derivatives of NECA

Since the early eighties the 4′-uronic acid ethyl ester analogue of adenosine, NECA, (**22**, Figure 2) was reported to be a potent coronary dilator and hypotensive,[89] and a good inhibitor of platelet aggregation induced by ADP.[25]

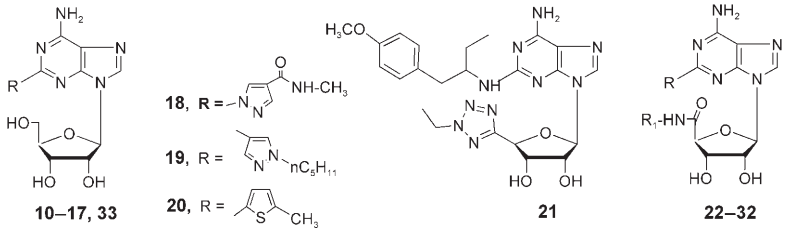
However, NECA showed little or no A_2-selectivity in either functional or binding studies (Table 1).[21,24,75] Hence, starting from the observation made by Bruns et al.[8] that 2-phenylaminoadenosine (CV 1808, **3**, Figure 1) was slightly A_2 versus A_1 selective, a series of 2-(arylalkylamino)-*N*-ethylcarboxyamidoadenosine was synthesized and evaluated for their A_1 and A_{2A} binding profile in rat brain membranes.[81] As in the case of arylalkylaminoadenosines, the phenylethylamino analogues of NECA **23** showed the highest rat A_{2A} AR affinity in the series and a greater than 2000-fold separation between A_2 (coronary vasodilation) and A_1 (negative chronotropic effect) receptor mediated events. Among them 2-{[4-(2-carboxyethyl)phenyl-

Table 1. Affinities of Ado derivatives in radioligand binding assays at rat and human A_1, A_{2A}, and A_3 ARs and effects on adenylate cyclase activity at human A_{2B} AR.



Compd	R	R_1	K_i (A_1)[a]	K_i (A_{2A})[a]	EC_{50} (A_{2B})[b]	K_i (A_3)[a]	Ref.[c]
			K_i or EC_{50} [nM]				
1a	H		115 r	2900 r			[67]
1-DeazaAdo[d]							
2	Cl		9.3 r	63 r	24000 r[66]	1890 r	[58]
2-ClAdo							
2a	Cl		226 r[67]	163 r[67]		2480[58]	
2-Cl-1-DeazaAdo[d]							
2b	Cl		300 r	20 r		1090 r	[58]
Thioribosyl analogue of **2**[d]							
3	NHC_6H_5		400 r	100 r			[80]
CV-1808							
4	$NH(CH_2)_2C_6H_5$		977 r	68 r			[80]
			530 h	62 h		310 h	[86]
5	$NH(CH_2)_2C_6H_{11}$		11700 r	22 r			[80]
6	$O(CH_2)_2C_6H_5$		130 r	17 r			[82]
			221 h	9.3 h	3490 h	54 h	[86]
7	$O(CH_2)_3C_6H_{11}$		2800 r	22 r			[82]
			1730 h	92 h		83 h	[86]
8	$S(CH_2)_2C_6H_5$			892 p			[84]
			550 r	49 r	>100 μM	64 r	[*unp.*]
			3700 h	590 h		1960 h	[86]
			99 h	85 h		289 h	[85]
9	$C\equiv C(CH_2)_2C_6H_5$		100 r	37 r			[38]
10	$C\equiv C{-}C_6H_5$		701 r	109 r			[38]
PEAdo			806 r	246 r	>100 μM h	28 r	[*unp.*]
			395 h	363 h		16 h	[77]
11	$C\equiv C(CH_2)_3CH_3$		98 r	2.2 r			[62]
HEAdo			111 r	5.2 r	≈100 μM h	24 r	[*unp.*]
			18 h	5.7 h		4.7 h	[77]
12	$C\equiv C{-}CH(OH)C_6H_5$		3.4 r	1.9 r			[38]
(*R,S*) PHPAdo			3.9 r	5.3 r	2400 h	0.98 r	[*unp.*]
			0.67 h	7.0 h		3.3 h	[77]
13	$C\equiv C{-}CH(OH)C_6H_5$		1.5 r	1.0 r		0.40 r	[*unp.*]
(*R*) PHPAdo			0.44 h	29 h	6200 h	5.0 h	[77]
14	$C\equiv C{-}CH(OH)C_6H_5$		2.5 r	1.6 r		24 r	[*unp.*]
(*S*) PHPAdo			0.67 h	1.8 h	920 h	1.4 h	[77]
15	(*E*) $CH{=}CH(CH_2)_3CH_3$		332 r	14 r			[23]
16	(*Z*) $CH{=}CH(CH_2)_3CH_3$		618 r	757 r			[23]
17	$(CH_2)_5CH_3$		>1 μM r	>10 μM r			[23]
18				1122 p			[46]
CVT-314							
19			5836 h	2895 h			[46]
20				692 p			[46]
21	(*S*-diastereomer)		356 h	1.0 h	2780 h[e]	100 h	[88]
22	H	C_2H_5	10 r[21]	7.8 r[21]		113 r[58]	
NECA			63 r	16 r	3100 h	10 h	[97]
			14 h	20 h	2400 h	6.2 h	[77]
22a[f]	H	C_2H_5	51 r	580 r[16]	16000 h[65]	703 r	[58]
1-DeazaNECA							
22b	H	CH_3	1100 r	330 r	45000 h	6.4 h	[97]
MECA							
22c	H	cC_3H_5	63 r	12 r	5300 h	108 h	[97]
NCPCA							
23	$NH(CH_2)_2C_6H_5$	C_2H_5	473 r	9.7 r			[24]
24	$NH(CH_2)_2$-*p*-$[(CH_2)_2CO_2H]C_6H_4$	C_2H_5	1400 r[81]	19 r[81]		584 r[58]	
CGS 21680			290 h	27 h	88800 h	67 h	[70]

Table 1. (Continued)



Compd	R	R_1	K_i (A_1)[a]	K_i (A_{2A})[a]	EC_{50} (A_{2B})[b]	K_i (A_3)[a]	Ref.[c]
			K_i or EC_{50} [nM]				
25	$S(CH_2)_2C_6H_5$	C_2H_5	>10 μM p	85 p			[84]
			380 r	15 r	> 100 μM	46 r	[*unp.*]
			189 h	24 h		86 h	[85]
26	$C\equiv C(CH_2)_3CH_3$	C_2H_5	130 r	2.2 r		26 r[58]	[21]
HENECA			160 r	1.0 r	6100 h	18 r	[*unp.*]
			60 h	6.4 h		2.4 h	[75, 77]
26a	$C\equiv C(CH_2)_3CH_3$	cC_5H_9	403 h	49 h	>100 μM h	16 h	[76]
26b	$C\equiv C(CH_2)_3CH_3$	$CH_2C_6H_5$	1700 h	720 h	>100 μM h	246 h	[76]
27	$C\equiv C–CH(OH)C_6H_5$	C_2H_5	2.5 r	0.9 r			[21, 31]
(*R*,*S*) PHPNECA			3.8 r	2.7 r	1100 h	7.7 r	[77]
			2.7 h	3.1 h		0.42 h	[75, 77]
27a	$C\equiv C–CH(OH)C_6H_5$	C_2H_5	5.9 r	2.6 r			[31]
(*R*) PHPNECA			2.7 r	16 r	2400 h	0.46 r	[*unp.*]
			1.9 h	39 h		5.5 h	[75, 77]
27b	$C\equiv C–CH(OH)C_6H_5$	C_2H_5	4.0 r	0.5 r			[31]
(*S*) PHPNECA			5.5 r	1.8 r	220 h	2.6 r	[*unp.*]
			2.1 h	2.0 h		0.75 h	[75, 77]
28	$C\equiv C–C_6H_5$	C_2H_5	698 r	120 r			[29]
PENECA			1000 r	267 r	>100 μM h	728 r	[*unp.*]
			560 h	620 h		6.2 h	[75, 77]
29	$C\equiv C–CH_2$-*p*-$(COOH)C_6H_{10}$	C_2H_5	292 r	20 r			[94]
30	$C\equiv C–CH_2$-*p*-$(COOCH_3)C_6H_{10}$	C_2H_5	28 r	5.5 r			[94]
ATD-146e or BMS-068645							
31	$C\equiv C–CH_2$-*p*-$(CH_2OAc)C_6H_{10}$	C_2H_5	14 r	6.2 r			[94]
32	$C\equiv C–CH_2$-*p*-$(CH_2OH)C_6H_{10}$	C_2H_5	0.84 r	0.48 r			[94]
33	$NHN{=}CHC_6H_{11}$		48000 h	270 h	>100 μM h	900 h	[187]
WCR-0470 or MRE-0470							

[a] Binding data from different laboratories at rat (r), human (h) or pig (p) A_1, A_{2A} and A_3 ARs, expressed as K_i [nM]. [b] Measurement of receptor-stimulated adenylate cyclase activity in CHO cells stably transfected with human recombinant A_{2B} AR, expressed as EC_{50} [nM]. [c] References in this column refer to the whole row; when reported values from different references appear in the same row, they are listed close to the data ([*unp.*] = unpublished data). [d] Compounds **1a**, **2a**, and **2b** do not have the NECA structure; see Figure 1. [e] Binding data. [f] Compound **22a** does not have the NECA structure; see Figure 2.

ethyl]amino}-5′-*N*-ethylcarboxamideadenosine (CGS 21680, **24**, Figure 2) was selected for extensive biological evaluation[40] and tritiation for use as an A_{2A}-selective ligand for receptor binding.[41]

Recently, some 2-arylalkylthio analogues of NECA were synthesized and tested in radioligand binding studies. Also in this series, the 2-phenylethylthio derivative **25** (Figure 2) proved to be the most potent and selective agonist at the pig and rat A_{2A} AR.[85]

The same observations that led to the synthesis and testing of 2-alkynyladenosines drove the synthesis and evaluation of 2-alkynyl derivatives of NECA, bearing from five to eight linear carbon atom chains.[62] Affinities for A_1 and A_{2A} ARs were determined in rat membranes by using radioligand competition assays. All compounds showed good A_1 and A_{2A} affinities (K_i in the nanomolar range) and moderate A_{2A} selectivity.

Further studies were carried out by Monopoli et al., using in vitro and in vivo models, to characterize the pharmacological profile of the selected 2-hexynyl derivative of NECA (HENECA, **26**, Table 1 and Figure 2).[27] In addition to the binding studies on both rat and bovine brain, which confirmed the moderate A_{2A} versus A_1 selectivity, HENECA administered intraperitoneally in conscious spontaneously hypertensive rats caused a dose-dependent reduction in systolic blood pressure with minimal reflex tachycardia. It also appeared to penetrate the central nervous system as shown by its protection against pentylenetetrazole-induced convulsions in rats.[27]

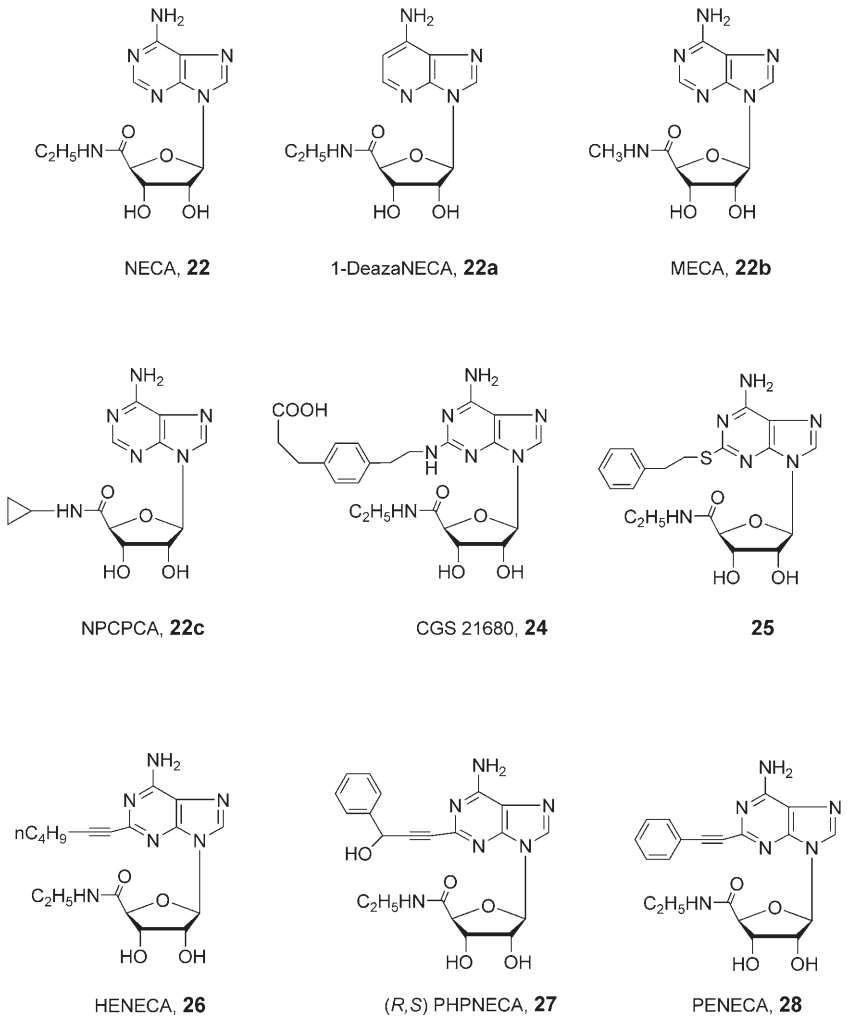


Figure 2. 5′-*N*-Alkylcarboxamidoadenosine derivatives.

In another study, carried out by Morelli et al., administration of HENECA i.p. induced Fos-like immunoreactivity in the rat nucleus accumbens shell, lateral septal nucleus, and dorso-medial striatum, similar to that induced by atypical neuroleptics.[90] The therapeutic potential of HENECA for the treatment of cardiovascular and psychotic diseases led to the synthesis of a series of 2-alkynyl, 2-cycloalkynyl, 2-aralkynyl, and 2-heteroaralkynyl derivatives of NECA, that were tested in binding and functional assays (Table 1) to assess their potency for the A_{2A} compared to A_1 ARs.[21,22]

The presence of an α-hydroxy group in the alkynyl chain of NECA derivatives accounts for the high A_2 agonist potency, leading to compound **27**, (*R*,*S*) PHPNECA (Figure 2), endowed with subnanomolar affinity in binding studies (K_i $A_1 = 2.5$ nM and K_i $A_{2A} = 0.9$ nM) and 16-fold more potent than NECA as platelet aggregation inhibitor. However, these analogues also possess good A_1 receptor affinity resulting in low A_{2A} selectivity.

As this compound bears a chiral carbon in the side chain, diastereomer separation was accomplished to obtain compounds **27a** and **27b**. Binding assays in rat membranes showed that the (*S*)-diastereomer **27b** is about fivefold more potent and selective than the (*R*)-diastereomer **27a** as agonist of the A_{2A} receptor subtype ((*S*)-PHPNECA, K_i $A_{2A} = 0.5$ nM; (*R*)-PHPNECA, K_i $A_{2A} = 2.6$ nM, Table 1).[31]

In 1998, cloning of the four human AR subtypes and their stable transfection into CHO cells allowed for comparative studies in a similar cellular background, using binding studies (A_1, A_{2A}, A_3) or adenylate cyclase activity assays (A_{2B}).[70] These transfected CHO cells were used for a screening of some nucleosides, previously considered A_{2A} AR selective (Table 1).

