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Medicinal Chemistry of A_3 Adenosine Receptor Modulators: Pharmacological Activities and Therapeutic Implications

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INTRODUCTION

The purine nucleoside adenosine is identified as a ubiquitous molecule regulator of different tissues and cell functions.[1] Adenosine is generated in the extracellular space by the breakdown of adenosine 5′-triphosphate (ATP) through a series of ectoenzymes, including apyrase and ecto-5′-nucleotidase.[2] Inside the cell, adenosine is phosphorylated to adenosine 5′-monophosphate (AMP) by adenosine kinase or degraded to inosine by adenosine deaminase.[3] Adenosine production from the hydrolysis of AMP is mediated by a cytosolic 5′-nucleotidase or by the hydrolysis of *S*-adenosylhomocysteine.[3] The levels of adenosine in the interstitial fluid are in the range 20−200 nM even if dramatically increased under metabolically unfavorable conditions.[4] Adenosine effects are widespread and closely associated with the expression of different adenosine receptor (AR) subtypes which can be coexpressed and serve as active modulators in the cell signaling transduction.[5] ARs are characterized by seven transmembrane domains connected by different intracellular and extracellular loops.[4] A_1AR stimulation through the interaction with various members of pertussis toxin-sensitive family of G proteins modulates different cellular effectors as adenylate cyclase (AC) and phospholypase C (PLC).[4] The A_{2A} and A_{2B}ARs through coupling with Gs proteins activate AC and increase cyclic AMP levels.[4] A_3ARs, via the interaction with Gi proteins, inhibit adenylate cyclase, decreasing cyclic AMP accumulation and protein kinase A (PKA) activity. In addition, A_3ARs, by coupling with Gq proteins, stimulate PLC, causing an increase of calcium levels from intracellular stores, and modulate the protein kinase C (PKC) activity.[6] From the molecular point of view, the presence of histidine residues at the C-terminus of A_3ARs is responsible for the cell signaling transduction mechanisms. In addition, the presence of serine and threonine residues is involved in the desensitization and down-regulation of the receptors.[7] There is considerable evidence for A_3ARs to modulate the regulatory pathways of the mitogen-activated kinases (MAPKs) that consist of the extracellular signal regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 kinases.[4] Different effects of A_3AR activation on the Akt/Ras/Raf/MEK/ERK signaling pathway modulation in different cells have been reported (Figure 1).[8] A strong link is well evident between A_3ARs and hypoxia inducible factor 1α (HIF-1α), signaling that represents the main transcription factor regulating the cellular responses in hypoxia.[9] It is well reported that the MAPK pathway is regulated by A_3ARs through a feedback mechanism that controls G-protein-coupled receptor kinase 2 (GRK2) activity and involves a specific receptor phosphorylation.[10] The activation of A_3ARs causes the accumulation of arrestin 3 in plasma membranes through the translocation correlated with receptor sensitivity to GRK-mediated phosphorylation.[10] It has been reported that a short time, about 10 min, of agonist exposure results in a rapid A_3AR internalization and in a functional desensitization as observed by the reduction of the inhibition of forskolin-stimulated AC.[11] A prolonged treatment, about 20 h, with the A_3AR agonists induces uncoupling of the receptor and functional desensitization associated with the receptor down-regulation.[11] Despite this A_3AR desensitization, the adenylate cyclase activity is not reduced as observed from experiments performed in the presence of forskolin stimulation. In addition, the removal of the agonists mediates, in about 35 min, a restoration of the receptor functionality and recycling to plasma membrane.[10] Among the four ARs, A_3ARs are the latest cloned and pharmacologically characterized: the amino acid sequence of the human A_3ARs (hA_3ARs) is 54%, 48%, and 44% identical in sequence to hA_1, hA_{2A}, hA_{2B}ARs, respectively.[12] Among the various species, rat A_3ARs (rA_3ARs) are significantly different from human, having 74% of identical sequence whereas 85% homology is shown between sheep and human A_3ARs. Moreover, while A_3ARs mRNA were found in rat testis, heart, and lung and at low levels in various brain areas,[13] a significant expression of human A_3ARs mRNA has been observed in many peripheral tissues with lower levels in the central nervous system and testis.[14] As a consequence, the pronounced species-dependent differences of A_3ARs in tissue distribution and in the pharmacology hamper the evaluation of the potential therapeutic characteristics in animal models.[2] The tissue distribution of A_3ARs has been well investigated and suggests that these receptors are primarily expressed in lung, liver, and immune cells. A minor expression of A_3ARs is reported in kidney, heart, brain, and gastrointestinal tissues.[12] The widespread distribution in different cells and tissues of the A_3ARs could suggest their potential involvement in various pathologies and the possible use as a selective pharmacological target (Figure 2).

The presence of A_3ARs has been studied by using different experimental approaches as radioligand binding, mRNA, Western blotting analysis, and functional assays in a variety of primary cell cultures, native tissues, and cell lines.[15−19] In binding assays, several agonist or antagonist radioligands have been widely used in the characterization of A_3ARs (see the related section below). From a thermodynamic point of view all ARs have been analyzed adding important findings as the thermodynamic discrimination of agonists from antagonists and the enthalpy−entropy compensation.[20,21] In particular, the thermodynamic discrimination of ARs is also confirmed for A_3ARs, showing that agonist binding is entropy-driven probably because of the disorganization of a solvation area around the

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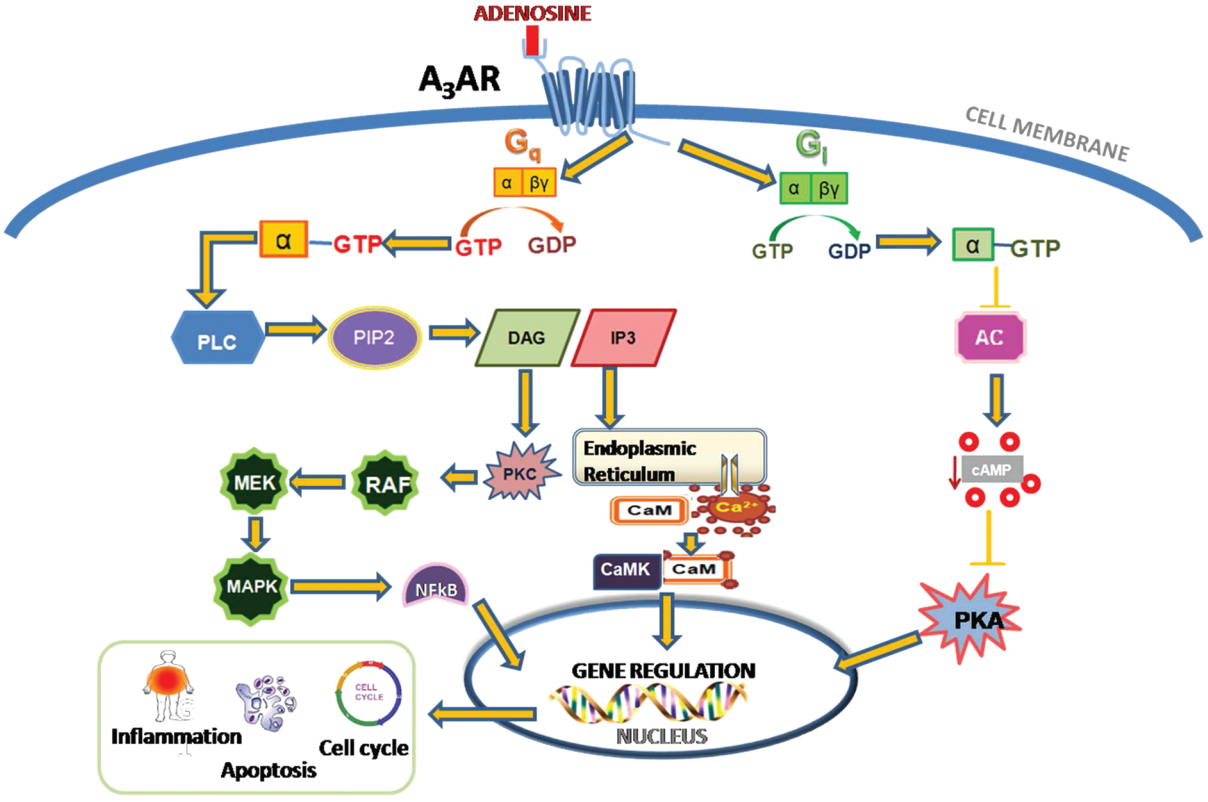

Figure 1. Schematic representation of signaling pathways mediated by A_3AR activation.

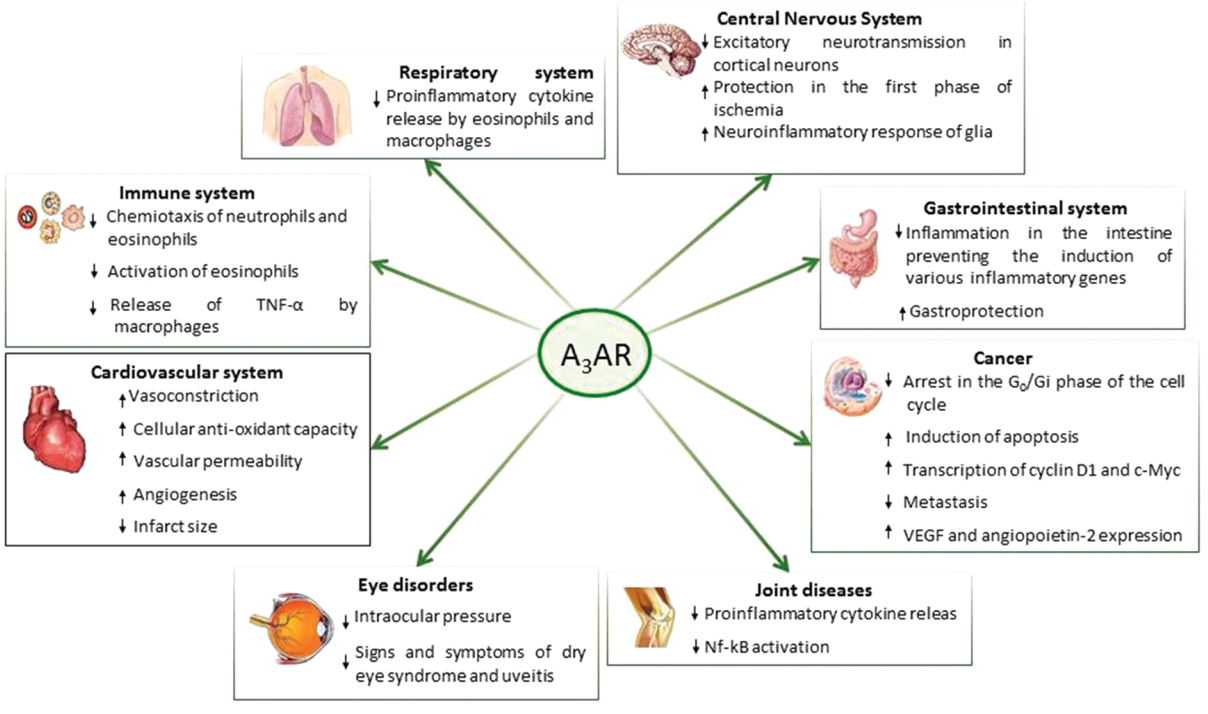

Figure 2. Schematic diagram illustrating the functional role of A_3ARs.

ligand–receptor interaction.[22,23] Moreover, the antagonist binding is mainly enthalpy driven for hydrogen bond formation and van der Waals interactions occurring between the ligands and the binding pocket.[22] Knowledge of the thermodynamic parameters could help the discovery and characterization of novel selective A_3AR agonists or antagonists.

A_3 ADENOSINE RECEPTOR AGONISTS

The vast majority of A_3AR agonists reported to date reflects the nucleoside structure of the endogenous orthosteric ligand, adenosine (**1**, Figure 3).[7,24−26] The most successful structural manipulations of the adenosine skeleton in enhancing A_3AR potency and selectivity involve N^6-, C^2-, and 5′-substitutions or

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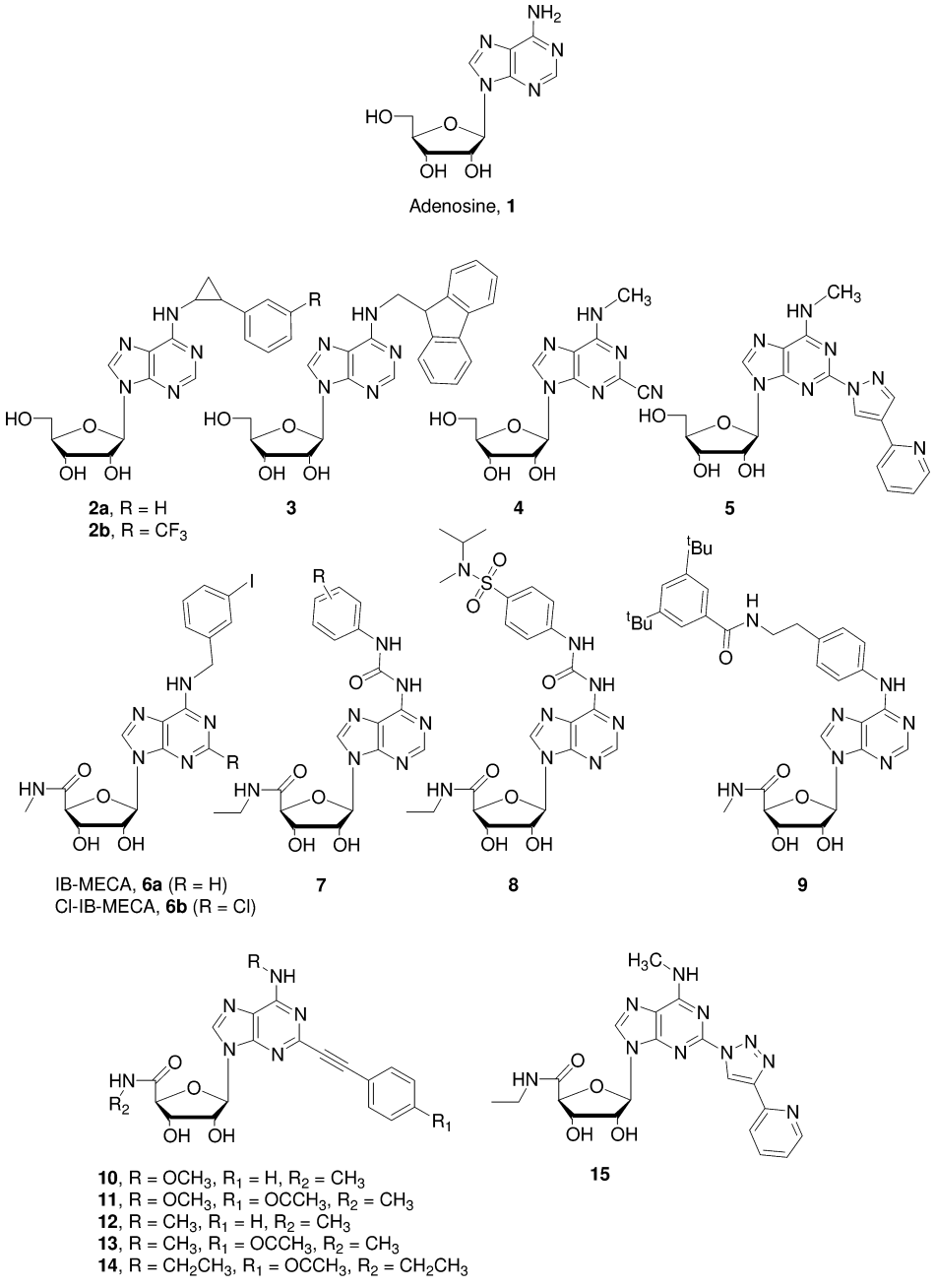

Figure 3. Representative collection of N^6-substituted (**2**, **3**), C^2-N^6-disubstituted (**4**, **5**), N^6-5′-disubstituted (**6a**, **7**−**9**), and C^2-N^6-5′-trisubstituted (**6b**, **10**−**15**) adenosine derivatives as A_3AR agonists.

suitable combinations of these. The ribose pentacycle has also been recognized as a possible object of structural modifications affecting both A_3AR binding affinity and efficacy. Recently, few examples of non-adenine nucleosides and non-nucleoside derivatives able to activate A_3ARs have been as well reported. In this section we intend to expand on and update our earlier reviews[26] and book chapters[62] on the examined topic.

***N*⁶-Substituted Adenosine Derivatives.** A large series of N^6-substituted adenosines has been reported by Gao et al. in 2003.[27] Addition of small groups such as a methyl or ethyl appeared preferred over larger or branched alkyl chains. N^6-Cyclobutyl and N^6-phenyl substitution resulted in potent A_3AR agonists even with higher affinity toward A_1AR subtype. N^6-Benzyl or N^6-phenylethyl substitution, although beneficial in terms of A_3AR vs A_1AR selectivity, determined a lower relative efficacy[28] at the A_3AR (55% and 84%, respectively). Among N^6-phenylethyl derivatives, the efficacy was partially or completely restored with modifications of the ethylenic bridge such as in compound **2a** (Figure 3) showing the best binding/activity profile of the series (see Table 1). Further SAR studies based on this molecule[29] led to the identification of N^6-(*trans*-2-(3-trifluoromethyl)phenyl)-1-cyclopropyl (**2b**) and N^6-(9-fluorenylmethyl) (**3**) as potent A_3AR agonists. Interestingly, the efficacy of these series was strictly dependent on the conformation properties of the N^6-substituent, as testified by the observation that N^6-(2,2-diphenylethyl)adenosine, unconstrained analogue of **3**, completely lost efficacy, behaving as an A_3AR antagonist (see compound **72** among the antagonists reported below).

***C*²-*N*⁶-Disubstituted Adenosine Derivatives.** Ohno et al. scrutinized different combination of C^2-N^6 disubstitution of

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Table 1. Binding Paremeters Expressed As Affinity Values (K_i, nM) and Selectivities of the Most Representative A_3AR Agonists

	K_i (nM)[a]				
	A_1AR	A_{2A}AR	A_3AR	A_1/A_3	A_{2A}/A_3
2a[29]	124 (h)	2530 (h)	0.86 (h)	144 (h)	2942 (h)
	10 (r)	2980 (r)	399 (r)		7.5 (r)
2b[29]	104 (h)	2370 (h)	1.9 (h)	55 (h)	1247 (h)
	16 (r)	459 (r)			
3[29]	14 (h)	145 (h)	0.91 (h)	15 (h)	161 (h)
	9.4 (r)	33 (r)			
4[28]	70 (h)	>10000 (h)	3.4 (h)	21 (h)	>2941 (h)
			>10000 (r)		
5[30]	3800 (h)	>5000 (h)	2 (h)	1900 (h)	>2500 (h)
6a[32,54]	51 (h)	2900 (h)	1.0 (h)	51 (h)	2900 (h)
	54 (r)	56 (r)	1.1 (r)	49 (r)	51 (r)
6b[37,54]	222 (h)	5360 (h)	1.4 (h)	159 (h)	3828 (h)
	820 (r)	470 (r)	0.33 (r)	2485 (r)	1424 (h)
8[35]	325 (h)	>1000 (h)	8.0 (h)	44 (h)	>125 (h)
9[36]	245 (h)	>10000 (h)	2.3 (h)	106 (h)	>4348 (h)
10[40]	9140 (h)	16300 (h)	1.9 (h)	4810 (h)	8579 (h)
11[40]	53800 (h)	10400 (h)	2.5 (h)	21520 (h)	4160 (h)
12[40]	32800 (h)	41700 (h)	0.44 (h)	75545 (h)	94773 (h)
13[40]	10200 (h)	7030 (h)	0.33 (h)	30909 (h)	21303 (h)
14[43]	558 (h)	4963 (h)	0.75 (h)	744 (h)	6617 (h)
15[44]	1640 (h)	>10000 (h)	1.8 (h)	911 (h)	>5555 (h)
16[43]	7300 (h)	>50000 (h)	5.8 (h)	1259 (h)	>8620 (h)
17[50]	1330 (h)	>10000 (h)	0.28 (h)	4750 (h)	>35714 (h)
18[50]	193 (h)	223 (h)	0.38 (h)	508 (h)	586 (h)
19[54]	20.2 (h)	475 (h)	0.25 (h)	81 (h)	1900 (h)
			1.86 (r)		
20[56]	260 (h)	2300 (h)	0.29 (h)	896 (h)	7931 (h)
			1.0 (r)		
21[59]	>10000 (h)	766 (h)	2.1 (h)	>4761 (h)	365 (h)
22[29,60]	>10000 (h)	5740 (h)	9.6 (h)	>1041 (h)	598 (h)
23[7]	37300 (r)	>10000 (r)	229 (r)	162 (r)	>44 (r)
24[7]	7.0 (h)	214 (h)	24 (h)		8.9 (h)