According to this screening, none of the prototypical AR agonists exhibits high affinity and selectivity for the human A_{2A} AR subtype. Both NECA (**22**) and CGS 21680 (**24**), which are available as radioligands for this subtype, have lower affinity at the human than at the rat receptor, whereas HENECA (**26**) showed high affinity also at human A_{2A} and A_3 receptors with a 10-fold and 25-fold selectivity over the A_1 subtype, respectively (K_i $A_1 =$ 60.0 nM, K_i $A_{2A} = 6.4$ nM, and K_i $A_3 = 2.4$ nM). The potency for A_{2B} receptor is comparable with that of NECA (HENECA: EC_{50} $A_{2B} =$ 6.1 μM versus NECA EC_{50} $A_{2B} = 2.4$ μM).[74]

It was also confirmed that (*R*,*S*) PHPNECA (**27**) is a highly potent, nonselective agonist at A_1, A_{2A}, and A_3 subtypes with a K_i in the low nanomolar range at the three subtypes, (Table 1 and Figure 1). In the A_{2B} functional assay it has been found that (*R*,*S*)-PHPNECA (EC_{50} $A_{2B} = 1.1$ μM) is 2-fold more potent than NECA. This was the first report in which the introduction of a bulky group in the 2-position of NECA led to a compound that showed good potency at the human A_{2B} subtype.[75,91,92] In fact, CGS 21680 (**24**) resulted in an agonist about 100-fold weaker than (*R*,*S*)-PHPNECA at the same subtype, with EC_{50} $A_{2B} = 89$ μM.[74] The (*S*)-diastereomer (*S*)-PHPNECA (**27b**) was the most potent A_{2B} agonist reported to date with an EC_{50} in the nanomolar range ($EC_{50} = 220$ nM, Table 1).

On the other hand, the substituent linked to the triple bond allowed modulation of selectivity at A_3 receptor. In fact, the presence of a phenyl ring conjugated to the triple bond is detrimental for all the subtypes with the exception of the A_3 receptor; hence, PENECA (**28**, Figure 2) showed high potency and good selectivity for the A_3 subtype.[75,93]

In conclusion, the affinity at the human and rat A_{2A} receptor is as follows PHPNECA ≥ HENECA > NECA > CGS 21680 > PENECA, but none of these compounds is at the same time selective towards both A_1 and A_3 receptor subtypes. Thus, so far, no satisfactorily A_{2A}-selective AR agonists are available.

In 2001 four new compounds (**29**–**32**, Table 1), whose structures are similar to that of 2-alkynyl derivatives of NECA previously well characterized by other authors,[20] were evaluated only by competitive binding assays employing the A_{2A} receptors in rat striatal membranes and A_1 receptors of rat cortex. Hence, the A_{2A} versus A_1 selectivity was evaluated, but no A_{2A} versus A_3 selectivity was reported.[94] As some 2-alkynyl derivatives of NECA had been previously reported to behave as potent A_3 agonists, affinity at this receptor should be measured before claiming selectivity for the reported compounds.

2.4. Ribose and purine modified NECA derivatives

Few modifications of ribose moiety of NECA have been reported in the last years.[76,95–97] Substitution of the ethyl group of N-alkylcarboxamido function by a cyclopropyl group seems to be the only well tolerated by the A_{2A} rat receptor (see compounds **22b** and **22c** in Figure 1 and Table 1, K_i A_{2A} = 330 nM and 12 nM, respectively).[97] On the other hand, replacing the same ethyl substituent in the 5′ position of HENECA with a cyclopentyl or benzyl group brought about a relevant decrease of affinity at all the receptor subtypes (see compounds **26a** and **26b** in Table 1, K_i A_{2A} = 49 nM and 720 nM, respectively).[76] Some deoxy and dideoxy derivatives of 5′-*N*-methylcarboxamidoadenosine (MECA, **22b**, Figure 2) have been described and the general effect of these modifications is a reduced affinity at all receptor subtypes.[95,96]

However, removing of the 3′-hydroxy group seems to be better tolerated by the A_{2A} AR than removing the corresponding group in the 2′-position (Cristalli et al., unpublished data).

The only purine modified analogue of NECA, which has been synthesized and tested so far, is 1-deazaNECA (**22a**, Figure 2 and Table 1).[58,68] As in the case of the other 1-deazaadenosine analogues, the affinity of **22a** at all ARs is reduced in comparison to that of the parent compound NECA **22**. However, in contrast to 2-chloro-1-deazaadenosine (**2a**), which was the only 1-deaza analogue showing slight A_{2A}-selectivity, the potency of **22a** at A_1, A_{2B}, and A_3 ARs is diminished by a factor of about 5, whereas that at the A_{2A} subtype is about 60-fold lower than that of NECA. Hence, 1-deazaNECA displayed a moderate A_{2A} AR-selectivity.

3. Structure–Activity Relationships of A_{2A} Adenosine Receptor Antagonists

In the last years, A_{2A} AR antagonists proved to be an attractive pharmacological tool considering their neuroprotective functions. In particular A_{2A} ARs are located in the striatum and are co-expressed with the dopamine D_2 receptors. It has been widely reported that blockade of A_{2A} ARs contrasts the catalepsy induced by dopamine receptors or by dopamine depletion, suggesting that A_{2A} AR antagonists could represent an alternative approach for the treatment of Parkinson's disease.[1b,98–101] In addition, A_{2A} AR antagonists seem to protect against cellular death induced by ischemia.[102–104] These pharmacological results strongly support the great efforts for developing potent and selective A_{2A} AR antagonists. In this field, several heterocyclic compounds have been studied as AR antagonists which could be classified into two families: a) xanthine derivatives and b) nitrogen polyheterocyclic derivatives.[105,106]

3.1. Xanthine derivatives

Considering that natural xanthines (for example, caffeine, theophylline) bind all the AR subtypes with the exception of the rat A_3 AR,[107] without any selectivity at micromolar concentrations (range 15–80 μM), this nucleus has represented the starting point for the discovery of AR antagonists. In particular a large number of modifications at the 1, 3, 7, and 8 positions have been performed to obtain potent and selective A_{2A} AR antagonists. The first xanthine derivative considered an A_{2A} AR antagonist was the 3,7-dimethyl-1-propargylxanthine (DMPX, **34**), but this compound was poorly active (K_i A_{2A} = 16 μM) and the selectivity against the A_1 and A_{2B} receptor subtypes was very low (0.3–3-fold). Nevertheless, this compound has been widely used in in vivo studies because of its good water solubility and bioavailability.[108–110] A further study on DMPX analogues yielded 8-unsubstituted 1-propargylxanthine (**35**) which displayed potency at the A_{2A} AR subtype in the high nanomolar range (K_i = 105 nM) and a good selectivity against A_1 ARs (45-fold; Figure 3 and Table 2).[111,112]

In a program focused on 1, 3, and 8-substituted xanthines, Suzuki and co-workers discovered the family of 8-styryl xanthines, which represented the first example of potent and selective A_{2A} AR antagonists. In particular the 1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine, ((*E*) KF17837, **36**), was highly potent at the A_{2A} AR subtype (K_i = 1 nM) and significantly selective against A_1 ARs (62-fold; Figure 3).[113,114]

In an extensive study on this class of compounds, Jacobson and colleagues, identified the 3-chlorostyrylcaffeine (CSC, **37**) that was less potent than **36** at the A_{2A} ARs (K_i = 54 nM) but more selective against the A_1 AR subtype (560-fold).[115]

However, several problems such as the poor water solubility[116] and the fact that these compounds rapidly photoisomerize when exposed to normal daylight in dilute solution, have initially limited the use of these compounds as pharmacological tools (Figure 4).[117]

This process is not present when styrylxanthines are applied perorally as solid substances, but during binding studies, performed in buffer solution and in the presence of light, the phenomenon occurs very rapidly. For instance, (*E*) KF17837 (**36**) becomes, after photoisomerization, a stable mixture of ~18% (*E*) (**36**) and ~82% (*Z*) (**38**) of the two isomers, giving in a binding study the following data: K_i A_{2A} = 7.9 nM, K_i A_1 = 390 nM.[117] Moreover, several styryl xanthines have been synthesized and different ratios of (*E*)/(*Z*) mixtures have been observed, but usually the (*Z*) isomer is predominant.[118]

On this class of compounds a great SAR profile has been generated, through modification of all the positions of the xan-

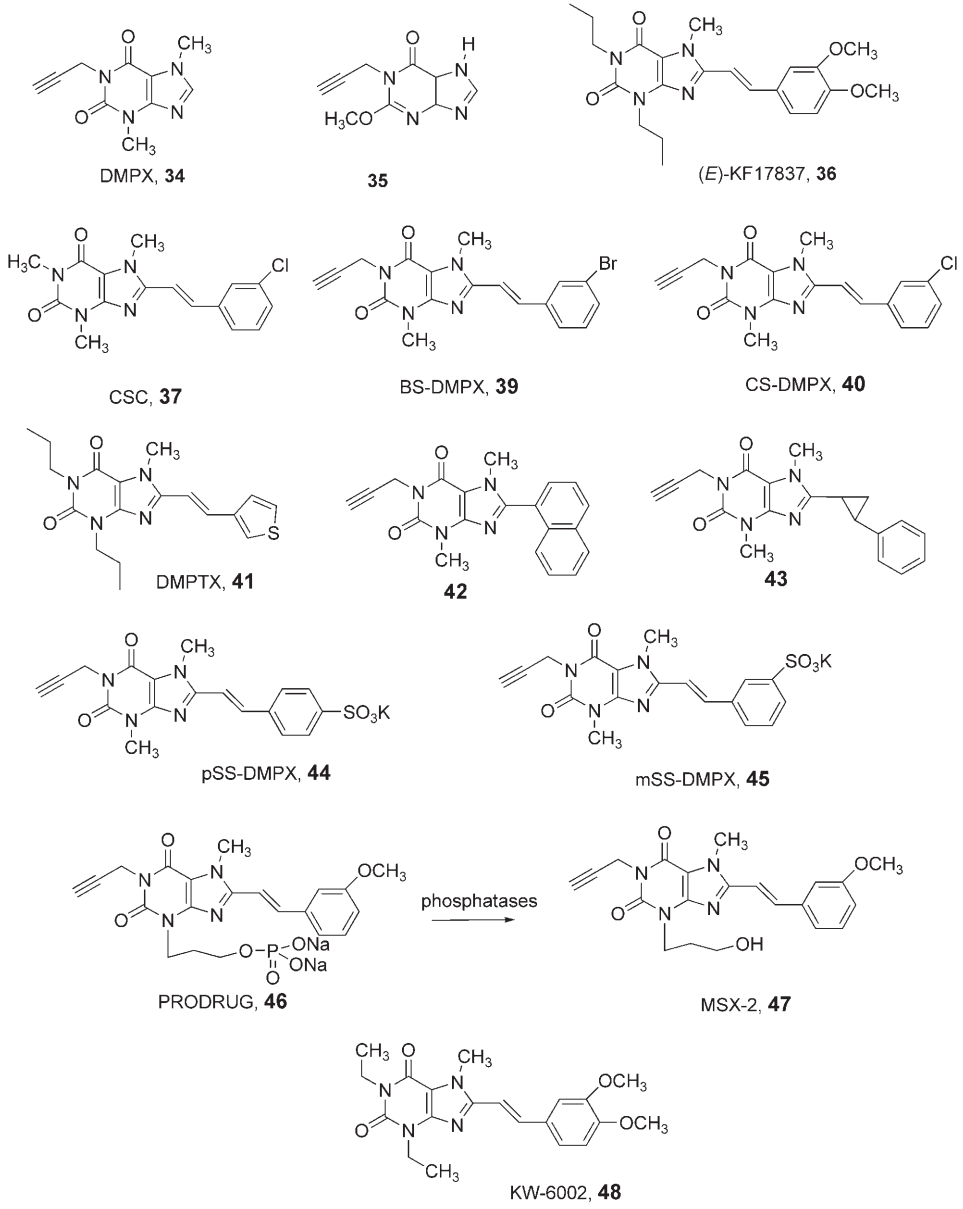


Figure 3. Xanthine derivatives as A_{2A} adenosine receptor antagonists.

thine nucleus. For example, the bioisosteric replacement of CH at the 8 position with nitrogen affords more potent and selective antagonists for the A_{2A} ARs, but the compounds were highly unstable in aqueous solution because of the presence of Schiff bases.[111] Also a substitution of ethenyl group with an azo structure has been followed, and the obtained compounds retain selectivity but present lower affinity (data not shown).[111] Substitution at the 1 position with a propargyl or n-propyl groups seems to increase affinity at the A_{2A} AR subtype with retention of selectivity, in particular two compounds, named BS-DMPX (3,7-dimethyl-1-propargyl-8-(3-bromostyryl)xanthine, **39**) and CS-DMPX (3,7-dimethyl-1-propargyl-8-(3-chlorostyryl)-xanthine, **40**), could be considered the most potent derivatives of this series.[140] Furthermore, methyl substitutions at the 3 and 7 positions seem to be optimal for both affinity and selectivity at this receptor subtype (Figure 3, Table 2).[120–122]

Regarding the 8 position, the presence of an aromatic ring attached to an ethenyl group seems to be essential for both affinity and selectivity.[121–123] The bioisosteric replacement of phenyl ring with a thienyl moiety led to a DPMTX ((*E*)-1,3-dipropyl-7-methyl-8-[2-(3-thienyl)-ethenyl]xanthine, **41**) which showed high affinity and selectivity.[121]

To overcome the problem of the photoisomerization, several derivatives in which the ethenyl group has been replaced with several constrained moieties such as 1-naphthyl (**42**) or racemic cyclopropyl (**43**) group have been synthesized, but a significant loss of potency and selectivity was observed.[124]

Two different approaches have been used aimed at improving the water solubility of styryl xanthines: introduction of polar groups on the phenyl ring, or preparation of phosphate prodrugs. Introduction of a sulfonic group in the phenyl ring *para* (**44**) or *meta* (**45**) positions led to water soluble derivatives but a significant loss of potency (20–30-fold) was observed (compare compound **45** with **40**).[125]

More interesting results have been obtained through the second approach, which involved the preparation of phosphate prodrugs. These studies allowed the discovery of the very highly water soluble (15 mM) prodrug **46**, which after phosphate cleavage (by phosphatases) led to MSX-2 (3-(3-hydroxypropyl)-8-(3-methoxystyryl)-1-propargylxanthine, **47**), endowed with high A_{2A} affinity and selectivity.[126] All these studies have clearly re-evaluated the class of styryl xanthines as A_{2A} AR antagonists. In fact, a compound named KW-6002 (1,3-diethyl-8-(3-methoxystyryl)-7-methylxanthine, **48**) has been selected for phase III clinical trials for treatment of Parkinson's disease.[127,128]

This compound showed a (*E*)/(*Z*) stable equilibrium ratio of 19:81 with good affinity and selectivity (Table 2) but most importantly a very interesting anticataleptic activity (0.03 mg kg^{-1}, p.o.) in the haloperidol model.[118]

Unfortunately, very recently, detailed studies performed on derivatives **47** and **48**, clearly indicate that photoisomerization occurs not only in dilute solution but also in the solid state. In

Table 2. Affinities of xanthine derivatives in radioligand binding assays at A_1, A_{2A}, A_{2B}, and A_3 ARs.

Compd	K_i [nM]				
	K_i (A_1)[a]	K_i (A_{2A})[a]	K_i (A_{2B})[a]	K_i (A_3)[a]	Ref.[b]
34 DMPX	45000 r	16000 r	2500 m 4130 h	> 10000 h	[110]
35	4700 r	105 r			[111]
36 (*E*)-KF17837	62 r	1 r			[113, 114]
37 CSC	28000 r	54 r			[115]
38 (*Z*)-KF17837	> 10000 r	860 r			[117]
39 BS-DMPX	1200 r	8.2 r			[119]
40 CS-DMPX	1300 r	13 r			[119]
41 DMPTX	561 r	19 r			[121]
42	980 r	380 r			[124]
43	4600 r	1700 r			[124]
44 pSS-DMPX	4900 r	240 r		> 100000 h	[125]
45 mSS-DMPX	8900 r	300 r		> 100000 h	[125]
47	900r	8 r			[126]
MSX-2	2500 h	5.0 h		> 10000 h	[126]
48	580 r	13 r			[127]
KW6002	2830 h	36 h	1800 h	> 3000 h	[128]

[a] Binding data from different species: rat (r), human (h) or mouse (m) A_1, A_{2A}, A_{2B} and A_3 ARs, expressed as K_i [nM]. [b] References in this column refer to the whole row; when reported values from different references appear in the same row, they are listed close to the data.

fact, upon irradiation of **48**, a dimer structure **49** (derived from [2+2] cycloaddition) was isolated, and proved to be totally inactive at the A_{2A} AR subtype. This new finding should be now considered as another limit for clinical use of styryl xanthines (Figure 4).[129]

3.2. Nitrogen polyheterocyclic systems

The great problems of xanthine derivatives (for example, poor water solubility and photoisomerization) stimulated the scientists to search for alternative heterocyclic derivatives as lead compounds. Several structures were investigated, including for example CGS 15943 (9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine, **50**)[130–133] and CP 66,713 (4-amino-8-chloro-1-phenyl-[1,2,4]triazolo[4,3-*a*]quinoxaline, **51**).[134] These compounds, although not selective, have represented the starting point for obtaining nonxanthine-based A_{2A} antagonists. A few years later, Gatta and co-workers[135,136] reported the synthesis of 8FB-PTP (**52**, 5-amino-8-(4-fluorobenzyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine), a bioisostere of CGS 15943 (**50**) in which the phenyl ring was replaced by a substituted pyrazole nucleus, which showed good affinity but no selectivity against A_{2A} ARs (Figure 5, Table 3).

An extensive study performed on the pyrazolo-triazolo-pyrimidine nucleus permitted determination of important characteristics related to A_{2A} potency and selectivity, such as the presence of a free amino group at the 5-position, of the furan ring, and the influence of the substituent on the pyrazole ring. In particular, substitutions at the 7-position afforded selective compounds, whereas the same substitution at the 8-position resulted in potent but not selective derivatives (for example, 7-*n*-butyl derivative **53** and the corresponding 8-substituted analogue **54**).[137–139] Moreover, replacement of the pyrazole ring with a triazole permits retention of affinity but a complete loss of selectivity.[140]

Optimization of the substitution at the N7 position led to the discovery of two compounds named SCH 58261[141] (5-amino-7-(β-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo-[1,5-*c*]pyrimidine, **55**) and SCH 63390 (5-amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine, **56**), which proved to be the most potent and selective A_{2A} AR antagonists.[139–141]

Unfortunately, the low water solubility and consequently the poor bioavailability, limited the use of these derivatives as pharmacological tools. (Figure 5, Table 3)

Taking into account this problem, many efforts were under-

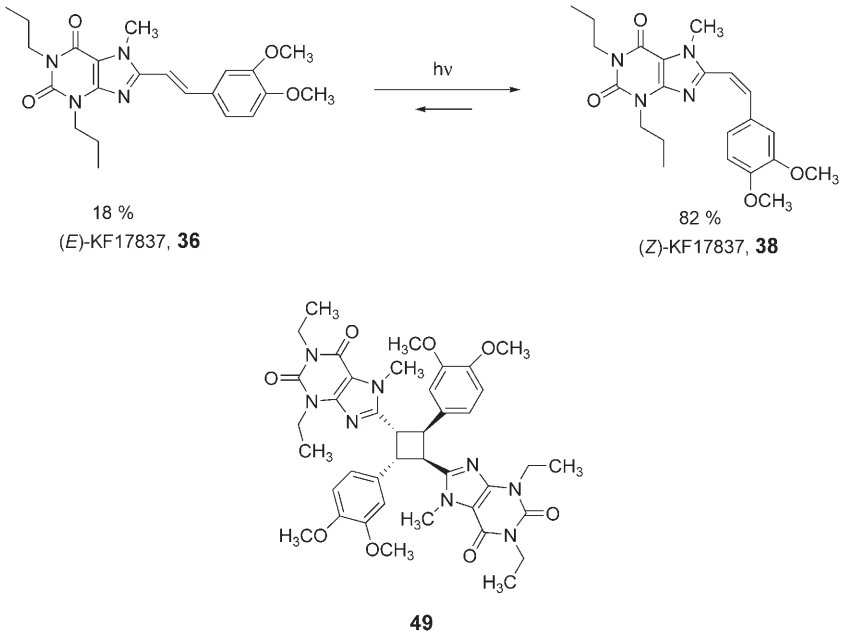


Figure 4. Photoisomerization of styrylxanthines and structure of the dimer **49**.

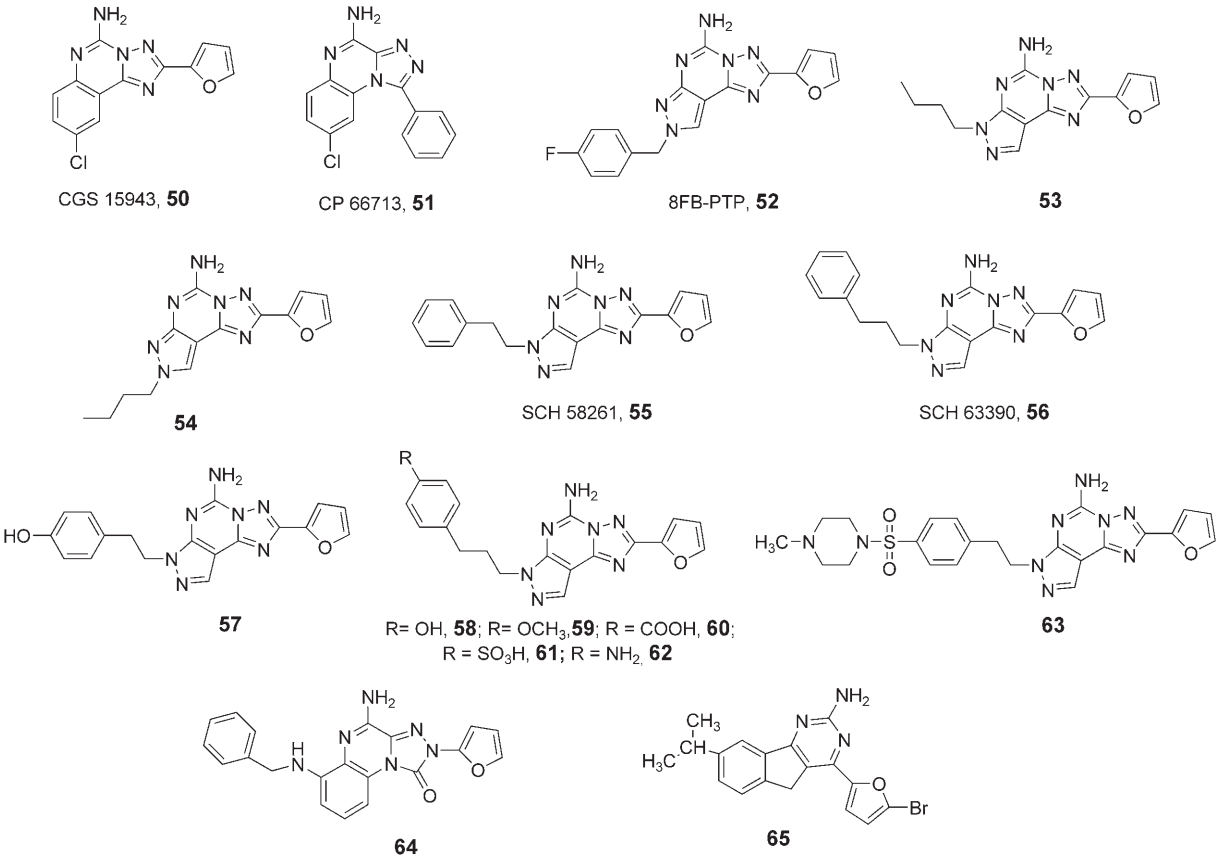


Figure 5. Tricyclic structures as A_{2A} adenosine receptor antagonists.

Table 3. Affinities of tricyclic derivatives in radioligand binding assays A_1, A_{2A}, A_{2B}, and A_3 ARs.

	K_i [nM]				
Compd	K_i (A_1)[a]	K_i (A_{2A})[a]	K_i (A_{2B})[a]	K_i (A_3)[a]	Ref.[b]
50	21 r	3.3 r			[130]
CGS15943	4.4 h	0.43 h	25 h	85 h	[133]
51	270 r	21 r			[134]
CP 66713					
52	3.3 r	1.2 r			[135]
8-FB-PTP					
53	236 r	8.9 r			[138]
54	30.4 r	2.4 r			[138]
55	121 r	2.3 r			[138]
SCH58261	549 h	1.1 h	> 10000 h	> 10000 h	[139]
56	504 r	2.4 r			[138]
SCH63390	350 h	1.2 h	> 10000 h	> 10000 h	[139]
57	444 r	1.7 r		> 10000 h	[142]
58	741 r	0.94 r			[142]
	1111 h	1.5 h		> 10000 h	[142]
59	1815 r	0.048 r			[143]
SCH442416	1111 h	0.5 h	> 10000 h	> 10000 h	[143]
60	4927 h	4.63 h	> 10000 h	> 10000 h	[144]
61	139 h	140 h	> 10000 h	> 10000 h	[144]
62	2160 h	0.22 h	> 10000 h	> 10000 h	[144]
63	369 h	3.8 h	> 10000 h	> 10000 h	[144]
64	15 b	6.5 b		> 10000 h	[145]
65	83 h	0.8 h			[148]

[a] Binding data from different species: rat (r), human (h) or bovine (b) A_1, A_{2A}, A_{2B} and A_3 ARs, expressed as K_i [nM]. [b] References in this column refer to the whole row; when reported values from different references appear in the same row, they are listed close to the data.

taken to increase the hydrophilicity of these derivatives by adding polar groups on the phenyl ring located on the side chain of the pyrazole nucleus. In particular, the introduction of a hydroxy function at the phenyl ring *para* position of compounds **55** and **56**, led to compounds **57** (5-amino-7-[β-(4-hydroxyphenyl)-ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) and **58** (5-amino-7-[3-(4-hydroxy-phenyl)propyl]-2-(2-furyl)pyra-zolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyr-imidine), which not only showed a slightly better hydrophilic char-acter, but also a significant in-crease in both affinity and selec-tivity.[142]

Interestingly, substitution of the phenolic group with a me-thoxy reduced the hydrophilicity of the compound, but an excep-tional potency and selectivity at the A_{2A} AR was observed. The compound, named SCH 442416

(5-amino-7-[3-(4-methoxyphenyl)propyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine, **59**) has been used as a tool for PET studies in its ^{11}C labeled form.[143]

Nevertheless, the introduction of oxygenated groups on the phenyl ring at the side chain was not enough to confer the necessary water solubility. For this reason several polar functions such as carboxylic (**60**) and sulfonic (**61**) moieties were introduced. As expected an increased solubility was observed, in particular with the sulfonic moiety, but a great loss of affinity and selectivity was the consequence. In contrast, the introduction of an amino group at the *para* position of the phenyl ring in the side chain (**62**) gave the best results in terms of affinity and selectivity but water solubility still remained poor. A good balance between solubility and affinity was obtained with the sulfonamido derivative **63**, when used as hydrochloride.[144]

In the last years, other classes of tricyclic compounds have been investigated with the aim of obtaining new tools; unfortunately, none of the reported compounds showed a better profile than the above mentioned derivatives. Only two classes of compounds, the triazolo-quinoxaline[145–147] and the indeno-pyrimidines[148] seem to possess promising requirements as A_{2A} AR antagonists.

In the triazolo-quinoxaline series, only compound **64** (4-amino-6-benzylamino-1,2-dihydro-2-1,2,4-triazolo[4,3-*a*]quinoxalin-1-one) showed an interesting binding profile. However, this nucleus seems to be very sensitive to any kind of modification. In fact, any modifications (for example, alkylation of amino group, replacement of amino group with carbonyl functions, or substitutions on the phenyl ring) reduced affinity at A_{2A} ARs, and in some cases, the affinity at the hA_3 receptors was predominant.[145–147]

In contrast, a very promising class of derivatives was the indeno-pyrimidines, in particular derivative **65**, which show affinity in the nanomolar range and good selectivity for the A_{2A} AR subtype. Unfortunately the lack of binding data at A_{2B} and A_3 receptors does not permit this compound to be considered an ideal candidate as A_{2A} AR antagonist.[148]

Nevertheless, these tricyclic structures presented several problems such as poor water solubility, and most importantly tangled synthetic preparation.