[a] K_i values are evaluated in human (h) or rat (r) tissues or cells. For detailed experimental conditions see the cited references.

the adenosine skeleton, evaluating the effect on ARs binding.[28] This study also highlighted the importance of C^2 substitution in modulating A_3AR activation efficacy. In particular, the introduction of a 2-CN group exerted opposite effect in relation to the kind of N^6-substitution. 2-Cyano-N^6-methyladenosine (**4**) was shown, indeed, to be a full agonist with improved affinity if compared with its C^2 unsubstituted analogue, while the same group at 2-position of derivative **2a** completely abolished A_3AR activation, resulting in a relatively potent antagonist. Other small groups, at the 2 position of N^6-methyladenosine (i.e., NH_2 or CF_3), instead decreased selectivity and affinity toward the A_3AR. Elzein et al. synthesized a series of 2-pyrazolyl-N^6-substituted adenosine derivatives as very potent and selective ligands for the A_3AR,[30] among which compound **5** (K_i = 2 nM) showed relevant selectivity for the A_3 versus A_1 and A_{2A} ARs (selectivity ratios of 1900 and >2500, respectively, Table 1) and was claimed for use in the treatment of cancer and neutropenia by CV Therapeutics.[31]

N^6-5′-Disubstituted Adenosine Derivatives. Potent and selective A_3AR agonists have been identified combining N^6-substitution with a 5′-uronamide function. The first A_3AR selective compounds combined a 5′-*N*-alkyluronamide with an N^6-benzyl group.[26] One of the most representative compound of this series, N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA, **6a**), was discovered in 1994.[32] After these findings, our research group prepared a series of N^6-arylcarbamoyl derivatives of NECA (5′-*N*-ethylcarboxamidoadenosine) with general structure **7** that exhibited good binding profiles as A_3AR agonists.[33−35] The most recent components of this series were functionalized at the N^6-position with a 4-(substituted)-sulfonamidophenylcarbamoyl moiety such as in compound **8**, which displayed good affinity and selectivity for the A_3AR.[35] The best results were observed with disubstitution of the sulfonamide group with small alkyl chains because of a slight enhancement of A_3AR vs A_1AR selectivity. The cardioprotective effects of a number of N^6-substituted adenosine-5′-*N*-methylcarboxamides, among them compound **9**, were recently demonstrated.[36] Although the exact nature of such cardioprotection mechanism would need further investigation, it has been observed that the pharmacologic effect was reversed by a selective A_3AR antagonist.

C^2-N^6-5′-Trisubstituted Adenosine Derivatives. The effect of different combinations of substitutions at the N^6- and C^2- positions of the adenine core of 5′-*N*-alkylcarbamoyladenosine derivatives has been largely investigated. The introduction of small groups (i.e., halogens, methylamino, or thiomethyl functions) at the C^2-position of **6a** generally increased both affinity and selectivity at the A_3AR subtype and led to the discovery of C^2-chloro-N^6-(3-iodobenyl)-5′-*N*-methylcarboxamidoadenosine (Cl-IB-MECA, **6b**) as a highly selective ligand, currently considered as the prototypical A_3AR agonist.[37] This compound has a K_i of 0.33 nM at the rA_3AR,

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with a K_i of 820 and 470 nM at rA_1 and rA_{2A} ARs, respectively. Cl-IB-MECA is known to be more selective for the rA_3AR than the hA_3AR (K_i values at the hARs: hA_1AR = 222 nM, $A_{2A}AR$ = 5360 nM, and A_3AR = 1.4 nM).[38] A further important contribution to the study of the effect of C^2-substitution has been furnished by Cristalli and co-workers who synthesized a wide series of 2-(ar)alkynyl derivatives of adenosine with variable affinity/selectivity profile at the ARs.[39] The best results in terms of affinity, selectivity, and intrinsic activity toward A_3AR have been achieved combining 2-arylalkynyl substitution with a methylcarboxamido function at the 4′-position of the ribose nucleus and small groups, such as a methyl[40] or a methoxy,[41] at the N^6-position (compounds **10**−**13**). *N*-Ethylcarboxamido derivatives of this series showed lower selectivity vs A_1AR and $A_{2A}AR$ subtypes if compared to the corresponding MECA-related compounds. N^6-Methyl substitution increased A_3ARs affinity from 3- to 8-fold if compared to unsubstitution, reaching subnanomolar values. Compound **12** stands out as one of the most potent and selective hA_3AR agonists so far reported. The authors highlighted the presence of an alkylcarboxamido group in the 4′-position to be essential to obtain full agonists at the A_3AR subtype, as adenosine-related analogues presented a profile from partial agonists to antagonists (see compound **71** of the antagonists section).[41] Molecular modeling studies of these derivatives have been recently performed.[42] A series of N^6-ethyl-2-alkynyl NECA derivatives has been later reported by Zhu et al. of which compound **14** exhibited hA_3AR affinity similar to that of **6a** or **6b** but with significantly improved hA_3/hA_1AR selectivity.[43]

Strictly in agreement with Cristalli's work, Cosyn et al. found relevant affinity for the A_3AR in a series of 2-triazolyl-N^6-methyladenosines,[44] although associated with reduced relative efficacy (see compound **75** of the antagonists section). Also this study confirmed that in order to maintain efficacy, a 5′-ethyluronamide was necessary such as in compound **15** displaying full agonism (efficacy of 90%) at the hA_3AR with a K_i of 1.8 nM and good selectivity over hA_1 (K_i = 1640) and $hA_{2A}ARs$ (displacement at 10 μM of 45%).

3′-OH Modification. Different studies highlighted the importance of both the 2′- and 3′-hydroxyl groups of the ribose nucleus to ensure efficacy and affinity of adenosine-related molecules at the hA_3AR. As a matter of fact, the replacement of either these moieties, especially the 2′-OH, with fluorine atoms determined a marked worsening of the binding profile.[45,46] DeNinno et al.[47] reinforced the hypothesis of a role of the 3′-OH group as a hydrogen bond donor, since 3′-NH_2 adenosine analogues, if properly modified at the 5′ and N^6 positions, preserved good affinity and selectivity at the A_3AR. The 3′-NH_2 group promoted as well as water solubility. Of this series, **CP608039**[47] (**16**, Figure 4) was chosen for clinical development as a full agonist and was able to bind hA_3ARs with a K_i of 5.8 nM with over 1000-fold selectivity versus the hA_1AR. Even greater selectivity was observed over the hA_{2A} and $hA_{2B}AR$ subtypes (K_i > 50 μM). Essentially, only small hydrogen bonding donors, like a hydroxyl or amino group, at the 3′-position of adenosine can be tolerated, since a series of 3′-acetamidoadenosine derivatives was shown to be totally devoid of affinity at the A_3AR.[48]

Modification of the Pentose Ring: 4′-Thio Derivatives. The bioisosteric replacement of the endocyclic oxygen of the pentose ring with a sulfur atom led Jeong at al. to the discovery of a series of N^6-substituted (2-Cl)-4′-thioadenosine-5′-uronamides[49,50] and N^6-substituted 4′-thioadenosines[51] as highly

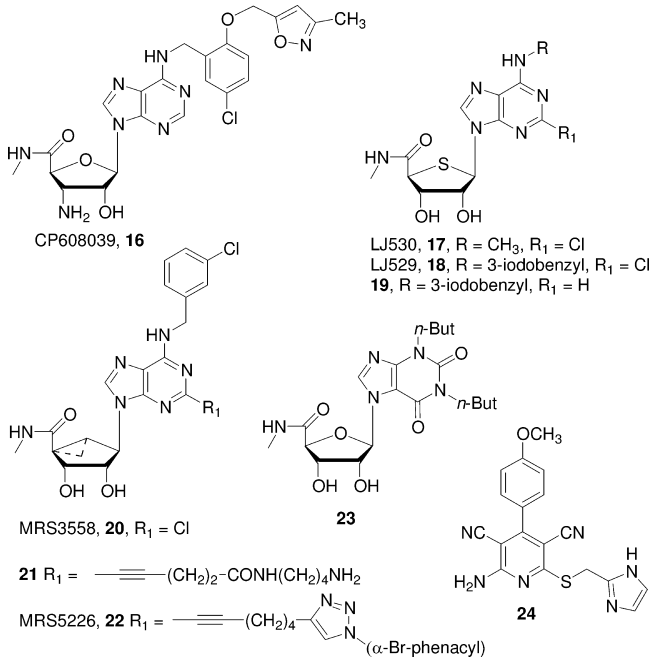

Figure 4. Representative collection of 3′-OH (**16**) or ribose (**17**−**22**) modified adenosine derivatives, nonadenine nucleosides (**23**), and non-nucleosides (**24**) as A_3AR agonists.

potent and selective agonists. Among all the synthesized 5′-uronamides, the 5′-methyluronamide function was the most helpful to promote A_3AR affinity and potency. LJ-530[50] (**17**, K_i = 0.28 nM) is one the most potent and selective compound of the series. The N^6-3-iodobenzyl analogues **18** also showed potent in vitro and in vivo anticancer activity.[52,53] The removal of the 2-Cl atom led to thio-IB-MECA (**19**), which is the most potent compound of the 4′-thioadenosine series, with high A_3AR affinity across species (K_i for the rat A_3AR of 1.86 nM), although such structural modification determined a decrease of A_3AR vs A_1AR selectivity.[54] Larger 5′-uronamide substituents occasionally led to reduction of efficacy, which was also observed in the 4′-thioadenosine series when a substituted benzyl group at the N^6 position was present.[51] For these regions, 4′-thioadenosines were also scrutinized as a source of adenosine-related antagonists for the A_3ARs (see the relevant section below).