To overcome these problems, the researchers have focused their attention on simplified analogues, in particular bicyclic systems. The first goal in this field was achieved by the Zeneca group with a compound named ZM 241385 (4-[2-[[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-*a*] [1,3,5]triazin-5-yl]amino]ethyl]phenol, **66**), which proved to be one of the most potent A_{2A} AR antagonists ever reported, with quite good water solubility (Figure 6, Table 4).[149–151]

In addition, ZM 241385 binds also with good affinity hA_{2B} ARs. In fact, its tritiated form is actually used in radioligand binding studies of this receptor subtype.[152]

Recently, a large series of derivatives bearing various substituents at the 5-position on the triazolo-triazine nucleus and its deaza analogues triazolo-pyrimidines have been synthesized.[153–157] In particular, derivative **67** showed great potency and selectivity for the A_{2A} AR as compared with the A_1 AR. Nevertheless, the lack of binding data at the A_{2B} and A_3 prevents a comparison of the derivatives with other fully characterized derivatives. Some of these derivatives, although not displaying very high affinity in binding studies, showed good oral efficacy in a rodent catalepsy model of Parkinson's disease.[153–157]

Several isosteres of the triazolo-triazine nucleus have been synthesized; in particular some oxazolo-pyrimidines (**68**)[158] and triazolo-pyrazines (**69**, **70**).[159,160] All these compounds showed good potency at the A_{2A} AR and good selectivity versus A_1 AR, in particular derivative **69**, but all these com-

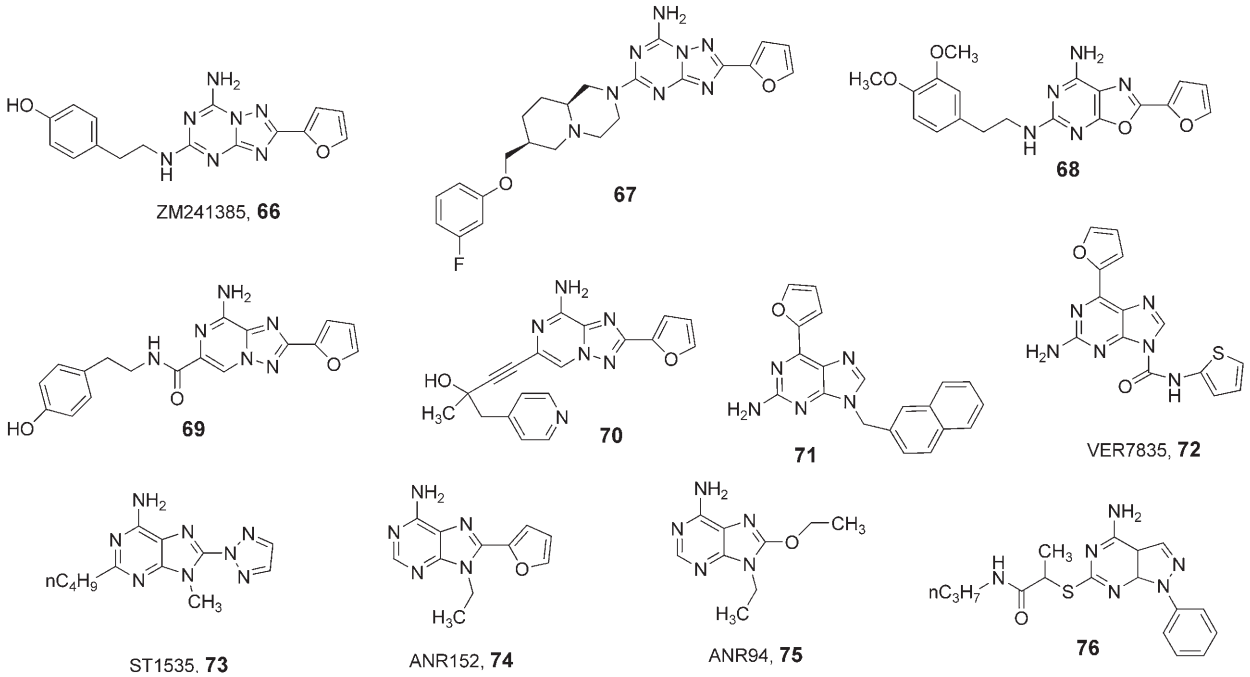


Figure 6. Simplified heterocyclic derivatives as A_{2A} adenosine receptor antagonists.

Table 4. Affinities of bicyclic derivatives in radioligand binding assays A_1, A_{2A}, A_{2B}, and A_3 ARs.

	K_i [nM]				
Compd	K_i (A_1)[a]	K_i (A_{2A})[a]	K_i (A_{2B})[a]	K_i (A_3)[a]	Ref.[b]
66	257 r	1.8 r	28 h[152]	743 h[128]	[152]
ZM241385	774 h[106]	1.6 h [106]	87 g[150]		
67	3300 p	0.2 p			[156]
68	1270 p	14 p			[158]
69	320 r	1 r			[160]
70	100 r	1.1 r			[159]
71	652 h	1.4 h			[161]
72	170 h	1.7 h	141 h	1931 h	[128]
VER7835					
73	71.8 h	6.6 h	352 h	> 10000 h	[162]
ST1525					
74	24 h	3.7 h	380 h	4700 h	[163]
ANR152					
75	2400 h	46 h	> 30000 h	21000 h	[163]
ANR94					
76	329 r	196 b			[164a]

[a] Binding data from different laboratories at rat (r), human (h) or porcine (p) A_1, A_{2A}, A_{2B} and A_3 ARs, expressed as K_i [nM]. [b] References in this column refer to the whole row; when reported values from different references appear in the same row, they are listed close to the data.

pounds have not been fully characterized at the four AR subtypes. Some purine derivatives recently reported by several groups seem to be very promising. Among this series two compounds, the 6-(2-furanyl)-9H-purin-2-amino derivatives **71**[161] and VER7835 (**72**)[128] show affinities in the low nanomolar range and a good level of selectivity against the other receptor subtypes. In this field also, various adenine derivatives have been reported as A_{2A} AR antagonists. ST1535 (**73**)[162] proved to be quite potent but low selectivity versus A_1 AR was observed.

Nevertheless, this compound has been selected for in vivo studies and shown to induce a dose-related increase in locomotor activity.

Very simple adenine derivatives with promising potency at the A_{2A} AR were also reported by Cristalli et al., in particular ANR152 (**74**) and ANR94 (**75**).[163] In this series, it should be underlined that whereas ANR152 (**74**) was more potent at A_{2A} with poor selectivity against A_1, the replacement of the furan ring with an ethoxy function (ANR94, **75**) led to a decrease in affinity but a significant increase of selectivity. Both these derivatives are able to ameliorate motor deficits in rat models of Parkinson's disease.

As simplified analogues some pyrazolo-pyrimidines have also been reported, but only one compound (**76**)[164a] showed promising binding data but proved to have both low selectivity and potency for the A_{2A} AR subtype. However, this compound could represent a good starting point in the search of new A_{2A} receptor antagonist leads. Recently, very simplified heterocyclic derivatives, such as benzothiazole[164b] and 1,2,4-triazole[164c] derivatives have been reported by the Roche group. These derivatives seem to be promising in the treatment of Parkinson's disease but the not exceptional affinity at the A_{2A} AR (micromolar or high nanomolar range) and incomplete biological characterization does not permit these compounds to be considered potent and selective A_{2A} AR antagonists.

Considering the great efforts made to find nonxanthine A_{2A} AR antagonists, it is necessary to underline that if the problem of affinity and selectivity has been completely solved, more work is necessary to obtain water soluble derivatives, and most important more structurally simplified analogues.

4. Receptor Topology of A_{2A} Adenosine Receptor and Comparison of Agonist and Antagonist Binding Domains

The human A_{2A} receptor shares 49% amino acid sequence identity with the human A_1 receptor, 58% with the human A_{2B} subtype, and only 41% with the human A_3 receptor. As with all other members of the A-GPCR family, the general topology of the human A_{2A} receptor is preserved, consisting of a typical 3–4 type helix–helix contact associated with optimal interactions between nearly parallel aligned helices (Figure 7).

The transmembrane region of rhodopsin, and probably all members of the A-GPCR family, is stabilized by a number of interhelical H-bonds and hydrophobic interactions, most of which are mediated by highly conserved residues in GPCRs.[165] For instance, in the case of the rhodopsin-based A_{2A} AR, Asn 24 (TM1) interacted with the backbone carbonyl groups of Ser 281 (TM7) and Asp 52 (TM2), as was observed in rhodopsin, which had interhelical H-bonds between the highly conserved Asn 55 (TM1) and the backbone carbonyl groups of Ala 299 (TM7) and Asp 83 (TM2). Another asparagine residue, Asn 78 (TM2), in rhodopsin formed H-bonds to Ser 127 (TM3), Thr 160 (TM4), and Trp 161 (TM4). The corresponding amino acid in the human A_{2A} AR, Ser 47 (TM2), showed the same hydrophilic interaction with Ser 94 (TM3) and Trp 129 (TM4). Concerning the highly conserved (D/E)R(Y/W) motif in GPCRs, the carboxylate of Glu 134 (TM3) in rhodopsin formed a salt bridge with the guanidium group of the adjacent Arg 135 (TM3), which was also associated with Glu 247 and Thr 251 in TM6. The corresponding amino acids in the human A_{2A} AR were Asp 101–Arg 102–Tyr 103. The equivalent interactions occurred in the A_{2A} AR, that is, the salt bridge of Arg 102 (TM3) with Asp 101 (TM3) and Glu 228 in TM6. For the NPXXY motif in TM7 of GPCRs, the hydroxy group of Tyr 306 (TM7) was close to Asn 73 (TM2) in rhodopsin, which was also highly conserved among GPCRs. The same result appeared with the modeled A_{2A} AR structure, that is, the OH group of Tyr 288 (TM7) in the A_{2A} AR was positioned in proximity to the side chain of Asn 42 (TM2).

Two important interhelical H-bonding interactions for highly conserved sequences took place in ARs but not in rhodopsin.

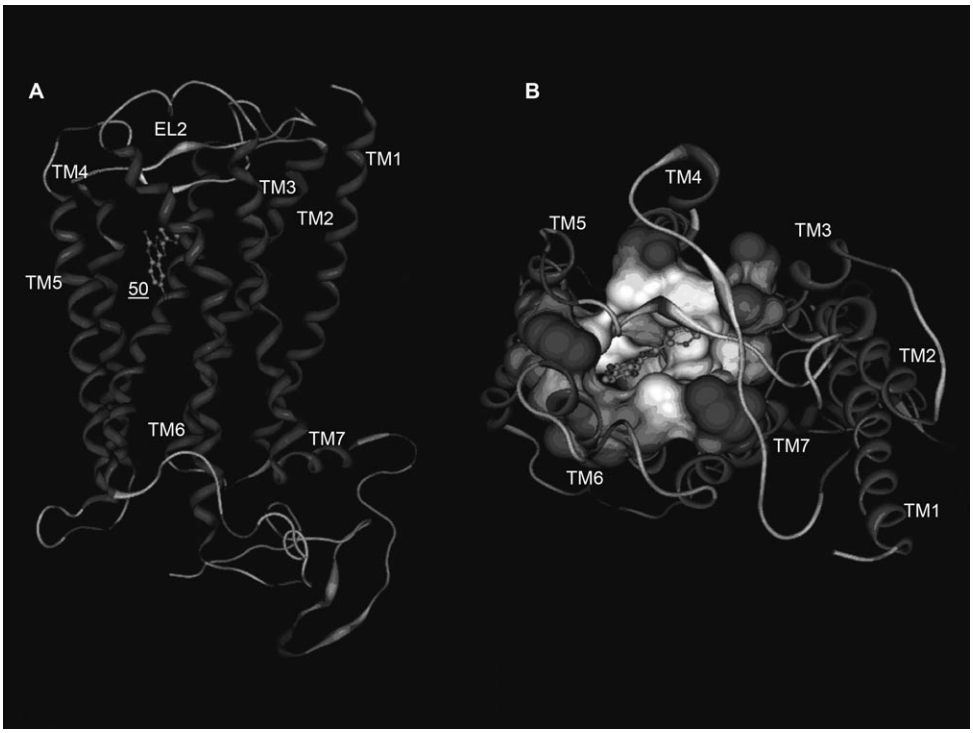


Figure 7. The human A_{2A} receptor model viewed from the membrane side (Panel A) and from the extracellular side (Panel B), showing the E2 loop folded into the binding crevice. Putative binding sites suggested by site-directed mutagenesis studies are delimited by the docked derivative **50**.

As previously proposed, there was H-bonding between the side chains of Glu 13 (TM1) and His 278 (TM7). Mutational results indicated that Glu 13 (TM1) and the corresponding residue in the A_1 AR, Glu 16, facilitated agonist binding.

Another residue, Asp 52 (TM2) in the A_{2A} AR, was highly stabilized by a H-bonding network among the highly conserved amino acids, Asn 280 (TM7), Ser 281 (TM7), and Asn 284 (TM7), which also formed H-bonds with Ser 91 (TM3).

Unlike rhodopsin, there were additional H-bonds in the ARs for Asp 52 (TM2) interacting with hydrophilic amino acids; whereas in rhodopsin, the amino acids that correspond to those participating in the H-bond network were all alanine residues except Asn 284 (TM7). The corresponding amino acid, Asp 55 (TM2) in the A_1 AR, was responsible for sodium binding.[166]

Among hydrophobic interactions, the conserved Trp 246 (TM6) was typically surrounded by hydrophobic residues from TMs 3, 6, and 7, as was observed for the human A_3 AR and another GPCR, the thyrotropin-releasing hormone receptor.[167] Those hydrophobic amino acids near Trp 246 were Val 84, Leu 85, Leu 87, Phe 242, Ala 243, and Pro 248. The hydrophilic aromatic residues His 250 and His 278 were also in proximity. The indole ring of Trp 246 (TM6) also formed an H-bond with Asn 280 (TM7), which was stabilized through H-bonding with Asp 52 (TM2). Agonist binding would cause a rotation of the Trp side chain, disrupting these interhelical interactions. Thus, the intramolecular contact network might be destabilized, facilitating the conformational change required to activate the A_{2A} AR. The experimental results reported by Jacobson and collaborators were consistent with this hypothesis:[168] the Trp 243 Ala (TM6) mutant A_3 AR displayed normal agonist binding but no activity in a functional assay.

Moreover, comparing the primary sequences of the four subtypes of ARs, several amino acid mutations are detectable, particularly in the putative ligand binding cavity (Figure 8).

As clearly demonstrated by site-directed mutagenesis studies, some of these mutations might play a role in the recognition process of both agonists and antagonists.[1b,167–169] To elucidate the pharmacological differences among all receptors subtypes, in the recent past several authors theoretically depicted the general topology of all four human ARs.[167,168,175] Ideally, a crystallographic determination of the human A_{2A} receptor structure would be a better method by which to analyze the conformational implications of all mutagenesis and pharmacological experiments; however, presently no structure is available.

Rhodopsin-based homology modeling is the most validated theoretical strategy to obtain structural information on the A-GPCR family.[167,168,175] Rhodopsin-based homology modeling is not an automatic method for obtaining a realistic structure for a given GPCR, but rather requires time-consuming custom treatment according to known pharmacological data.

Recently, both agonists and antagonists have been docked in the human A_{2A} receptor model. It is to be emphasized that, in general, docking of agonists to GPCR models is subject to even greater uncertainty than antagonists as the template consists of the rhodopsin inactive state. Often multiple modes of docking of a given agonist or antagonist ligand are observed, and the selection of preference of one docking mode in such cases must be based on diverse pharmacological data, rather than on computational results alone. Nevertheless, the increasing level of refinement of rhodopsin-based homology modeling, for example in the addition of the extracellular loops, has yielded useful insight and results.

4.1. Antagonist binding requirements

As recently reported by Jacobson and collaborators,[169] the major mode of interaction of antagonists with the A_{2A} AR consisted of hydrophobic interactions. For instance, 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-*c*]quinazoline (CGS15943, **50**), a potent and nonselective adenosine antagonist was used as a modeling template in previous studies.[169–176] According to

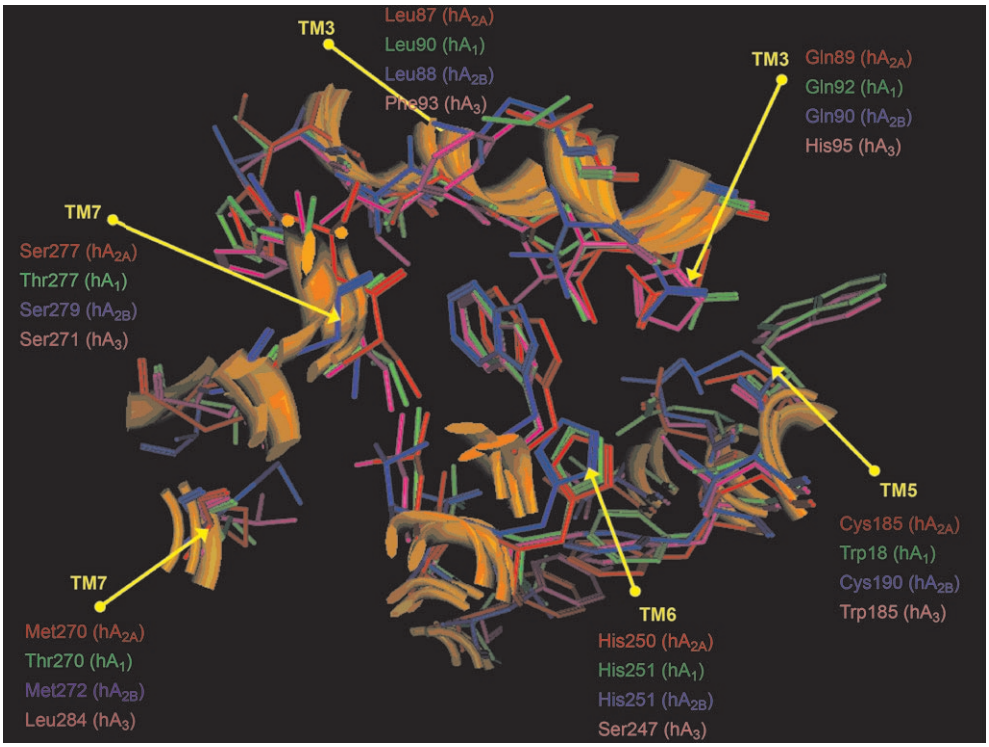


Figure 8. Mutational analysis for selected residues of the ARs with respect the hypothetical ligand binding viewed from the extracellular side. Extracellular loop 2 (EL2) has been voluntarily omitted. Side chains of some amino acids important for ligand recognition are highlighted. Hydrogen atoms are not displayed. Less conserved amino acids are highlighted.

molecular modeling studies,[169] large hydrophobic pockets consisting mostly of residues at TMs 3, 6, and 7 interacted with the ligand (Figure 9).

Hydrophobic amino acids that participated in these interactions with the ligand were Leu 85 (TM3), Ile 135 (TM4), Leu 167 (EL2), Phe 168 (EL2), Phe 182 (TM5), Val 186 (TM5), Trp 246 (TM6), and Leu 249 (TM6) near the quinazoline ring, and Ile 80 (TM3), Val 84 (TM3), and Ile 274 (TM7) in proximity to the furan ring.

One important hydrophilic interaction was an H-bond formed between the exocyclic amino group at the 5-position and Asp 253 (TM6). Additional weak H-bonding between the side chain of Asn 181 (TM5) and N6 of the CGS15943 served to increase the thermal stability of the complex. This docking result was consistent with our previously reported Ala mutant receptors Phe 182 Ala, His 250 Ala, Asn 253 Ala, Ile 274 Ala, and His 278 Ala all of which lost the high-affinity binding of both A_{2A} AR agonists and antagonists. The aromatic residue His 250 also appeared to be a required component of this mainly hydrophobic pocket. H-bonding to this residue was not essential, as indicated by retention of function in Phe and Trp mutant receptors.[177]

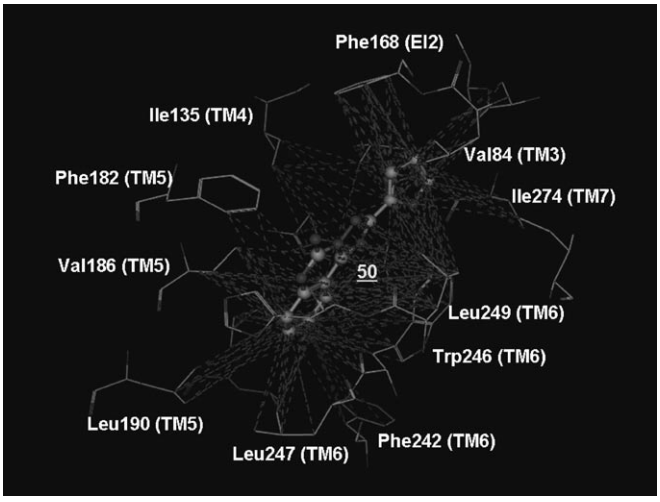


Figure 9. Detailed interaction with derivate **50** in the putative human A_{2A} binding site. The most important amino acids involved in hydrophobic interaction (dashed lines) are shown and labeled.

Another interesting modeling result by Jacobson and collaborators[169] concerns the docking of a series of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives developed by Baraldi and co-workers as potent and selective nonxanthine A_{2A} antagonists.[138] In particular, the addition of the 4-aminophenylpropyl group at the N7-position of the triazolopyrimidine ring produced a highly A_{2A}-selective antagonist **62** ($K_i = 0.22$ nM, hA1/hA2A = 9820), which did not significantly interact with either A_{2B} or A_3 ARs.[144] Its derived structure–activity relationships indicate that the tricyclic structure of the pyrazolotriazolopyrimidine, the presence of the furan ring, the exocyclic 5-amino group, and the arylalkyl substituent on the nitrogen at the 7-position are probably essential for both affinity and selectivity for the A_{2A} AR subtype. Additional hydrophobic interactions between the 4-aminophenylpropyl moiety and the hydrophobic pocket at TM4 and TM5, and H-bonding of the 4-amino group with Asn 145 (EL2), could contribute to the increase in A_{2A} AR affinity.

The A_{2A} AR sequence alignment indicated that most of the amino acids in the putative binding site within 5 Å of the A_{2A}-selective antagonist **62** were conserved among ARs. Highly conserved amino acids were Leu 85 (TM3), Thr 88 (TM3), Gly 136 (TM4), Pro 139 (TM4), Phe 168 (EL2), Met 177 (TM5), Phe 182 (TM5), Trp 246 (TM6), Asn 253 (TM6), Ile 274 (TM7), and His 278 (TM7). However, in the docked complex the amino acids located near the N5, N7, and N8 positions of the triazolo-[1,5-*c*]pyrimidine varied. In particular, hydrophilic amino acids

such as Leu 167 and His 250 in the A_{2A} receptor (Gln 167 and Ser 247 in the A_3 subtype) near the putative binding region for N5 substituents would be expected to increase the selectivity for the A_3 subtype through additional H-bonding with carbamoyl groups. On the other hand, the bulky and aromatic side chains such as Leu 167 and His 250 in the A_{2A} AR made it easy to accommodate an unsubstituted N5-amino group. His 95 in the A_3 AR near the 8-position binding region had a more hydrophobic character than did the homologous Ser in the A_1, A_{2A}, and A_{2B} ARs, possibly explaining the fact that the hydrophobic factor at the N8-position was important for A_3 AR binding.[178]

4.2. Agonist binding requirements

According to Jacobson and collaborators' molecular modeling studies,[169] agonist binding was significantly different than antagonist binding in the region of the ribose ring, as expected from the requirement for a ribose ring in the agonist but not in the antagonist. As expected from the structural differences between the agonists and antagonists, a characteristic feature of agonist binding was additional H-bond formation of the 3′-OH with His 278 (TM7) and of the 5′-amide group of NECA with Thr 88 (TM3) and Ser 277 (TM7) as shown in Figure 10.

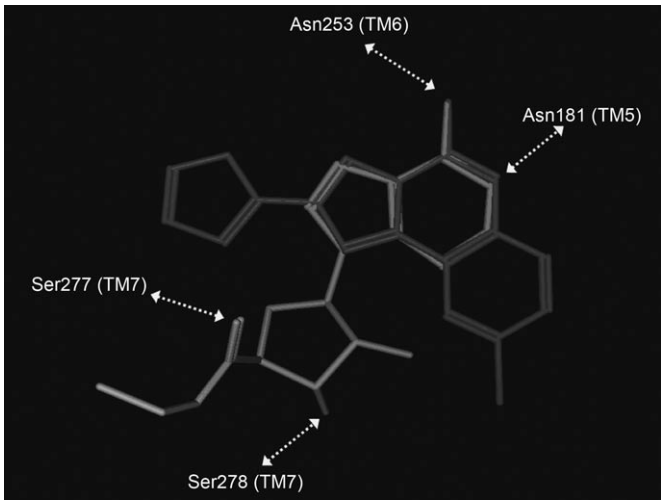


Figure 10. Superimposition of the bound conformations of the A_{2A} antagonist **50** (dark grey) and agonist **22** (light grey) in the putative binding sites.

The anticonformation of the glycosidic bond of NECA (**22**) and other agonists was energetically favorable as an active conformation for A_3 AR binding;[168,169] this was supported by both a molecular modeling study and a binding preference for the methanocarba-ring system in the (*N*)-conformation, which favors the anticonformation.[169–179]

As generally accepted, the putative ribose-binding region is probably involved in receptor activation.[169] An interesting result concerned the conserved Trp 243 (TM6) side chain in the A_3 AR, which was involved in recognition of the classical (non-nucleoside) A_3 AR antagonists but not adenosine-derived ligands and which displayed a characteristic movement-counterclockwise rotation as viewed from the exofacial side exclusively upon docking of agonists.[168,169] It has been similarly speculated for the A_{2A} AR that a significant distinction between agonists and antagonists was whether ligand binding could effect the movement of Trp 246 (TM6) side chain.

Additional binding of the ribose 5′-substituents shown in the agonist complex might induce the movement of the side-chain of Trp 246. That conformational change might disrupt the H-bonding network of Trp 246 with Asn 280 (TM7), which participated in the H-bonding network of Asp 52 (TM2), and hydrophobic interactions of Trp 246 with hydrophobic residues from TMs 3, 6, and 7, thus facilitating a conformational change upon receptor activation. If the A_{2A} AR behaves like the A_3 subtype,[168] then flexibility of the ribose moiety and specific recognition elements at the 3′- and 5′-positions to permit the movement of TM6 would be important for agonism at the A_{2A} AR. It also correlated with recent studies[180] based on electron paramagnetic resonance and fluorescence spectroscopy, which suggested an outward movement of the cytoplasmic end of TMs 3 and 6, and an anticlockwise rotation of TM6 around its helical axis as viewed from the extracellular side.

Finally, two of the three most conserved prolines in GPCRs, Pro 248 and Pro 285 in human A_{2A}, occur on TM6 and TM7. Pro 248 is in proximity to the binding site, that is, Trp 246 (TM6). Pro 285 is near Asp 284 (TM7), which associated with Asp 52 (TM2), the putative sodium-binding site, through H-bonding. It was proposed that in rhodopsin the proline corresponding to Pro 248 in human A_{2A} acts as a flexible hinge, straightening TM6 upon light-induced activation.[181] Thus, these two proline residues that are conserved among GPCRs would facilitate the agonist-induced movement of TM6 and subsequently TM7 to rearrange intracellular loop (IL) 3 and helix 8, which are known to be important for the receptor-G protein interface.[182]

5. Therapeutic Application of A_{2A} AR Agonists and Adenosine Derivatives in Clinical Trials

Adenosine induces coronary arteriolar vasodilatation associated with a hyperemic coronary flow due to stimulation of A_{2A} AR on arteriolar vascular smooth muscle cells, causing vasorelaxation.[183] However, intravenous administration of Ado, carried out for use in myocardial perfusion imaging procedures is associated with a high incidence of side effects, such as chest pain, dyspnea, and facial flushing, that result in patient discomfort.[184,185] More serious side effects of Ado administration, although less frequently reported, are heart block and bronchoconstriction in asthmatic patients.

Both animal and human studies have shown that the negative dromotropic action of Ado[185] and the chest pain[186] are due to activation of A_1 AR. On the other hand, A_3 AR activation may be responsible for the bronchoconstriction that occurs when adenosine is administered to asthmatic patients.[187] Coronary vasodilators, such as adenosine and dipyridamole, commonly used in pharmacological stress testing stimulate adenosine A_{2A} receptors. However, both agents also nonselectively

stimulate A_1, A_{2B}, and A_3 receptor subtypes, resulting in a high incidence of adverse events. Research efforts continue in an attempt to develop novel pharmacological stress agents with fewer unwanted side effects, more selective A_{2A} receptor-agonist effects, and which can be administered as a bolus instead of by infusion to produce selective vasodilatation with a rapid onset and short duration of action. Hence, a compound capable of producing coronary vasodilatation through activation of A_{2A} AR, but that is devoid of A_1- and A_3-agonist activity would have advantages over Ado for use in myocardial perfusion imaging studies.

Currently, three A_{2A} AR agonists have begun phase III studies. Two of them, regadenoson (also known as CVT-3146, **18**)[188–190] and binodenoson (also known as MRE-0470 or WRC-0470, **33**) are therapeutically evaluated as pharmacologic stress agents (Figure 11).[191]

Regadenoson is a low affinity A_{2A} agonist, which produces a response that is of equivalent magnitude but more rapid in termination than that caused by a high-affinity agonist. Hence, it may prove to be superior to currently available high-affinity agonists as coronary vasodilators during myocardial imaging with radionuclide agents. Binodenoson is reported to be a selective agonist at the adenosine A_{2A} receptor versus A_1 and A_{2B} subtypes, whereas its selectivity against A_3 is only 3-fold.[187,191] This compound has been studied predominantly in the catheterization laboratory setting, producing dose-related increases in coronary blood flow velocity, with mean maximal coronary vasodilatory responses equivalent to those produced by intracoronary adenosine. Administration of this agent would result in myocardial perfusion images similar to those of nonselective AR stimulation, accompanied by fewer or less severe symptoms and adverse events.

The third A_{2A} agonist in clinical trials is the 2-alkynyl derivatives of NECA ATL-146e or BMS-068645 (Figure 11, **30**).[94] This compound proved to be effective in the treatment of the acute spinal cord injury (SCI) while avoiding the adverse effects of steroid agents.[192] Moreover, it has been demonstrated that chronic A_{2A} AR activation in diabetic rats by ATL-146e ameliorates histological and functional changes in kidneys induced by diabetes and causes reduced inflammation associated with diabetic nephropathy.[193]

Other A_{2A} agonists, including CGS-21680, have also been investigated in preclinical trials. There is evidence that exogenous agents such as an adenosine A_{2A} receptor agonist increases neovascularization in the early stages of wound repair by increasing both EPC recruitment (vasculogenesis) and local vessel sprouting (angiogenesis).[194,195]

A_{2A} receptor agonists may also represent a novel therapeutic approach in preventing organ injury following trauma/hemorrhagic shock.[196]

Other potential therapeutic applications of selective A_{2A} AR agonists are in the treatment of eye diseases, such as glaucoma,[197] in inducing sleep by increasing GABA release,[198] in inflammation,[199] and in neurodegenerative diseases.[200,5b]

However, clinical evaluation of some A_1 and A_{2A} AR agonists has been discontinued. Major problems include side effects due to the wide distribution of ARs, low brain penetration, which is important for the targeting of CNS diseases, short half-life of compounds, or a lack of effects, in some cases perhaps attributable to receptor desensitization or to low receptor density in the targeted tissue. More detail can be found in some recent reviews dealing with pharmacology of purinergic receptors.[201,202]

6. Therapeutic Applications of A_{2A} Adenosine Receptor Antagonists and Compounds in Clinical Trials

As already described, the great interest in the field of A_{2A} AR antagonists is related to their application in neurodegenerative disorders and in particular in Parkinson's disease.[203] This class of compounds could be classified as non-dopaminergic anti-Parkinsonian symptomatic agents.