Modification of the Pentose Ring: Methanocarba-(bicyclo[3.1.0]hexane) Ring Systems. Nucleoside analogues containing a rigid scaffold in place of the ribose ring have been explored as ligands for the ARs. A structure widely investigated by Jacobson and co-workers, for this purpose, is the methanocarba (bicyclo[3.1.0]hexane) ring system,[55] which is in line with receptor docking of the ribose ring, indicating that the endocyclic oxygen would not be required for interaction with ARs. In methanocarba analogues, a fused cyclopropane ring constrains the accompanying cyclopentane moiety to mimic the conformation of a rigid furanose ring held in either a Northern (N) or Southern (S) conformation. These ribose modifications were combined with modifications that had been previously shown to support A_3AR affinity. (N)-Methanocarba-adenosine was favored in A_3AR binding by 150-fold over the (S)-conformation and by 2.5-fold over adenosine. The (N)-methanocarba-N^6-(3-iodobenzyl)adenosine and its 2-chloro derivative had K_i values of 4.1 and 2.2 nM at A_3ARs, respectively, and were highly selective partial agonists. 5′-Alkyluronamide modification of the (N)-methanocarba

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nucleus increased by 6-fold the hA_3AR affinity. Of this series, MRS3558[56] (**20**) has the pharmacological profile of a full agonist with subnanomolar A_3AR potency. The utility of **20** in treating lung injury was shown in a model of ischemia reperfusion lung injury.[57] Gao et al. synthesized a radioiodinated form of the 3-iodobenzyl analogues of **20**, which was selected for radiolabeling because of its high A_3AR affinity across species, with nanomolar affinity at both rat and human A_3ARs.[58] Further SAR extension on **20**-related molecules[38] confirmed that a 5′-uronamide moiety would be essential to promote full agonism at the A_3AR, since 5′-OH substitution has been exploited to develop A_3AR antagonists. Moreover, 5′-uronamide in the (N)-methanocarba series, as in the ribose series, proved to counter the loss of efficacy associated with substitutions of the N^6-position. The introduction of 2-alkynyl chains of varying length gave rise to potent and selective agonists such as compound **21**.[59] The terminal reactive carboxylates, esters, or amino groups on the C^2-alkynyl chain of such derivatives have been exploited for conjugation with biotin or fluorescent cyanine dye in view of possible imaging employments. To the same purpose, a series of 2-dialkynyl (N)-methanocarba adenosines were later prepared[60] in which the distal alkyne was shown to react selectively with alkyl-/arylazides by click cycloaddition to form triazole derivatives of general structure **22**. These molecules displayed functional properties from partial to full agonists at the A_3AR. Among the latter, the substitution of the triazole nitrogen with a 4-(α-bromophenacyl) group led to the most potent and selective compound (MRS5226,[60] K_i = 9.6 nM).

Non-Adenine Nucleosides and Non-Nucleosides as A_3 Adenosine Receptor Agonists. The substitution of the adenine ring of MECA with a 1,3-disubstituted xanthine, as in compound **23** (*N*-ethyl-1,3-dibutylxanthine-7-β-D-ribofuronamide) led to the discovery of non-adenine A_3AR agonist with fair selectivity.[7] Moreover, a series of non-nucleoside agonist ligands with variable selectivity profile at the ARs were reported,[61] among which the pyridine-3,5-dicarbonitrile **24**, although having higher affinity at the A_1AR and potency at the $A_{2B}AR$, displayed an interesting affinity also at the A_3AR, which could be considered for the development of more selective A_3AR ligands in this series.

A_3 ADENOSINE RECEPTOR ANTAGONISTS

Initial attempts to obtain A_3AR antagonists focused on broad screening of different heterocyclic systems. Some xanthine or purine analogues were examined first, but none of the tested compounds showed significant affinity or selectivity at rA_3AR.[26] During subsequent evaluations, a large number of compounds with high potency and selectivity in antagonizing the hA_3AR were recognized as being generally characterized by remarkable structural diversity as nitrogen-containing aromatic monocyclic/bicyclic/tricyclic systems and, more recently, nucleoside-derived antagonists. The SAR of adenosine antagonists at the A_3AR was recently reviewed.[24−26,62] In this section, we summarized the leading achievements of the field expanding on and updating our earlier reviews[26] and book chapters[62] on the examined topic.

Monocyclic Systems. *Pyridine Derivatives.* After the first evidence that 1,4-dihydropyridines exerted affinity to ARs, Jacobson and co-workers exploited this nucleus as a template for probing SAR profile at A_3AR.[26] The replacement of the methyl ester at the 5-position of nifedipine with the larger 4-NO_2-benzyl ester, combined with the introduction of a phenyl ring and a phenylethynyl moiety at the 6- and 4-positions, respectively, led to compound **25** (MRS1334,[26] Figure 5, Table 2) in which the antagonism at L-type calcium channels was shifted to the A_3AR. The AR binding profile of 3,5-diacyl-2,4-dialkylpyridines obtained from the oxidation of the corresponding 1,4-dihydropyridines has also been examined.[26] The most potent compounds of this series, unlike the dihydropyridines, were substituted at the 4-position with small alkyl groups (**26**, MRS1505).[26] Potent fluorinated and hydroxylated pyridine derivatives have also been reported, and an extension of this research performed by Jacobson and co-workers led to a series of *N*-alkylpyridinium salts with improved water solubility.[26]

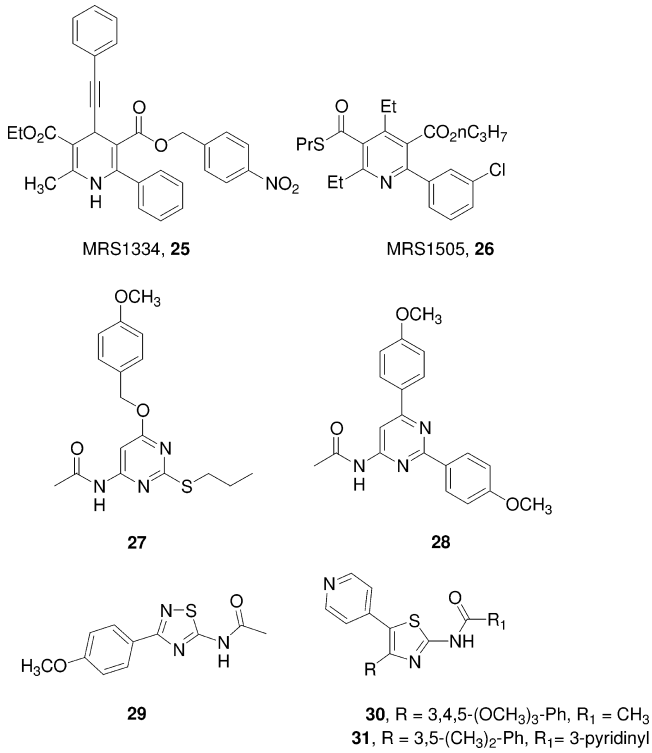

Figure 5. Monocyclic systems as A_3AR antagonists.

Pyrimidines. As a part of the endogenous ligand (adenosine), the pyrimidine core has been particularly exploited, as such or within bi- and tricyclic systems displaying ARs antagonism.

A series of 4-amino-6-hydroxy-2-mercaptopyrimidine derivatives has been synthesized and biologically evaluated as A_3AR antagonists.[63] The stepwise lead optimization resulted in compounds with very potent affinity and selectivity at the hA_3AR such as **27**. Several important attractive interactions have been highlighted when representative molecules were docked into a model of A_3AR. Compound *N*-[2,6-bis(4-methoxyphenyl)pyrimidin-4-yl]acetamide (**28**) was recently identified as a potent hA_3AR antagonist among two regioisomeric series of diaryl-2- or 4-amidopyrimidines. The structural determinants supporting the binding profiles of the series were evaluated through an exhaustive computational investigation.[64,65]

Thiazoles and Thiadiazoles. These derivatives were originally identified by simplifying the bicyclic heterocyclic ring system of isoquinolines and quinazolines (see below) with several monocyclic rings.[26] Derivative *N*-[3-(4-methoxyphenyl)-[1,2,4]thiadiazol-5-yl]acetamide (**29**) was later claimed as a potent A_3AR antagonist with a K_i of 0.79 nM.[66] The SAR studies revealed that a 5-(pyridine-4-yl) moiety on a 2-aminothiazole ring was also optimal for enhancing receptor potency and

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Table 2. Binding, Selectivities, and Functional Parameters of the Most Representative A_3AR Antagonists[a]