The most promising compound of this series is represented by KW6002 (Istradefylline, **48**).[204–206] This compound developed by Kyowa is now undergoing phase III clinical trials. Other compounds are at present under clinical investigation, in particular the non-xanthine A_{2A} antagonists V2006[207] developed by Biogen Idec Inc. and various SCH58261 analogues[208] registered by Schering–Plough are undergoing phase II clinical trials. New entries in this field, including compounds developed by Adenosine Therapeutics,[209] Neurocrine Biosciences, and Almirall Prodesfarma,[210] have been selected as candidates for clinical studies (preclinical phase). On these bases we may assume that at the present KW6002 (**48**) is the more advantageous candidate as an anti-Parkinson's agent but if some emerging compound could prove to be useful as monotherapy and not in combination with dopamine (such as for KW6002), new frontiers could be opened in this field.

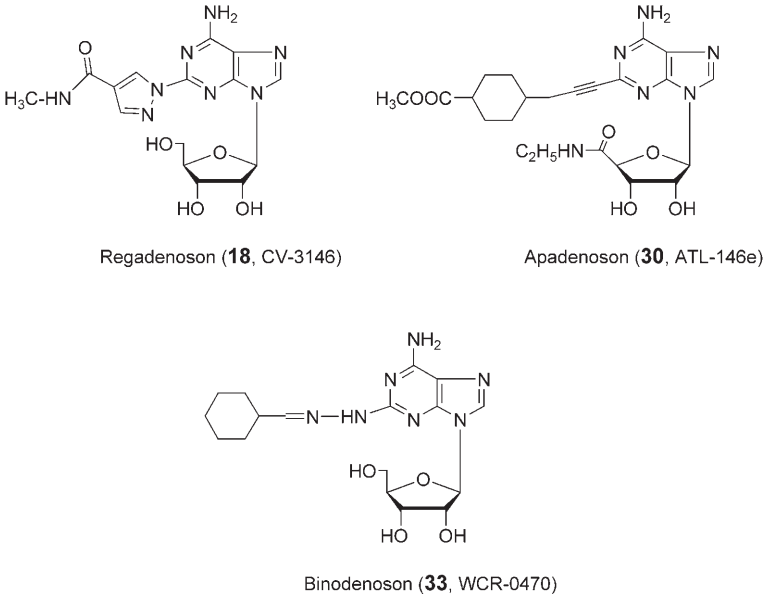


Figure 11. Adenosine derivatives in clinical trials.

7. Summary and Outlook

In conclusion, although medicinal chemistry has produced a number of putative A_{2A} AR agonists, detailed characterization of these compounds at the four cloned human ARs has revealed that none of them exhibits both high affinity and selectivity for the human A_{2A} subtype. Hence, there is a strong need for more selective agonists at this subtype because of their potential therapeutic applications in myocardial perfusion imaging studies and as anti-aggregatory, anti-inflammatory, antipsychotic, and anti-Huntington's disease agents. Moreover, it has been demonstrated that the neuroprotective effects of A_{2A} receptor antagonists are not restricted to dopaminergic neurons. In fact, there is evidence that in rodents A_{2A} receptor antagonists conferred significantly better outcomes in terms of neuronal survival, particularly in the cerebral cortex, although the mechanism underlying this neuroprotective effect is poorly understood. Hence, even though the great number of nonxanthine A_{2A} AR antagonists, synthesized so far, allowed the problems of affinity and selectivity in this class of AR ligands to be solved, more work is necessary to obtain water soluble and structurally simplified analogues.

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Keywords: A_{2A} adenosine receptor · A_{2A} agonists and antagonists · nitrogen heterocycles · nucleosides

[1] a) For a recent review on medicinal chemistry and pharmacology of adenosine receptors, see: *Adenosine Receptors: Chemistry and Pharmacology* (Eds.: G. Cristalli, R. Volpini), *Curr. Top. Med. Chem.* **2003**, *3*(4), 355 – 469; b) K. A. Jacobson, Z. G. Gao, *Nat. Rev. Drug Discovery* **2006**, *5*, 247 – 264.

[2] A. N. Drury, A. Szent-Gyorgyi, *J. Physiol.* **1929**, *68*, 213 – 217.

[3] J. P. DiMarco, T. D. Sellers, R. M. Berne, G. A. West, L. Belardinelli, *Circulation* **1983**, *68*, 1254 – 1263.

[4] L. Belardinelli, A. Pelleg in *Purine in cellular signaling: targets for new drugs* (Eds.: K. A. Jacobson, J. W. Daly, V. Manganiello), Springer, New York, **1990**, pp. 95 – 99.

[5] a) B. B. Fredholm, *Med. Biol.* **1977**, *55*, 262 – 267; b) B. B. Fredholm, R. A. Cunha, P. Svenningsson, *Curr. Top. Med. Chem.* **2003**, *3*(4), 413 – 426.

[6] J. Premont, M. Perez, J. Bockaert, *Mol. Pharmacol.* **1977**, *13*, 662 – 670.

[7] J. W. Daly, P. Butts-Lamb, W. Padgett, *Cell. Mol. Neurobiol.* **1983**, *3*, 69 – 80.

[8] R. F. Bruns, G. H. Lu, T. A. Pugsley, *Mol. Pharmacol.* **1986**, *29*, 331 – 346.

[9] C. Maenhaut, J. Van Sande, F. Libert, M. Abramowicz, M. Parmentier, J. J. Vanderhaegen, J. E. Dumont, G. Vassart, S. Schiffmann, *Biochem. Biophys. Res. Commun.* **1990**, *173*, 1169 – 1178.

[10] Y. Chern, K. King, H. L. Lai, H. T. Lai, *Biochem. Biophys. Res. Commun.* **1992**, *185*, 304 – 309.

[11] J. S. Fink, D. R. Weaver, S. A. Rivkees, R. A. Peterfreund, A. E. Pollack, E. M. Adler, S. M. Reppert, *Brain Res. Mol. Brain Res.* **1992**, *14*, 186 – 195.

[12] T. J. Furlong, K. D. Pierce, L. A. Selbie, J. Shine, *Brain Res. Mol. Brain Res.* **1992**, *15*, 62 – 66.

[13] C. Ledent, J. M. Vaugeois, S. N. Schiffmann, T. Pedrazzini, M. El Yacoubi, J. J. Vanderhaeghen, J. Costentin, J. K. Heath, G. Vassart, M. Parmentier, *Nature* **1997**, *388*, 674 – 678.

[14] F. Meng, G. Xie, D. Chalmers, C. Morgan, S. J. Watson, Jr., H. Akil, *Neurochem. Res.* **1994**, *19*, 613 – 621.

[15] K. Pfleger, E. Seifen, H. Schondorf, *Biochem. Pharmacol.* **1969**, *18*, 43 – 51.

[16] M. Rockwell, M. H. Maguire, *Mol. Pharmacol.* **1966**, *2*, 574 – 584.

[17] G. Cristalli, S. Costanzi, C. Lambertucci, G. Lupidi, S. Vittori, R. Volpini, E. Camaioni, *Med. Res. Rev.* **2001**, *21*, 105 – 128.

[18] M. H. Maguire, D. M. Nobbs, R. Einstein, J. C. Middleton, *J. Med. Chem.* **1971**, *14*, 415 – 420.

[19] R. Marumoto, Y. Yoshioka, O. Miyashita, S. Shima, K. Imai, *Chem. Pharm. Bull.* **1975**, *23*, 759 – 774.

[20] G. Cristalli, C. Lambertucci, S. Taffi, S. Vittori, R. Volpini, *Curr. Top. Med. Chem.* **2003**, *3(4)*, 387 – 401.

[21] G. Cristalli, R. Volpini, S. Vittori, E. Camaioni, A. Monopoli, A. Conti, S. Dionisotti, C. Zocchi, E. Ongini, *J. Med. Chem.* **1994**, *37*, 1720 – 1726.

[22] G. Cristalli, E. Camaioni, S. Vittori, R. Volpini, P. A. Borea, A. Conti, S. Dionisotti, E. Ongini, A. Monopoli, *J. Med. Chem.* **1995**, *38*, 1462 – 1472.

[23] S. Vittori, E. Camaioni, E. Di Francesco, R. Volpini, A. Monopoli, S. Dionisotti, E. Ongini, G. Cristalli, *J. Med. Chem.* **1996**, *39*, 4211 – 4217.

[24] G. Cristalli, S. Vittori, R. D. Thompson, W. L. Padgett, D. Shi, J. W. Daly, R. A. Olsson, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1994**, *349*, 644 – 650.

[25] N. J. Cusack, S. M. O. Hourani, *Br. J. Pharmacol.* **1981**, *72*, 443 – 447.

[26] I. Antonini, G. Cristalli, P. Franchetti, M. Grifantini, S. Martelli, F. Petrelli, *J. Pharm. Sci.* **1984**, *73*, 366 – 369.

[27] A. Monopoli, A. Conti, C. Zocchi, C. Casati, R. Volpini, G. Cristalli, E. Ongini, *Arzneim.-Forsch.* **1994**, *44*, 1296 – 1304.

[28] G. Cristalli, R. Volpini, S. Vittori, E. Camaioni in *Adenosine and Adenine Nucleotides: from Molecular Biology to Integrative Physiology* (Eds.: L. Belardinelli, A. Pelleg), Kluwer Academic Publishers, Philadelphia, **1995**, pp. 140 – 148.

[29] G. Cristalli, E. Camaioni, S. Vittori, R. Volpini, *Nucleosides Nucleotides* **1995**, *3–5*, 449 – 453.

[30] G. Cristalli, E. Camaioni, E. Di Francesco, R. Volpini, S. Vittori in *Perspectives in Receptor Research* (Eds.: D. Giardinà, S. Piergentili, M. Pigini), Pharmaco Chemistry Library, Elsevier Science Publishers, Amsterdam, **1996**, pp. 165 – 180.

[31] E. Camaioni, E. Di Francesco, S. Vittori, R. Volpini, G. Cristalli, *Bioorg. Med. Chem.* **1997**, *5*, 2267 – 2275.

[32] M. Viziano, E. Ongini, A. Conti, C. Zocchi, M. Seminati, D. Pocar, *J. Med. Chem.* **1995**, *38*, 3581 – 3585.

[33] R. Munshi, A. S. Clanachan, H. P. Baer, *Biochem. Pharmacol.* **1988**, *37*, 2085 – 2089.

[34] L. E. Brackett, J. W. Daly, *J. Pharmacol. Exp. Ther.* **1991**, *257*, 205 – 213.

[35] B. K. Trivedi, C. J. Blankley, J. A. Bristol, H. W. Hamilton, W. C. Patt, W. J. Kramer, S. A. Johnson, R. F. Bruns, D. M. Cohen, M. J. Ryan, *J. Med. Chem.* **1991**, *34*, 1043 – 1049.

[36] T. Abiru, T. Yamaguchi, Y. Watanabe, K. Kogi, K. Aihara, A. Matsuda, *Eur. J. Pharmacol.* **1991**, *196*, 69 – 76.

[37] A. Matsuda, M. Shinozaki, T. Yamaguchi, H. Homma, R. Nomoto, T. Miyasaka, Y. Watanabe, T. Abiru, *J. Med. Chem.* **1992**, *35*, 241 – 252.

[38] T. Abiru, T. Miyashita, Y. Watanabe, T. Yamaguki, H. Machida, A. Matsuda, *J. Med. Chem.* **1992**, *35*, 2253 – 2260.

[39] H. Homma, Y. Watanabe, T. Abiru, T. Murayama, Y. Nomura, A. Matsuda, *J. Med. Chem.* **1992**, *35*, 2881 – 2890.

[40] A. J. Hutchison, R. L. Webb, H. H. Oei, G. R. Ghai, M. B. Zimmerman, M. Williams, *J. Pharmacol. Exp. Ther.* **1989**, *251*, 47 – 55.

[41] M. F. Jarvis, R. Schulz, A. J. Hutchison, U. H. Do, M. A. Sills, M. Williams, *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888 – 893.

[42] R. A. Olsson, S. Kusachi, R. D. Thompson, D. Ukena, W. Padgett, J. W. Daly, *J. Med. Chem.* **1986**, *29*, 1683 – 1689.

[43] J. W. Daly, W. Padgett, R. D. Thompson, S. Kusachi, W. J. Bugni, R. A. Olsson, *Biochem. Pharmacol.* **1986**, *35*, 2467 – 2481.

[44] A. J. Hutchison, M. Williams, R. de Jesus, R. Yokoyama, H. H. Oei, G. R. Ghai, R. L. Webb, H. C. Zoganas, G. A. Stone, M. F. Jarvis, *J. Med. Chem.* **1990**, *33*, 1919 – 1924.

[45] D. R. Borcherding, N. L. Lentz, P. M. Weintraub, M. W. Dudley, R. Secrest, P. R. Kastner, N. P. Peet, *Nucleosides Nucleotides* **1999**, *18*, 2175 – 2191.