	K_i (nM)[b]					
	A_1AR	A_{2A}AR	A_3AR	A_3AR IC_{50} (nM)[c]	A_1/A_3	A_{2A}/A_3
			Monocyclic System			
25[26]	>100000 (r)	>100000 (r)	2.69 (h) 3850 (r)	nd	>26 (r)	>26 (r)
26[26]	41400 (r)	24100 (r)	7.9 (h) 814 (r)	nd	51 (r)	30 (r)
27[63]	2443 (h)	>10000 (h)	1.8 (h)	2.7 (h)	1357 (h)	>5555 (h)
28[65]	>100 (h)	>100 (h)	3.6 (h)	3.6 (h)[d]	>28 (h)	>28 (h)
29[66]	>10000 (h)	>10000 (h)	0.79 (h) >10000 (r)	nd	>12658 (h)	>12658
30[67]	>10000 (h)	>10000 (h)	0.41 (h)	9.6 (h)[d]	>24390 (h)	>24390 (h)
31[68]	>666 (h)	>826 (h)	0.36 (h) 1.6 (r)	nd	>1850 (h)	>2294 (h)
			Bicyclic System			
32[26]	>10000 (r)	>10000 (r)	561 (h) 5500 (r)	nd	>1.8 (r)	>1.8 (r)
33[26,123]	>10000 (r)	>10000 (r)	17 (h) 4200 (r)	nd	>588 (h)	>588 (h)
34[26]	>10000 (r)	>10000 (r)	4.0 (h)	7.9 (h)		
35[70]	>10000 (h)	>10000 (h)	0.78 (h)	8.3 (h)	>12820 (h)	>12820 (h)
36[26]	8000 (h)	833 (h)	26 (h)	nd	308 (h)	32 (h)
37[71]	430 (h)	8050 (h)	6.0 (h)	148 (h)	72 (h)	1342 (h)
38[26]	>10000 (h)	>10000 (h)	47 (h)	nd	>213 (h)	>213 (h)
39[72]	1037 (h)	3179 (h)	0.18 (h)	2.4 (h)	5761 (h)	17661 (h)
40[72]	>10000 (h)	>10000 (h)	2.9 (h)	8.2 (h)	>3448 (h)	>3448 (h)
41[73]	>1000 (h)	>1000 (h)	1.2 (h)	5.2 (h)	>833 (h)	>833 (h)
			Tricyclic System			
42[26]	231 (h) 53 (r)	25 (h) 10 (r)	0.59 (h)	1.7 (h)[d] 7.2 (h)[e]	391 (h)	42 (h)
43[90]	>10000 (h)[f]	>10000 (h)[f]	8.2 (h)[f]	nd	>1219 (h)	>1219 (h)
44[74]	>10000 (h)	>10000 (h)	2.1 (h)	nd	>4762 (h)	>4762 (h)
45[76]	>1000 (h)	>1000 (h)	9.0 (h)	34 (h)	>111 (h)	>111 (h)
46[26]	>10000 (h)	>10000 (b)	4.7 (h)	nd	>2128 (h)	>2128 (h)
47[26]	>10000(h)	>10000 (h)	0.80 (h)	nd	>12500 (h)	>12500 (h)
48[26]	>10000 (h)	>10000 (h)	2.1 (h)	nd	>4762 (h)	>4762 (h)
49[77]	2700 (h)	>10000 (h)	1.6 (h)	3.1 (h)	1687 (h)	>6250 (h)
50[78]	114 (h)	>10000 (h)	3.3 (h)	nd	34 (h)	>3030 (h)
51[16]	1100 (h) >10000 (r)	140 (h) 1990 (r)	0.29 (h) >10000 (r)	4.5 (h)	3793 (h)	483 (h)
52[82]	350 (h)	100 (h)	0.01 (h)	0.7 (h)	35000 (h)	10000 (h)
53[86]	>30000 (h)	>100000 (h)	2.1 (h)	nd	>14286 (h)	>47619 (h)
54[86]	562 (h)	778 (h)	0.11 (h)	nd	5109 (h)	7072 (h)
55[86]	>30000 (h)	>100000 (h)	0.24 (h)	nd	>125000 (h)	>416667 (h)
56[87]	>100000 (h)	>100000 (h)	2.1 (h)	nd	>47619 (h)	>47619 (h)
57a[89]	800 (h)	500 (h)	15 (h)	75 (h)	53 (h)	33 (h)
57b[89]	743 (h)	200 (h)	10 (h)	50 (h)	74 (h)	20 (h)
58[89]	923 (h)	222 (h)	110 (h)	nd	8.4 (h)	2 (h)
59[89]	129 (h)	68 (h)	61 (h)	nd	2.1 (h)	1.1 (h)
60[26]	>10000 (h)	>10000 (h)	0.95 (h)	0.61 (h)	>10526 (h)	>10526 (h)
61[92]	50 (h)	119 (h)	4 (h)	nd	12 (h)	30 (h)
62[92]	>10000 (h)	>10000 (h)	35 (h)	nd	>286 (h)	>286 (h)
63[93]	>10000 (h)	>10000 (h)	2.2 (h)	nd	>4545 (h)	>4545 (h)
64[94]	>1000 (h)	>1000 (h)	0.80 (h)	5.0 (h)	>1250 (h)	>1250 (h)
65[94]	>1000 (h)	>1000 (h)	3.5 (h)	18 (h)	>286 (h)	>286 (h)
66[96]	1640 (h) 440 (r)	1280 (h) 2100 (r)	2.3 (h)	nd	713 (h)	556 (h)
67[99]	1800 (h)	470 (h)	0.20 (h)	270 (h)[e]	9000 (h)	2350 (h)
68[100]	2525 (h)	>5000 (h)	1.5 (h)	23 (h)	1329 (h)	>3333 (h)
69[100]	3023 (h)	1520 (h)	1.9 (h)	15 (h)	1591 (h)	800 (h)

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Table 2. continued

	K_i (nM)[b]					
	A_1AR	A_{2A}AR	A_3AR	A_3AR IC_{50} (nM)[c]	A_1/A_3	A_{2A}/A_3
			Nucleoside-Derived A_3AR Antagonist			
70[26]	>100000 (h)	>100000 (h)	650 (h)	nd	>154 (h)	>154 (h)
71[41]	437 (h)	2960 (h)	2.3 (h)	nd	190 (h)	1287 (h)
72[29]	50 (h)	510 (h)	3.9 (h)	nd	12.8 (h)	131 (h)
	44 (r)	75 (r)	538 (r)		12 (r)	7.2 (r)
73[45]	4640 (h)	>10000 (h)	75 (h)	nd	61 (h)	>133 (h)
	1350 (r)					
74[103]	>10000 (h)	>10000 (h)	32 (h)	0.16 (h)	>312 (h)	>312 (h)
75[44]	335 (h)	>10000 (h)	1.3 (h)	nd	258 (h)	>7692 (h)
76[104]	1000 (h)	16 (h)	16 (h)	5.0 (h)	62 (h)	1 (h)
77[105]	12100 (h)	29800 (h)	29 (h)	nd	417 (h)	1028 (h)
78[107]	>3000 (h)[c]			8.2 (h)		
79[107]	>3000 (h)[c]			12 (h)		
80[110]	5870 (h)	>10000 (h)	29 (h)	nd	202 (h)	>345 (h)
81[110]	6220 (h)	>10000 (h)	16 (h)	nd	389 (h)	625 (h)
			321 (r)			
82[111]	>10000 (h)	>10000 (h)	9.3 (h)	nd	>1075 (h)	>1075 (h)
83[110]	>10000 (h)	>10000 (h)	1.7 (h)	nd	>5882 (h)	>5882 (h)
			6.2 (r)			
84[110]	2490 (h)	341(h)	4.2 (h)	nd	593 (h)	81 (h)
			3.9 (r)			
85[115]	1150 (h)	>10000 (h)	13 (h)	nd	88 (h)	>769 (h)
			83 (r)			
86[46]	110 (h)	>10000 (h)	4.3 (h)	nd	26 (h)	>2325 (h)
87[38]	3040 (h)	1080 (h)	1.4 (h)	nd	2171 (h)	771 (h)
88[38]	1760 (h)	1600 (h)	0.73 (h)	nd	2411 (h)	2192 (h)

[a]The values are evaluated in human (h), bovine (b), rat (r) tissues or cells. For detailed experimental conditions see the cited references. [b]K_i (nM) from competition binding experiments, unless otherwise specified. [c]IC_{50} (nM) from cyclic AMP assays, unless otherwise specified. [d]K_B value from cyclic AMP assays. [e]IC_{50} (nM) from GTPγS assays. [f]IC_{50} (nM) from competition binding experiments.

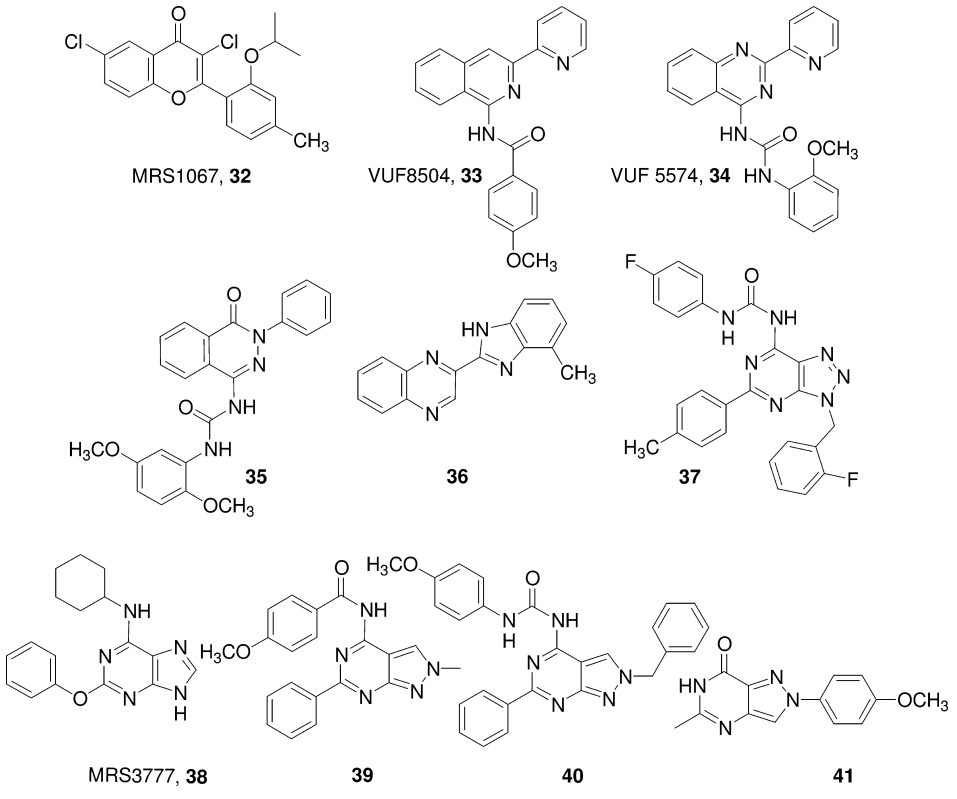

Figure 6. Bicyclic systems as A_3AR antagonists.

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selectivity.[67] Compound *N*-[4-(3,4,5-trimethoxyphenyl)-5-pyridin-4-ylthiazol-2-yl]acetamide **30** showed subnanomolar affinity at the hA_3AR with over 20000-fold selectivity against hA_1AR and $hA_{2A}AR$ and 8000-fold selectivity versus $hA_{2B}AR$. The introduction of an aromatic acyl substituent at the 2-position of the thiazole ring, such as benzoyl, nicotinoyl (compound **31**), or isonicotinoyl, led to compounds with good human and rat A_3AR affinity as well as selectivity over hA_1AR and $hA_{2A}AR$. Note that 5-(pyridine-4-yl)-2-aminothiazoles (**30** and **31**) are among the few molecules that display potent antagonist activity against both rat and human A_3ARs. Compound **30** was shown to selectively block the rat A_3AR in vivo.[67] In addition, in an in vivo rat model, compound **31** inhibited IB-MECA-induced plasma protein extravasation in the skin of rats, demonstrating a good oral absorption profile and bioavailability.[68] It also increased the antiasthmatic effect of dexamethasone by combination therapy, and these results suggested that the A_3AR antagonist could become a new type of antiasthma drug as an enhancer of steroids.

Bicyclic Systems. *Flavonoid Derivatives.* Some flavonoids revealed micromolar affinity at hA_3AR after binding screening of different phytochemicals.[26] MRS1067 (**32**, Figure 6) resulted from SAR optimization of the flavone nucleus as the most potent and selective compound of this series at hA_3AR subtype, while none of the tested derivatives showed significant affinity at the rA_3AR .

Isoquinolines, Quinazolines, Phthalazines, and Quinoxalines. In 1998 Ijzerman and co-workers identified a series of 3-(2-pyridinyl)isoquinoline derivatives with A_3AR affinity.[26] Different substituents at the 1- and 3-positions were introduced, and the best results were obtained with the 3-(2-pyridinyl)-1-(4-methoxybenzoylamino)-derivative VUF8504[26] (**33**). Later, the effect of an additional endocyclic nitrogen was estimated by synthesizing bioisosteric quinazoline derivatives. An increased affinity was obtained by substitution of the 1-amide spacer with a urea moiety. VUF5574[26] (**34**) showed affinity at hA_3AR in the nanomolar range, while it was ineffective at A_1AR and $A_{2A}AR$ subtypes.