[46] J. A. Zablocki, V. Palle, B. Blackburn, E. Elzein, G. Nudelman, S. Gothe, Z. Gao, Z. Li, S. Meyer, L. Belardinelli, *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 343 – 360.
[47] S. Kusachi, R. D. Thompson, R. A. Olsson, *J. Pharmacol. Exp. Ther.* **1983**, *227*, 316 – 321.
[48] R. Maramoto, S. Shima, K. Omura, M. Tanabe, S. Fujiwara, Y. Furukawa, *J. Takeda Res. Lab.* **1985**, *44*, 220 – 230.
[49] S. Kusachi, R. D. Thompson, J. W. Bugni, N. Yamada, R. A. Olsson, *J. Med. Chem.* **1985**, *28*, 1636 – 1643.
[50] S. Kusachi, R. D. Thompson, N. Yamada, D. T. Daly, R. A. Olsson, *J. Med. Chem.* **1986**, *29*, 989 – 996.
[51] H. W. Hamilton, M. D. Taylor, R. P. Steffen, S. J. Haleen, R. F. Bruns, *Life Sci.* **1987**, *41*, 2295 – 2302.
[52] M. Ueeda, R. D. Thompson, L. H. Arroyo, R. A. Olsson, *J. Med. Chem.* **1991**, *34*, 1334 – 1339.
[53] M. Ueeda, R. D. Thompson, L. H. Arroyo, R. A. Olsson, *J. Med. Chem.* **1991**, *34*, 1340 – 1344.
[54] K. Niiya, R. A. Olsson, R. D. Thompson, S. K. Silvia, M. Ueeda, *J. Med. Chem.* **1992**, *35*, 4557 – 4561.
[55] K. Niiya, R. D. Thompson, S. K. Silvia, R. A. Olsson, *J. Med. Chem.* **1992**, *35*, 4562 – 4566.
[56] R. A. Mathôt, E. M. van der Wenden, W. Soudijn, A. P. IJzerman, M. Danhof, *Br. J. Pharmacol.* **1995**, *116*, 1957 – 1964.
[57] E. M. van der Wenden, J. K. von Frijtag Drabbe-Künzel, R. A. Mathôt, M. Danhof, A. P. IJzerman, W. Soudijn, *J. Med. Chem.* **1995**, *38*, 4000 – 4006.
[58] S. M. Siddiqui, K. A. Jacobson, J. L. Esker, M. E. Olah, X. D. Ji, N. Melman, K. N. Tiwari, J. A. Secrist III, S. W. Schneller, G. Cristalli, G. L. Stiles, C. R. Johnson, A. P. IJzerman, *J. Med. Chem.* **1995**, *38*, 1174 – 1188.
[59] S. Vittori, A. Lorenzen, C. Stannek, S. Costanzi, R. Volpini, A. P. IJzerman, J. K. von Frijtag Drabbe-Künzel, G. Cristalli, *J. Med. Chem.* **2000**, *43*, 250 – 260.
[60] D. Ukena, E. Böhme, U. Schwabe, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 36 – 42.
[61] B. K. Trivedi, R. F. Bruns, *J. Med. Chem.* **1989**, *32*, 1667 – 1673.
[62] G. Cristalli, A. Eleuteri, S. Vittori, R. Volpini, M. J. Lohse, K.-N. Klotz, *J. Med. Chem.* **1992**, *35*, 2363 – 2368.
[63] R. Volpini, C. Lambertucci, S. Taffi, S. Vittori, K.-N. Klotz, G. Cristalli, *Collect. Symp. Ser.* **2005**, *7*, 297 – 300.
[64] K. A. Jacobson, J. W. Daly, *Nucleosides Nucleotides* **1991**, *10*, 1029 – 1038.
[65] M. De Zwaart, R. Link, J. K. von Frijtag Drabbe-Künzel, G. Cristalli, K. A. Jacobson, A. Townsend-Nicholson, A. P. IJzerman, *Nucleosides Nucleotides* **1998**, *17*, 969 – 985.
[66] A. J. Bridges, R. F. Bruns, D. F. Ortwine, S. R. Priebe, D. L. Szotek, B. K. Trivedi, *J. Med. Chem.* **1988**, *31*, 1282 – 1285.
[67] G. Cristalli, M. Grifantini, S. Vittori, W. Balduini, F. Cattabeni, *Nucleosides Nucleotides* **1985**, *4*, 625 – 639.
[68] G. Cristalli, P. Franchetti, M. Grifantini, S. Vittori, K. N. Klotz, M. J. Lohse, *J. Med. Chem.* **1988**, *31*, 1179 – 1183.
[69] A. S. Robeva, R. L. Woodard, X. D. Ji, Z. Gao, S. Bhattacharya, H. E. Taylor, D. L. Rosin, J. Linden, *Drug Dev. Res.* **1996**, *39*, 243 – 252.
[70] K.-N. Klotz, J. Hessling, J. Hegler, B. Owman, B. Kull, B. B. Fredholm, M. J. Lohse, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 1 – 9.
[71] B. B. Fredholm, A. P. IJzerman, K. A. Jacobson, K.-N. Klotz, J. Linden, *Pharmacol. Rev.* **2001**, *53*, 527 – 552.
[72] K.-N. Klotz, H. Vogt, H. Tawfik-Schlieper, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 196 – 201.
[73] X.-D. Ji, D. K. J. E. von Lubitz, M. E. Olah, G. L. Stiles, K. A. Jacobson, *Drug Dev. Res.* **1994**, *33*, 51 – 59.
[74] G. Cristalli, E. Camaioni, S. Costanzi, S. Vittori, R. Volpini, K.-N. Klotz, *Drug Dev. Res.* **1998**, *45*, 176 – 181.
[75] K.-N. Klotz, E. Camaioni, R. Volpini, S. Kachler, S. Vittori, G. Cristalli, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *360*, 103 – 108.
[76] R. Volpini, E. Camaioni, S. Costanzi, S. Vittori, K.-N. Klotz, G. Cristalli, *Nucleosides Nucleotides* **1999**, *18*, 2511 – 2520.
[77] R. Volpini, S. Costanzi, C. Lambertucci, S. Taffi, S. Vittori, K.-N. Klotz, G. Cristalli, *J. Med. Chem.* **2002**, *45*, 3271 – 3279.
[78] R. Volpini, S. Costanzi, C. Lambertucci, S. Vittori, G. Cristalli, *Curr. Pharm. Des.* **2002**, *8*, 2285 – 2298.
[79] M. Ohno, Z.-G. Gao, P. Van Rompaey, S. Tchilibon, S.-K. Kim, B. A. Harris, A. S. Gross, H. T. Duong, S. Van Calenbergh, K. A. Jacobson, *Bioorg. Med. Chem.* **2004**, *12*, 2995 – 3007.
[80] J. E. Francis, R. L. Webb, G. R. Ghai, A. J. Hutchison, M. A. Moskal, R. de Jesus, R. Yokoyama, S. L. Rovinski, N. Contardo, R. Dotson, B. Barclay, G. A. Stone, M. F. Jarvis, *J. Med. Chem.* **1991**, *34*, 2570 – 2579.
[81] H. H. Stein, P. Somani, R. N. Prasad, *Ann. N. Y. Acad. Sci.* **1975**, *255*, 380 – 389.
[82] J. W. Daly, W. L. Padgett, S. I. Secunda, R. D. Thompson, R. A. Olsson, *Pharmacology* **1993**, *46*, 91 – 100.
[83] A. Hasan, T. Hussain, S. J. Mustafa, P. C. Srivastava, *Bioconjugate Chem.* **1994**, *5*, 364 – 369.
[84] G. Cristalli, U.S. Patent no 60/184,475, **2000**.
[85] R. Volpini, S. Costanzi, C. Lambertucci, F. R. Portino, S. Taffi, S. Vittori, K.-N. Klotz, G. Cristalli, *ARKIVOC* **2004**, *V*, 301 – 311.
[86] Z.-G. Gao, L. K. Mamedova, P. Chen, K. A. Jacobson, *Biochem. Pharmacol.* **2004**, *68*, 1985 – 1993.
[87] J. M. Caddell, A. M. Chapman, B. E. Cooley, B. P. Downey, M. P. LeBlanc, M. M. Jackson, T. M. O'Connell, H.-M. Phung, T. D. Roper, S. Xie, *J. Org. Chem.* **2004**, *69*, 3212 – 3215.
[88] M. P. Bosch, F. Campos, I. Niubo, G. Rosell, J. L. Diaz, J. Brea, M. I. Loza, A. Guerriero, *J. Med. Chem.* **2004**, *47*, 4041 – 4053.
[89] R. N. Prasad, D. S. Bariana, A. Fung, M. Savic, K. Tietje, H. H. Stein, H. Brondyk, R. S. Egan, *J. Med. Chem.* **1980**, *23*, 313 – 319.
[90] A. Pinna, J. Wardas, G. Cristalli, M. Morelli, *Brain Res.* **1997**, 759, 41 – 49.
[91] C. Lambertucci, R. Volpini, S. Costanzi, S. Taffi, S. Vittori, G. Cristalli, *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*(5 – 8), 809 – 812.
[92] S. Vittori, S. Costanzi, C. Lambertucci, F. R. Portino, S. Taffi, R. Volpini, K.-N. Klotz, G. Cristalli, *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*-(1 – 2), 471 – 481.
[93] S. Vittori, R. Volpini, C. Lambertucci, S. Taffi, K.-N. Klotz, G. Cristalli, *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*(1 – 2), 935 – 938.
[94] J. M. Rieger, M. L. Brown, G. W. Sullivan, J. Linden, T. L. Macdonald, *J. Med. Chem.* **2001**, *44*, 531 – 539.
[95] K. A. Jacobson, S. M. Siddiqui, M. E. Olah, X. D. Ji, N. Melman, K. Bellamkonda, Y. Meshulam, G. L. Stiles, H. O. Kim, *J. Med. Chem.* **1995**, *38*, 1720 – 1735.
[96] R. Volpini, E. Camaioni, S. Vittori, L. Barboni, C. Lambertucci, G. Cristalli, *Helv. Chim. Acta* **1998**, *81*, 145 – 152.
[97] M. De Zwaart, A. Kourounakis, H. Kooijman, A. L. Spek, R. Link, J. K. von Frijtag Drabbe-Künzel, A. P. IJzerman, *J. Med. Chem.* **1999**, *42*, 1384 – 1392.
[98] M. Olah, G. Stiles, *Pharmacol. Ther.* **2000**, *85*, 55 – 75.
[99] S. Ferrè, G. Von Euler, J. Johansson, B. B. Fredholm, K. Fuxe, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 7238 – 7241.
[100] E. Ongini, B. B. Fredholm, *Trends Pharmacol. Sci.* **1996**, *17*, 364 – 372.
[101] F. Impagnatiello, E. Bustin, E. Ongini, A. Monopoli, *Emerging Ther. Targets* **2000**, *4*, 635 – 664.
[102] P. J. Richardson, H. Kase, P. J. Jenner, *Trends Pharmacol. Sci.* **1997**, *18*, 338 – 344.
[103] A: Monopoli, G. Lozza, A. Furlani, A. Mattavelli, E. Ongini, *NeuroReport* **1998**, *9*, 3955 – 3959.
[104] A, Melani, M. Gianfriddo, M. G. Vannucchi, S. Cipriani, P. G. Baraldi, M. G. Giovannini, F. Pedata, *Brain Res.* **2006**, 470 – 480.
[105] B. Cacciari, G. Pastorin, G. Spalluto, *Curr. Top. Med. Chem.* **2003**, *3*, 403 – 411.
[106] S. Moro, Z. G. Gao, K. A. Jacobson, G. Spalluto, *Med. Res. Rev.* **2006**, *26*, 131 – 159
[107] H. O. Kim, X. D. Ji, N. Melman, M. E. Olah, G. Stiles, K. A. Jacobson, *J. Med. Chem.* **1994**, *37*, 3373 – 3382.
[108] J. W. Daly, W. L. Padgett, M. T. Shamin, *J. Med. Chem.* **1986**, *29*, 1305 – 1308.
[109] T. W. Seale, K. A. Abla, M. T. Shamin, J. W. Carney, J. W. Daly, *Life Sci.* **1988**, *43*, 1671 – 1684.
[110] O. M. Abo-Salem, A. M. Hayallah, A. Bilkei-Gorzo, B. Filipek, A. Zimmer, C. E. Muller, *J. Pharmacol. Exp. Ther.* **2004**, *308*, 358 – 366.
[111] C. E. Muller, B. Stein, *Curr. Pharm. Des.* **1996**, *2*, 501 – 530.
[112] C. E. Muller, D. Deters, A. Dominik, M. Pawlowski, *Synthesis* **1998**, 1428 – 1436.
[113] J. Shimada, F. Suzuki, H. Nonaka, A. Ishii, S. Ichikawa, *J. Med. Chem.* **1992**, *35*, 2342 – 2345.