In 2007, Morizzo et al. reported a new series of 2-amino/2-oxoquinazoline-4-carboxamido derivatives resulting from an in silico molecular simplification approach of the 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one template, extensively investigated by the same authors in the search for A_3AR antagonists (see the relevant section below).[69] Very recently, an analogous strategy, applied to the same tricyclic skeleton, was confirmed to be effective for the identification of structurally simplified A_3AR antagonists with the advantage of less complex synthetic routes.[70] This study promoted the 2-phenylphthalazin-1(2*H*)-one ring system to a new versatile core suitable for molecular manipulation. Substitution of the 4-position with different amido and ureido moieties led to compound **35** possessing the best binding profile of the series. The 2-(4-methyl-1*H*-benzoimidazol-2-yl)-quinoxaline **36** deserves to be mentioned for the novelty of the design strategy applying a 3D database searching approach.[26]

Adenines and Adenine-like Derivatives. The first class of A_3AR selective antagonists with a bicyclic structure strictly related to adenine was claimed in 2005 by Biagi and co-workers.[71] The authors described the synthesis of a series of N^6-ureido-substituted-2-phenyl-9-benzyl-8-azaadenines whose adenine-like structure was responsible for the antagonist activity while a N^6-phenylcarbamoyl group ensured selectivity at the A_3AR. The structure–activity relationship studies were performed on the systematic optimization of substituents at the 2-, 6-, and 9-positions of the bicyclic scaffold and guided the desired enhancement of A_1/A_3AR selectivity (compound **37**).

On the basis of the finding that the known differentiation agent "reversine" (2-(4-morpholinoanilino)-N^6-cyclohexyladenine) exerted a moderate antagonist activity at the hA_3AR (K_i of 0.66 μM), Jacobson and co-workers developed a series of reversine analogues, focusing the attention on the substitution pattern at the 2- and N^6-position of the adenine scaffold.[26] One of most interesting compounds in terms of hA_3AR affinity and selectivity combines the N^6-cyclohexyl moiety of reversine with a 2-phenyloxy group (compound **38**). Few derivatives tested in binding assays to the rA_3AR seemed to reflect the species dependence of the affinity typical of most known A_3AR antagonists, shown to be inactive at 10 μM.

The pyrazolo[3,4-*d*]pyrimidine nucleus represents a novel bicyclic scaffold structurally related to adenine, which has been recently examined in view of identifying A_3AR antagonists.[72] The SAR profile of this series would indicate that amido (**39**) or ureido moieties (**40**) at the 4-position along with a phenyl ring at the 6-position are essential for promoting A_3AR affinity and selectivity. The N^2-position seems to be characterized by a good degree of steric tolerance, since both the small methyl group (**39**) and the bulkier benzyl moiety (**40**) are well tolerated. Compound **39**, standing out for the subnanomolar affinity at the A_3AR and the high selectivity versus the remaining AR subtypes, has been suggested as a promising lead compound for the development of adjuvant agents in glioma chemotherapy. The compound proved, indeed, to counter the A_3AR selective agonist-mediated proliferation (i.e., Cl-IB-MECA and IB-MECA) in human glioma U87MG cells. A series of junction isomers of **39** and **40** (i.e., 2-arylpyrazolo[4,3-*d*]pyrimidin-7-one derivatives), originating from the molecular simplification of the tricyclic pyrazolo[3,4-*c*]quinolin-4-one skeleton (see tricyclic systems), was reported by Lenzi and co-workers in 2009.[73] Aryl/arylalkyl substitution at the 5-position of such derivatives was poorly tolerated for A_3AR binding affinity, while small groups at the same position were shown to enhance ligand–receptor interaction. In addition, the substitution of the 2-phenyl ring with a 4-methoxy group led to the most potent compounds of the series (**41**).

Tricyclic Systems. *Triazoloquinazoline.* Jacobson and co-workers first demonstrated that the acylation of the 5-amino group of the AR antagonist 9-chloro-2-(2-furanyl)[1,2,4]-triazolo[1,5-*c*]quinazoline-5-amine (CGS15943, hA_1 K_i = 3.5 nM; hA_{2A} K_i = 0.4 nM; hA_{2B} IC_{50} = 44 nM; hA_3 K_i = 95 nM) enhances both hA_3AR affinity and selectivity.[26] Compound MRS1220[26] (**42**, Figure 7) showed subnanomolar affinity at the hA_3AR with ~400- and ~40-fold selectivity vs A_1AR and $A_{2A}AR$ subtypes, respectively. The removal of the 9-Cl atom along with the replacement of the 5-phenylacetamido and the 2-furyl moieties with a linear alkyl chain and a 4-Br-phenyl ring, respectively, led Okamura et al. to the discovery of derivative **43** as a potent and selective A_3AR antagonist.[26]

Pyrazolo[3,4-c]/[4,3-c]quinolines. The binding affinity at bovine A_1AR and $A_{2A}AR$ and at human cloned A_3ARs of some 2-arylpyrazolo[3,4-*c*]quinolin-4-ones, 4-amines, and 4-substituted amino derivatives have been reported by Colotta et al. in 2000.[26] The 4-benzoylamido derivative **44** exhibited one of the best binding profiles of the series as A_3AR antagonist. The same group reported some extensions of the SAR study about this class of compounds, highlighting that bulky and lipophilic (hetero)aroylamino groups or a benzylcarbamoyl residue at the 4-position was quite tolerated for hA_3AR binding potency and

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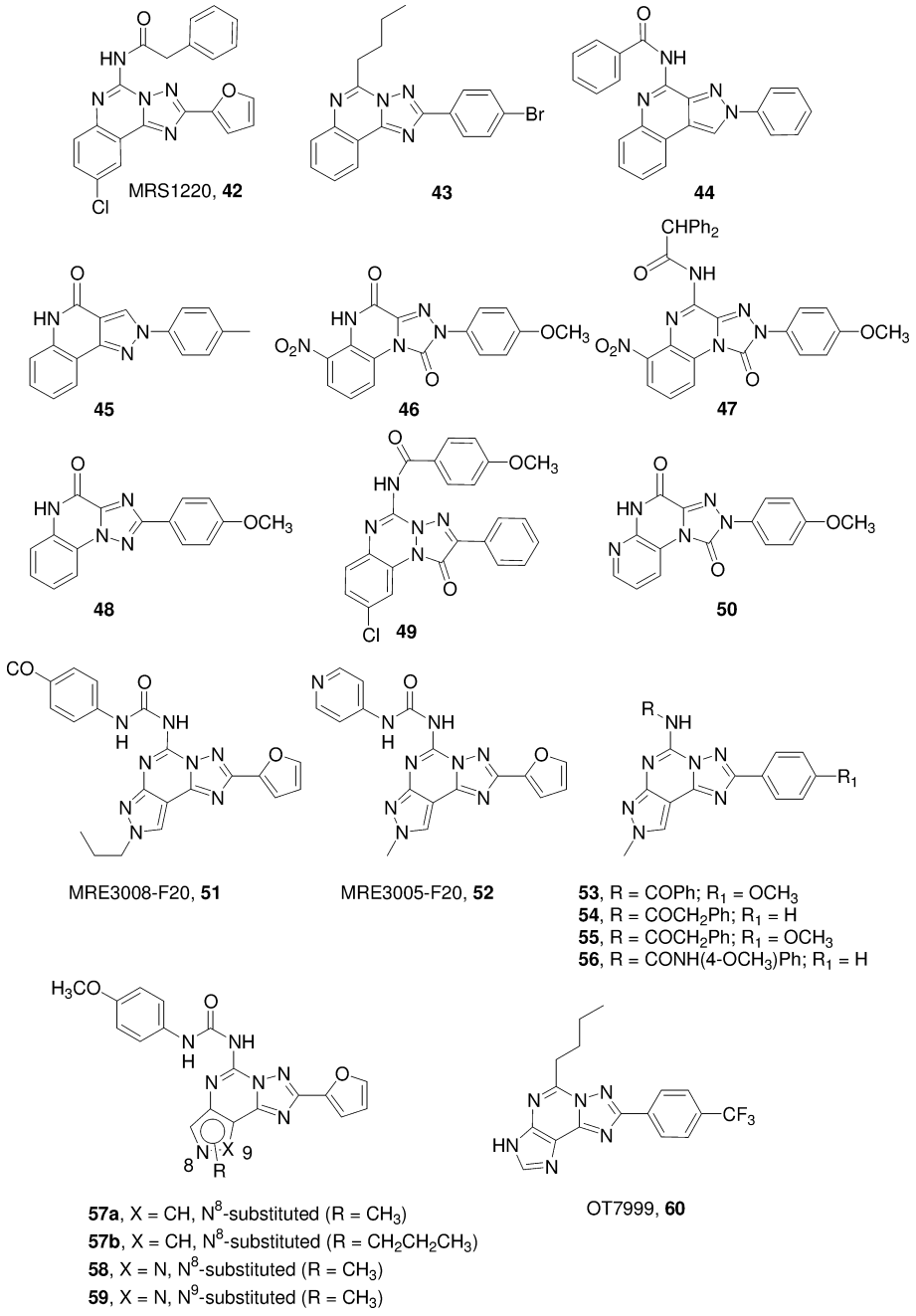

Figure 7. Representative collection of tricyclic systems as A_3AR antagonists.

selectivity.[74,75] Although displaying micromolar affinity at the rA_3AR, selected compounds of these series were tested in an in vitro rat model of cerebral ischemia and proved to prevent the irreversible failure of synaptic activity induced by oxygen and glucose deficiency in the hippocampus, thus confirming that A_3AR antagonists may substantially increase the tissue resistance to ischemic damage. The synthesis and the affinity profile at ARs of a series of 2-phenyl-2,5-dihydropyrazolo[4,3-*c*]quinolin-4-ones, conceived as structural isomers of the parent 2-arylpyrazolo[3,4-*c*]quinoline derivatives, have also been reported.[76] Some of the compounds synthesized showed A_3AR affinity in the nanomolar range and good selectivity as evaluated in radioligand binding assays at hARs. In particular, substitution at the 4-position of the 2-phenyl ring with a methyl, methoxy, or chlorine group and the presence of a 4-oxo functionality gave good activity and selectivity (compound **45**).

Triazolo[4,3-a]/[1,5-a]quinoxalines. Interesting studies performed in the past decade by Colotta and co-workers highlighted that the 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one is an attractive scaffold for obtaining potent and selective hA_3AR antagonists.[26] An intensive synthetic work based on the systematic substitution of the 2-, 4-, and 6-positions of the tricyclic template along with molecular modeling investigations performed to rationalize the experimental SAR findings led to the identification of the optimal structural requirements for A_3AR affinity and selectivity. In particular, introduction in the triazoloquinoxaline moiety of a 4-oxo (compound **46**) or 4-*N*-amido (compound **47**) function afforded selective and/or potent A_3AR antagonists, indicating that a C═O group, either

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extranuclear or nuclear, was necessary for A_3AR affinity. This suggested the probable engagement of this site of the molecule in a hydrogen bond with the A_3AR binding site. Hindering and lipophilic 4-acylamino moieties were shown to enhance A_3AR affinity (compound **47**). Substitution of the 4′-position of the 2-phenyl ring with a methoxy or a nitro group and 6-nitro substitution, as well as the combination of these substituents, afforded nanomolar A_3AR affinity and selectivity. For these reasons, 1-oxo, 6-nitro, and 4-amino groups have been supposed to be involved in hydrogen bond anchoring to the binding site.

Some 2-aryl-8-chloro-1,2,4-triazolo[1,5-*a*]quinoxaline derivatives have been synthesized and tested in radioligand binding assays at bA_1 (bovine A_1) and bA_{2A} adenosine receptors and at hA_1 and hA_3 adenosine receptors.[26] The SAR profiles of these compounds were in agreement with those previously reported for 2-aryl-1,2,4-triazolo[4,3-*a*]quinoxalines and 2-arylpyrazolo-[3,4/4,3-*c*]quinolines, suggesting similar AR binding modes. These studies provided some interesting compounds, among them the 2-(4-methoxyphenyl)-1,2,4-triazolo[1,5-*a*]quinoxalin-4-one (**48**), which is the most potent and selective hA_3AR antagonist of this series.

1,2,3-Triazolo[1,2-a][1,2,4]benzotriazinones. The structural manipulation of tricyclic ligands of the central benzodiazepine receptor led Da Settimo and co-workers to the identification of a series of 2-phenyl[1,2,3]triazolo[1,2-*a*][1,2,4]benzotriazin-1-ones, among which compound **49** stands out for its remarkable potency and selectivity at the A_3AR (K_i values at the A_1AR, A_{2A}AR, A_3AR of 2700, >10000, 1.6 nM, respectively, and IC_{50} from cAMP assay at the A_{2B}AR of > 1000 nM).[77] Interestingly, also the triazolobenzotriazinone nucleus presents isomeric analogy with the above-described triazoloquinoxalinone series (compounds **46**−**48**).

Pyrido[2,3-e]-1,2,4-triazolo[4,3-a]pyrazines. A series of 2-arylpyrido[2,3-*e*]-1,2,4-triazolo[4,3-*a*]pyrazin-1-one derivatives, both 4-oxo- and 4-amino-substituted, was designed applying the isosteric replacement of the typical nitrobenzene moiety of the 1,2,4-triazolo[4,3-*a*]quinoxalin-1-one derivatives with a pyridine ring.[78] Thus, by replacement of the 6-NO_2 group of compound **46** with an endocyclic nitrogen, still capable of hydrogen bonding but devoid of unfavorable steric hindrance, compound **50** was identified.

Pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines. The importance of the pyrazolotriazolopyrimidine (PTP) nucleus for the development of AR antagonists has been widely reviewed.[79−81] The first example of AR antagonist, containing the PTP scaffold, was reported by Gatta and co-workers.[81] A large number of compounds originated from the structure−activity optimization work based on the systematic substitution at the C^2, C^5, C^9, N^7, and N^8 positions.[81] The most potent and selective compounds at the hA_3AR subtype were derived from the combination of a small alkyl chain at the N^8-pyrazole position with a (substituted)phenylcarbamoyl residue at the N^5-position. Compound **51** is one of the most representative components of this class with high affinity (K_i = 0.29 nM against [^{125}I]AB-MECA binding to human receptors expressed in HEK293 cells) and good selectivity over the other hARs and has been also developed into a specific radioligand.[16] The bioisosteric replacement of the phenyl ring of the 5-phenylcarbamoyl moiety with a 4-pyridyl moiety[82] provided high water solubility while enhancing hA_3AR affinity. Compound MRE-3005-F20[82] (**52**) and the corresponding HCl salt indeed showed an excellent binding profile with K_i values at hA_3AR in the picomolar range (40 and 10 pM, respectively). Receptor modeling ascribed this increase of affinity, compared to neutral aryl carbamate derivatives, to strong electrostatic interactions between the pyridinium moiety and the side chain carbonyl oxygen atoms of Asn274 and Asn278, both located on TM7. Additional studies suggested the involvement of Tyr254 in an H-bond with the pyridyl ring to explain enhancement of receptor affinity and selectivity.[83] Several modeling investigations have been performed in the past years in order to elucidate the binding motif for PTPs and to identify key ligand−receptor interactions.[83−85]

Recently, some interesting extensions of SAR studies about PTPs have been reported, the most innovative regarding substitution at the 2-position where a 2-furyl group has been for a long time considered as essential for the binding at all AR subtypes.[81] Cheong et al. evaluated the effect of the replacement of the 2-furyl ring, often responsible for epatotoxicity (due to its metabolic cleavage by the cytochrome P450 enzymes in the liver), with a 4-(substituted)phenyl ring, known to be safer from a metabolic point of view.[86] This study demonstrated for the first time that a phenyl ring at the 2-position of PTPs induces better affinity and/or selectivity toward hA_3 receptor if compared to the 2-furyl congeners. Consistent with the previous SAR studies, the presence of the N^8-methyl group was shown to be preferred over bulkier substituents and N^5-benzoyl/phenylacetyl/phenylcarbamoyl substitutions induced higher A_3AR affinity/selectivity than observed in the case of N^5-unsubstitution (see compounds **53**−**56**).[87] A predictive QSAR study (CoMFA) on the new series of 2-aryl PTP derivatives provided new insights on the steric and electrostatic factors, especially related to the C^2-position, affecting hA_3 AR affinity.[88] In order to explore the role of the nitrogen at the 7-position of PTPs, we recently performed a new synthetic strategy for the preparation of pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives, which can be considered as 7-deaza analogues of the parent PTPs (see compounds **57a**,**b**). We also synthesized a novel series of N^8/N^9-substituted-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidines as junction isomers of the reference compounds (see compounds **58** and **59**).[89] The removal of the nitrogen at the 7-position significantly affected both A_{2A}AR and A_3AR affinities. The introduction of a 5-phenylcarbamoyl moiety, to promote A_3AR affinity and selectivity, appeared somewhat effective only in the pyrrole series of which the urea derivatives **57a** and **57b** were distinguished as relatively high affinity (K_i of 15 and 10 nM, respectively) and potent (IC_{50} = 75 and 50 nM, respectively) hA_3AR antagonists. The comparison between the new N^8-substituted pyrazolo[3,4-*e*]triazolopyrimidines (general structure **58**) and their structural isomers N^8-substituted pyrazolo[4,3-*e*]triazolopyrimidines showed how the shift of the pyrazole nitrogen from the 7- to the 9-position exerted, as a main effect, a remarkable decrease of hA_3AR affinity and selectivity of the 5-urea derivatives. When small alkyl chains were shifted from the N^8- to N^9-position (general structure **59**), the general effect was a random increase of affinity/potency at the AR subtypes. In contrast, N^9-arylalkyl substitution appeared generally detrimental for AR affinity. Thus, steric hindrance around this position seems poorly tolerated, in accordance with previous findings.[85]

Triazolopurines. Okamura at al.[90] reported a series of 1,2,4-triazolo[5,1-*i*]purines as A_3AR antagonists highlighting the structural similarity between the new class of compounds and the previously identified triazoloquinazoline derivatives (see above). These investigations led to potent and selective hA_3 ligands, the most representative of which, OT-7999[91] (**60**),

demonstrated significant reduction of intraocular pressure in cynomolgus monkeys at 2−4 h following topical application (500 μg).

"Tricyclic" Xanthines. Natural xanthines (caffeine and theophylline) show in general low affinity for the A_3AR subtype; nevertheless, the annulation of xanthine derivatives as a successful approach for the development of AR antagonists has been extensively argued.[26] Some pyrido[2,1-*f*]purine-2,4-dione derivatives, which could be considered as tricyclic xanthine derivatives, have been reported to exert low nanomolar hA_3 affinity.[92] The most potent compound of this series is the 1-benzyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione derivative **61** (Figure 8) with a K_i of 4.0 ± 0.3 nM at hA_3ARs. The

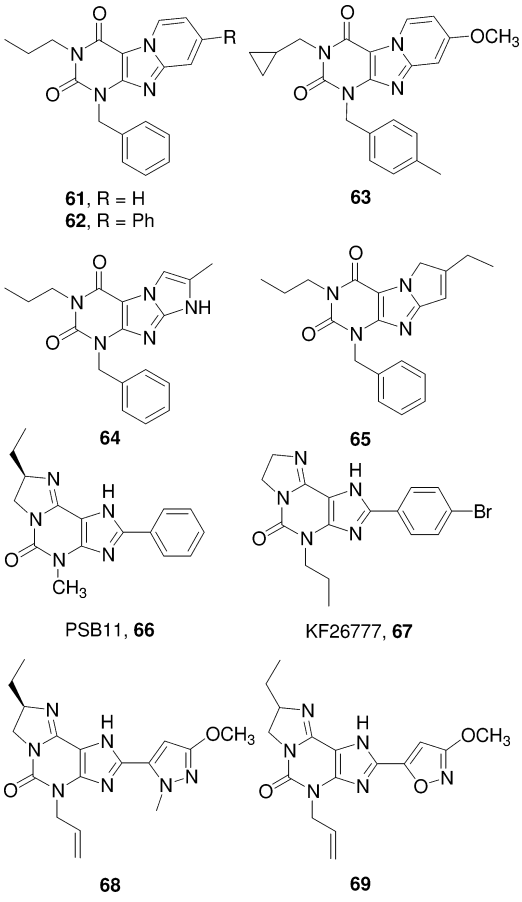

Figure 8. Fused xanthine derivatives as tricyclic antagonists of A_3ARs.