[114] H. Nonaka, M. Ichimura, M. Takeda, Y. Nonaka, J. Shimada, F. Suzuki, K. Yamaguchi, H. Kase, *Eur. J. Pharmacol. Mol. Pharmacol. Sect.* **1994**, *267*, 335 – 341.
[115] K. A. Jacobson, C. Gallo-Rodriguez, N. Melman, B. Fischer, M. Maillard, A. van Bergen, P. M. J. van Galen, Y. J. Karton, *J. Med. Chem.* **1993**, *36*, 1333 – 1342.
[116] E. K. Jackson, W. A. Herzer, F. Suzuki, *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1304 – 1310.
[117] Y. Nonaka, J. Shimada, H. Nonaka, N. Koike, N. Aoki, H. Kobayashi, H. Kase, K. Yamaguchi, F. Suzuki, *J. Med. Chem.* **1993**, *36*, 3731 – 3733.
[118] J. Shimada, N. Koike, H. Nonaka, S. Shiozaki, K. Yanagawa, T. Kanda, H Kobayashi, M. Ichimura, J. Nakamura, H. Kase, F. Suzuki, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2349 – 2352.
[119] C. E. Muller, U. Geis, J. Hipp, U. Schobert, W. Frobenius, M. Pawlowski, F. Suzuki, J. Sandoval-Ramirez, *J. Med. Chem.* **1997**, *40*, 4396 – 4405.
[120] M. T. Shamin, D. Ukena, W. L. Padgett, J. W. Daly, *J. Med. Chem.* **1989**, *32*, 1231 – 1237.
[121] M. R. Del Giudice, A. Borioni, C. Mustazza, F. Gatta, S. Dionisotti, C. Zocchi, E. Ongini, *Eur. J. Med. Chem.* **1996**, *31*, 59 – 63.
[122] R. H. Erickson, R. N. Hiner, S. W. Feeney, P. R. Blake, W. J. Rzeszotarski, P. Hicks, D. G. Costello, M. E. Abreu, *J. Med. Chem.* **1991**, *34*, 1431 – 1435.
[123] K. A. Jacobson, D. Shi, C. Gallo-Rodriguez, M. Manning, C. E. Muller, J. W. Daly, J. L. Neumeyer, L. Kiriasis, W. Pfeiderer, *J. Med. Chem.* **1993**, *36*, 2639 – 2644.
[124] C. E. Muller, U. Schobert, J. Hipp, U. Geis, W. Frobenius, M. Pawlowski, *Eur. J. Med. Chem.* **1997**, *32*, 709 – 719.
[125] C. E. Muller, J. Sandoval-Ramirez, U. Schobert, U. Geis, W. Frobenius, K. N. Klotz, *Bioorg. Med. Chem.* **1998**, *6*, 707 – 719.
[126] R. Sauer, J. Maurinsh, U. Reith, F. Fulle, K. N. Klotz, C. E. Muller, *J. Med. Chem.* **2000**, *43*, 440 – 448.
[127] L. J. Knutsen, S. M. Weiss, *Curr. Opin. Invest. Drugs* **2001**, *2*, 668 – 673 KW-6002 (Kyowa Hakko Kogyo).
[128] S. M. Weiss, K. Benwell, L. A. Cliffe, R. J. Gillespie, A. R. Knight, J. Lerpiniere, A. Misra, R. M. Pratt, D. Rivell, R. Uptan, C. T. Daurish, *Neurology* **2003**, *61*, S101-S106.
[129] J. Hockemeyer, J. C. Burbiel, C. E. Muller, *J. Org. Chem.* **2004**, *69*, 3308 – 3318.
[130] J. E. Francis, W. D. Cash, S. Psychoyos, G. Ghai, P. Wenk, R. C. Friedmann, C. Atkins, V. Warren, P. Furness, J. L. Hyun, G. A. Stone, M. Desai, M. Williams, *J. Med. Chem.* **1988**, *31*,1014 – 1020.
[131] M. Williams, J. Francis, G. Ghai, A. Braunwalder, S. Psychoyos, G. A. Stone, W. D. Cash, *J. Pharmacol. Exp. Ther.* **1987**, *241*, 415 – 420.
[132] Y. C. Kim, X. Ji, K. A. Jacobson, *J. Med. Chem.* **1996**, *39*, 4142 – 4148.
[133] P. G. Baraldi, B. Cacciari, R. Romagnoli, G. Spalluto, S. Moro, K. N. Klotz, E. Leung, K. Varani, S. Gessi, S. Merighi, P. A. Borea, *J. Med. Chem.* **2000**, *43*, 4768 – 4780.
[134] R. Sarges, H. R. Howard, R. G. Browne, L. A. Lebel, P. A. Seymour, B. K. Koe, *J. Med. Chem.* **1990**, *33*, 2240 – 2254.
[135] F. Gatta, M. R. Del Giudice, A. Borioni, P. A. Borea, S. Dionisotti, E. Ongini, *Eur. J. Med. Chem.* **1993**, *28*, 569 – 577.
[136] S. Dionisotti, A. Conti, D. Sandoli, C. Zocchi, F. Gatta, E. Ongini, *Br. J. Pharmacol.* **1994**, *112*, 659 – 665.
[137] P. G. Baraldi, S. Manfredini, D. Simoni, L. Zappaterra, C. Zocchi, S. Dionisotti, E. Ongini, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2539 – 2544.
[138] P. G. Baraldi, B. Cacciari, G. Spalluto, M. J. Pineda, C. Zocchi, S. Dionisotti, E. Ongini, *J. Med. Chem.* **1996**, *39*, 1164 – 1171.
[139] P. G. Baraldi, B. Cacciari, R. Romagnoli, K. N. Klotz, G. Spalluto, K. Varani, S. Gessi, S. Merighi, P. A. Borea, *Drug Dev. Res.* **2001**, *53*, 225 – 235.
[140] P. G. Baraldi, B. Cacciari, G. Spalluto, M.J Pineda, C. Zocchi, S. Ferrara, S. Dionisotti, *Il Farmaco* **1996**, *51*, 297 – 300.
[141] C. Zocchi, E. Ongini, A. Conti, A. Monopoli, A. Negretti, P. G. Baraldi, S. Dionisotti, *J. Pharmacol. Exp. Ther.* **1996**, *276*, 398 – 404.
[142] P. G. Baraldi, B. Cacciari, G. Spalluto, M. Bergonzoni, S. Dionisotti, E. Ongini, K. Varani, P. A. Borea, *J. Med. Chem.* **1998**, *41*, 2126 – 2133.
[143] S. Todde, R. M. Moresco, P. Simonelli, P. G. Baraldi, B. Cacciari, G. Spalluto, K. Varani, A. Monopoli, M. Matarrese, A. Carpimnelli, F. Magni, M. Galli Kienle, F. Fazio, *J. Med. Chem.* **2000**, *43*, 4359 – 4362.
[144] P. G. Baraldi, B. Cacciari, R. Romagnoli, G. Spalluto, A. Monopoli, E. Ongini, K. Varani, P. A. Borea, *J. Med. Chem.* **2002**, *45*, 115 – 126.
[145] V. Colotta, D. Catarzi, F. Varano, L. Cecchi, G. Filacchioni, C. Martini, L. Trincavelli, A. Lucacchini, *J. Med. Chem.* **2000**, *43*, 1158 – 1164.
[146] V. Colotta, D. Catarzi, F. Varano, G. Filacchioni, C. Martini, L. Trincavelli, A. Lucacchini, *Bioorg. Med. Chem.* **2003**, *11*, 5509 – 5518.
[147] V. Colotta, D, Catarzi, F. Varano, L. Cecchi, G. Filacchioni, C. Martini, L. Trincavelli, A. Lucacchini, *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 39 – 41.
[148] J. J. Matasi, J. P. Cadwell, J. Hao, B. Neustadt, L. Arik, C. J. Foster, J. Lachowicz, D. B. Tulshian, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1333 – 1336.
[149] P. W. R. Caulkett, G. Jones, M. McPartlin, N. D. Renshaw, S. K. Stewart, B. Wright, *J. Chem. Soc. Perkin Trans. 1* **1995**, 801 – 808.
[150] S. M. Poucher, J. R. Keddie, P. Singh, S. M. Stoggall, P. W. R. Caulkett, G. Jones, M. G. Collis, *Br. J. Pharmacol.* **1995**, *115*, 1096 – 1102.
[151] M. De Zwaart, R. C. Vollinga, M. W. Beukers, D. F. Sleegers, J. K. von Frijtag Drabbe-Künzel, M. De Groote, A. P. IJzerman, *Drug Dev. Res.* **1999**, *48*, 95 – 103.
[152] X. D. Ji, K. A. Jacobson, *Drug Des. Discovery* **1999**, *16*, 217 – 226.
[153] C. B. Vu, D. Pan, B. Peng, G. Kumaravel, D. Phadke, T. Engber, C. Huamg, J. Reilly, S. Tam, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4831 – 4834.
[154] C. B. Vu, P. Shields, B. Peng, G. Kumaravel, X. Jin, D. Phadke, J. Wang, T. Engber, E. Ayyub, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4835 – 4838.
[155] C. B. Vu, B. Peng, G. Kumaravel, G. Smits, X. Jin, D. Phadke, T. Enber, C. Huang, J. Reilly, S. Tam, D. Grant, G. Hetu, L. Chen, J. Zhang, R. C. Petter, *J. Med. Chem.* **2004**, *47*, 4291 – 4299.
[156] B. Peng, G. Kumaravel, G. Yao, L. Sha, H. Van Vlijmen, T. Bohnert, C. Huang, C. B. Vu, C. L. Ensinger, H. Chang, T. M. Engber, E. Whalley, R. C. Petter, *J. Med. Chem.* **2004**, *47*, 6218 – 6229.
[157] C. B. Vu, D. Pan, B. Peng, G. Kumaravel, G. Smits, X. Jin, D. Phadke, T. Engber, C. Huang, J. Reilly, S. Tam, D. Grant, G. Hetu, R. C. Petter, *J. Med. Chem.* **2005**, *48*, 2009 – 2018.
[158] M. H. Holschbach, D. Bier, S. Stusgen, W. Wutz, W, Sihve, H. H. Coenen, R. A. Olsson, *Eur. J. Med. Chem.* **2006**, *41*, 7 – 15.
[159] G. Yao, S. Haque, L. Sha, G. Kumaravel, J. Wang, T. M. Engber, E. T. Whalley, P. R. Conlon, H. Chang, W. F. Kiesman, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 511 – 515.
[160] J. E. Dowling, J. T. Vessels, S. Haque, H. X. Chang, K. van Vloten, G. Kumaravel, T. Engber, X. Jin, D. Phadke, J. Wang, E. Ayyub, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4809 – 4813.
[161] E. Kiselgof, D. B. Tulshian, L. Arik, H. Zhang, A. Fawzi, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2119 – 2122.
[162] P. Minetti, M. O. Tinti, P. Carminati, M. Castorina, M. A. Di Cesare, S. Di Serio, G. Gallo, O. Ghirardi, F. Giorgi, L. Giorgi, G. Piersanti, F. Bartoccini, G. Tarzia, *J. Med. Chem.* **2005**, *48*, 6887 – 6896.
[163] A. Pinna, R. Volpini, G. Cristalli, M. Morelli, *Eur. J. Pharmacol.* **2005**, *512*, 157 – 164.
[164] a) M. Chebib, D. McKeveney, R. J. Quinn, *Bioorg. Med. Chem.* **2000**, *8*, 2581 – 2590; b) "Benzothiazole derivatives": A. Flhor, C. Riemer, WO 2005 EP07592 20050713, **2006**; c) A. Alanine, L. Anselm, L. Steward, S. Thomi, W. Vifian, M. D. Groaning, *Bioorg. Med. Chem.* **2004**, *14*, 817 – 821.
[165] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, T. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739 – 745.
[166] H. Barbhaiya, R. McClain, A. IJzerman, S.A Rivkees, *Mol. Pharmacol.* **1996**, *50*, 1635 – 1642.
[167] A.-O. Colson, J. H. Perlman, ; A. Jinsi-Parimoo, D. R. Nussenzveig, R. Osman, M. C. Gershengorn, *Mol. Pharmacol.* **1998**, *54*, 968 – 978.
[168] Z.-G. Gao, S.-K. Kim, T. Biadatti, Q. Chen, K. Lee, D. Barak, S. G. Kim, C. R. Johnson, K. A. Jacobson, *J. Med. Chem.* **2002**, *45*, 4471 – 4484.
[169] S.-K. Kim, Z. G. Gao, P. Van Rompaey, A. S. Gross, A. Chen, S. Van Calenbergh, K. A. Jacobson, *J. Med. Chem.* **2003**, *46*, 4847 – 4859.
[170] S. Moro, F. Deflorian, G. Spalluto, K. A. Jacobson, *Chem. Commun.* **2003**, *21*, 2949 – 2956.
[171] S. Moro, G. Spalluto, K. A. Jacobson, *Trends Pharmacol. Sci.* **2005**, *26*, 44 – 51.
[172] B. Cacciari, G. Pastorin, C. Bolcato, G. Spalluto, M. Bacilieri, S. Moro, *Mini-Rev. Med. Chem.* **2005**, *5*, 1053 – 1060.
[173] S. Moro, F. Deflorian, M. Bacilieri, G. Spalluto, *Curr. Med. Chem.* **2006**, *13*, 639 – 645.
[174] S. Moro, F. Deflorian, M. Bacilieri, G. Spalluto, *Curr. Pharm. Des.* **2006**, *12*, 2175 – 2185.

[175] S. Moro, M. Bacilieri, F. Deflorian, G. Spalluto, *New J. Chem.* **2006**, *30*, 301 – 308.
[176] S. Moro, A.-H. Li, K. A. Jacobson, *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 1239 – 1248.
[177] J. Kim, J. Wess, ; A. M. van Rhee, T. Schöneberg, K. A. Jacobson, *J. Biol. Chem.* **1995**, *270*, 13 987 – 13 997.
[178] P. G. Baraldi, B. Cacciari, S. Moro, G. Spalluto, G. Pastorin, T. D. Ros, K.-N. Klotz, K. Varani, S. Gessi, P. A. Borea, *J. Med. Chem.* **2002**, *45*, 770 – 780.
[179] K. Lee, R. G. Ravi, X-d. Ji, V. E. Marquez, K. A. Jacobson, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1333 – 1337.
[180] D. L. Farrens, C. Altenbach, K. Yang, W. L. Hubell, H. G. Khorana, *Science* **1996**, *274*, 768 – 770.
[181] L. Shi, ; G. Liapakis, ; R. Xu, F. Guarneri, J. A. Ballesteros, J. A. Javitch, *J. Biol. Chem.* **2002**, *277*, 40 989 – 40 996.
[182] J. Liu, B. R. Conklin, N. Blin, J. Yun, J. Wess, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11 642 – 11 646.
[183] A. L. Tucker, J. Linden, *Cardiovasc. Res.* **1993**, *27*, 62 – 67.
[184] L. Belardinelli, *Drug Dev. Res.* **1993**, *28*, 263 – 267.
[185] B. D. Bertolet, L. Belardinelli, E. A. Franco, W. W. Nichols, R. A. Kerensky, J. A. Hill, *Circulation* **1996**, *93*, 1871 – 1876.
[186] V. Rankumar, G. L. Stiles, M. A. Beaven, H. Ali, *J. Biol. Chem.* **1993**, *268*, 16 887 – 16 890.
[187] P. L. Martin, R. J. Barrett, J. Linden, W. M. Abraham, *Drug Dev. Res.* **1997**, *40*, 313 – 324.
[188] R. C. Hendel, T. M. Bateman, M. D. Cerqueira, A. E. Iskandrian, J. A. Leppo, B. Blackburn, J. J. Mahmarian, *J. Am. Coll. Cardiol.* **2005**, *46*, 2069 – 2075.
[189] A. K. Dhalla, M.-Y. Wong, W.-Q. Wang, I. Biaggioni, L. Belardinelli, *J. Pharmacol. Exp. Ther.* **2006**, *316*, 695 – 702.
[190] M. D. Cerqueira, *Am. J. Cardiol.* **2004**, *94(suppl)*, 33D – 42D.
[191] J. E. Udelson, G. V. Heller, F. J. Wackers, A. Chai, D. Hinchman, P. S. Coleman, V. Dilsizian, M. DiCarli, R. Hachamovitch, J. R. Johnson, R. J. Barrett, R. J. Gibbons, *Circulation* **2004**, *109*, 457 – 464.
[192] D. O. Okonkwo, T. B. Reece, J. J. Laurent, A. S. Hawkins, P. I. Ellman, J. Linden, I. L. Kron, C. G. Tribble, J. R. Stone, J. A. Kern, *J. Neurosurg. Spine* **2006**, *4*, 64 – 70.
[193] A. S. Awad, L. Huang, H. Ye, E. T. Duong, W. K. Bolton, J. Linden, M. D. Okusa, *Am. J. Physiol. Renal Physiol.* **2006**, *290*, F828/F837.
[194] M. C. Montesinos, J. P. Shaw, H. Yee, P. Shamamian, B. N. Cronstein, *Am. J. Pathol.* **2004**, *164*, 1887 – 1892.
[195] A. Desai, C. Victor-Vega, S. Gadangi, M. C. Montesinos, C. C. Chu, B. N. Cronstein, *Mol. Pharmacol.* **2005**, *67*, 1406 – 1413.
[196] G. Hasko, D.-Z. Xu, Q. Lu, Z. H. Nemeth, J. Jabush, T. L. Berezina, S. B. Zaets, B. Csoka, E. A. Deitch, *Crit. Care Med.* **2006**, *34*, 1119 – 1125.
[197] T. Konno, T. Uchibori, A. Nagai, K. Kogi, N. Nakahata, *Eur. J. Pharmacol.* **2005**, *518*, 203 – 211.
[198] Z.-Y. Hong, Z.-L. Huang, W.-M. Qu, N. Eguchi, Y. Urade, O. Hayaishi, *J. Neurochem.* **2005**, *92*, 1542 – 1549.
[199] G. W. Sullivan, *Curr. Opin. Invest. Drugs* **2003**, *4*, 1313 – 1319.
[200] T. W. Stone, *Neurol. Res.* **2005**, *27*, 161 – 168.
[201] L. Yan, J. C. Burbiel, A. Maaß, C. E. Müller, *Expert Opin. Emerging Drugs* **2003**, *8*, 537 – 576.
[202] G. Burnstock, *Pharmacol. Rev.* **2006**, *58*, 58 – 86.
[203] T. H. Johnston, J. M. Brotchie, *Curr. Opin. Invest. Drugs* **2006**, *7*, 25 – 32.
[204] R. A. Hauser, J. P. Hubble, D. D. Truong, *Neurology* **2003**, *61*, 297 – 303.
[205] M. A. Schwarzschild, X. K. Oztas, J. P. Petzer, K. Castagnoli, N. Castagnoli, Jr., J. F. Chen, *Neurology* **2003**, *61*, S55 – S61.
[206] W. Bara-Jimenez, A. Sherzai, T. Dimitrova, A. Favit, F. Bibbiani, M. Gillespie, M. J. Morris, M. M. Mouradian, T. N. Chase, *Neurology* **2003**, *61*, 293 – 296.
[207] Biogen IDEC Inc.: Vernalis and Biogen Idec to collaborate on research for Parkinson's disease, (Press release, June **2004**): http://biogen.com/news/BiogenIDECPR_045.htm.
[208] Schering–Plough Corp.: Schering–Plough product pipeline: Worldwide prescription products, (Company website, November **2005**): http://www.sch-plough.com/pdf/productpipeline.pdf.
[209] Adenosine Therapeutics LLC: Product pipeline for 2005, (Company website **2005**): http://www.adenrx.com/pipe.html.
[210] Neurocrine Biosciences Inc.: Research program for 2005, (Company website **2005**): http://www.neurocrine.com/html/res_researchMain.html.

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