replacement of the benzyl nucleus at the 1-position with a methyl moiety determined a dramatic loss of both affinity and selectivity. The series[93] of 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones carrying a methoxy group at the 8-position, a cyclopropylmethyl group at the N^3-position, and substituted benzyl groups at the 1-position revealed a general preservation of hA_3AR affinity and a relevant enhancement of the overall selectivity (compound **63**). The effect of the replacement of the pyridine ring of the pyrido[2,1-*f*]purine-2,4-dione core with different five-membered heterocycles (i.e., pyrrole or imidazole) was later examined by our group.[94] Among the evaluated tricycles, the imidazo[2,1-*f*]purine-2,4-dione derivatives were 2- to 10-fold more potent than the corresponding pyrrolo[2,1-*f*]purine-2,4-dione derivatives. The best results were obtained with the introduction of small alkyl chains at the 7 position (**64**, **65**). A SAR extension of this project has been next realized performing new substitutions alternatively at the 1-, 3-, and 8-positions of the reference compound **64**, and the binding disposition of these molecules was analyzed by means of a docking approach using a mixed pharmacophoric−molecular modeling procedure.[95] The synthesis and biological evaluation of an analogue series of fused xanthine derivatives (imidazo[2,1-*i*]purin-5-ones) have been investigated by Müller and coworkers.[96] Compound **66** exhibited a K_i of 2.3 nM for the hA_3AR and good selectivity vs the other AR subtypes. The radiolabeled derivative of this compound exhibited a K_D of 4.9 nM (B_{max} = 3500 fmol/mg of protein).[97] An important innovation of such series, in comparison with xanthines, is a significant increase of water solubility due to the introduction of a basic nitrogen atom which can be protonated in physiological conditions. The analogue (8*R*)-ethyl-4-methyl-2-(2,3,5-trichlorophenyl)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one[98] (PSB-10), showed inverse agonist activity in binding studies in hA_3 CHO cells (IC_{50} = 4 nM). The 2-(4-bromophenyl) derivative named KF-26777[99] (**67**) with subnanomolar affinity at the hA_3AR (K_i = 0.2 nM) and high selectivity over A_1, A_{2A}, and A_{2B} ARs (9000-, 2350-, 3100-fold, respectively) was indicated to be of potential interest for the treatment of brain ischemia and inflammatory diseases such as asthma. In a recent study[100] we evaluated the effect of the replacement of the 2-phenyl ring of **66** and congeners with differently substituted five-membered heterocycles, in particular 1,3- and 1,5-disubstituted pyrazoles or 3-substituted isoxazoles (**68** and **69**). The 2-heterocyclic substitution proved to induce excellent affinity and selectivity toward hA_3AR subtype. Docking of the most potent compound (**68**) in complex with hA_3AR furnished a general survey of the hypothetical binding mode of the newly described derivatives.[100]

Nucleoside-Derived A_3 Adenosine Receptor Antagonists. The attempts of the scientific community focused on the design of A_3AR antagonists with nucleoside structure are aroused by a general limitation in characterizing non-purine heterocyclic antagonists, most of which, although having high affinity and selectivity for the hA_3AR, show low or no affinity toward the rA_3AR (with the exception of 5-(pyridine-4-yl)-2-aminothiazoles;[67,68] see above). This hampers drug development, considering the difficulty of employing animal models for drug testing. The structural manipulation at different positions of adenosine provided a large number of ligands of the A_3AR exerting different levels of potency, selectivity, and intrinsic efficacy. The exact combination of modifications was shown to affect the balance between full agonism, partial agonism, and antagonism.[101] Some nucleoside-derived antagonists (i.e., compounds **83**−**85**; see below) were shown to display affinity toward human and rat A_3AR in the same range of concentration, thus allowing the use of small animal models for future drug development.

A series of 8-alkynyladenosines reported by Volpini et al. in 2001[26] represents the first example of adenosine analogues, with the intact ribose moiety, which behave as selective antagonists at hA_3AR (compound **70**, Figure 9). The same group recently reported a series of 2-arylalkynyl-N^6-methoxyadenosine derivatives, among which the 2-pyridyl derivative **71** showed a profile as an hA_3AR antagonist.[41]

It has been demonstrated that the introduction of a benzyl or a 3-iodobenzyl moiety at the N^6- position and a Cl atom at the 2-position of adenosine synergistically contribute to reduction of intrinsic efficacy of the corresponding nucleoside derivatives. Thus, N^6-(3-iodobenzyl)adenosine acts as partial agonist

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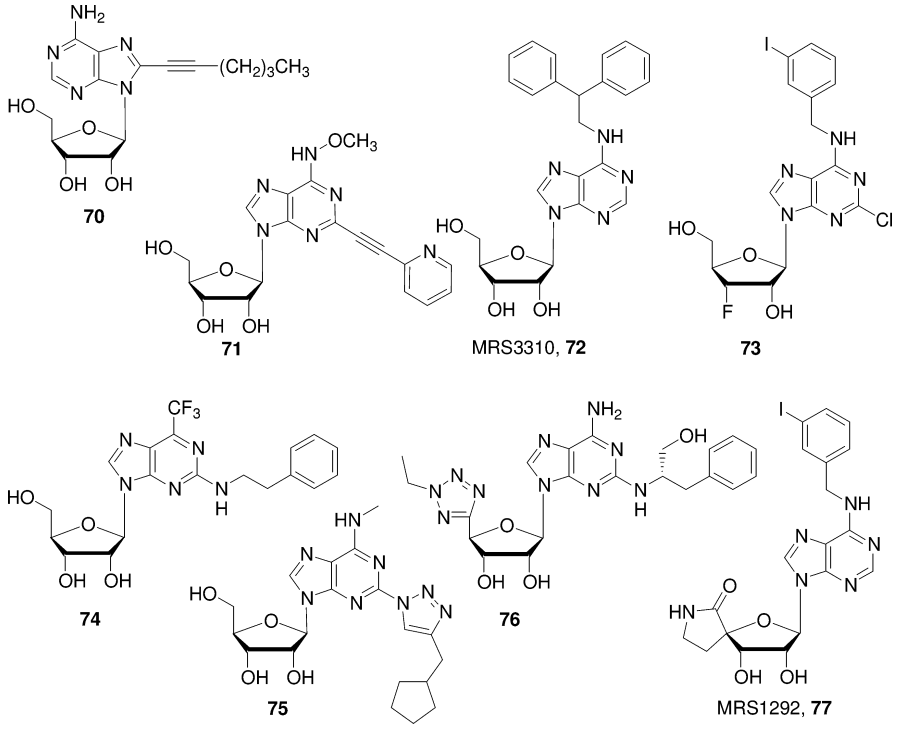

Figure 9. Nucleoside-derived A_3AR antagonists.

(46% A_3AR efficacy) while the 2-chloro-N^6-(3-iodobenzyl)-adenosine behaves as a potent but nonselective A_3AR antagonist ($K_i(A_3AR)$ = 1.8 nM, $EC_{50}(A_{2B}AR)$ > 10 000 nM, $K_i(A_{2A}AR)$ = 197 nM, $K_i(A_1AR)$ = 16.8 nM).[102] As mentioned above, the efficacy of the latter compound is completely restored by the replacement of the 4′-hydroxymethylene group with a 5′-methyluronamide function (Cl-IB-MECA). N^6-Substitution of adenosine affected intrinsic efficacy, as adenosine was shown to reverse its agonistic activity into antagonism when a N^6-2,2-diphenylethyl moiety was introduced (MRS3310, **72**).[29,56]

Some 2′- and 3′-fluoro substituted adenosines have been investigated as A_3AR ligands.[45] While the introduction of a fluorine atom at the 2′-position compromised both A_3AR binding and activation, the 3′-fluoro substitution generally resulted in partial agonism. Compound **73** is one of the few ligands of the series in which the presence of the fluorine at the 3′-position led to a total loss of hA_3AR efficacy.

A patent by Solvay[103] claimed the possibility of treating allergic diseases with a series of new nucleoside derivatives that are high affinity A_3AR antagonists. One of the most important compounds is compound **74** (Figure 9), which showed low nanomolar affinity for A_3AR with high selectivity over the other ARs, and its potent antagonistic activity has been assessed in functional models (pA_2 = 9.8). These findings highlight the importance of substitutions at the 2- and 6-positions of adenosine for receptor activation. Interestingly, these molecules are the only examples of A_3AR antagonists structurally related to adenosine in which the 6-amino group was completely removed and replaced by a trifluoromethyl moiety. A further example of N^6-C^2-disubstituted adenosine antagonist is represented by 2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl)-N^6-methyladenosine **75**, displaying hA_3AR affinity with 260-fold binding selectivity versus the hA_1AR. This corroborates the assessment according to which hindered groups at the 2-position promote A_3AR binding preventing receptor activation.[44] Researchers at GlaxoSmithKline recently confirmed this observation with the identification of the novel ligand (2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-{[(1*S*)-2-hydroxy-1-(phenylmethyl)ethyl]amino}-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol, **76**.[104] This compound displayed comparable affinity in binding assays at the $A_{2A}AR$ and A_3AR (K_i = 15.8 nM), behaving, however, as a potent agonist at $hA_{2A}AR$ subtype (pEC_{50} = 9.0 ± 0.2) and as a competitive antagonist at hA_3AR (pA_2 = 8.3 ± 0.04). Lower affinity for A_1AR and $A_{2B}AR$ has been detected ($pK_i \leq 6$). In addition, potent inhibitory effects on the generation of reactive oxygen species from human neutrophils and eosinophils and on the degranulation of human granulocytes subsequent to treatment with compound **76** have been as well described. These findings provide a useful tool for the understanding of the involvement of A_{2A} and A_3 ARs in inflammation processes. While in **76** the typical 4′-CH_2OH was replaced by a substituted tetrazole ring, MRS1292[105] (**77**) was obtained by the introduction of spirolactam moiety at the same position. This compound behaves as an A_3AR antagonist with a K_i from binding assays of 29.3 nM.[105] The presence of the additional ring induced a conformational restriction which could affect the capacity of the endocyclic 5′-uronamide to realize a hydrogen bond required for the receptor activation.[102] Compound **77** was shown to inhibit A_3AR-mediated shrinkage of human nonpigmented ciliary epithelial cells and reduce mouse intraocular pressure acting as a cross-species A_3AR antagonist. This would suggest the possible employment of such ligands in the treatment of glaucoma.[106] Further examples of conformationally constrained analogues of known nucleoside-based A_3AR agonists have been synthesized and tested for their binding affinity as well as for their agonist and/or antagonist activity at the ARs.[107] Among these, 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleosides (locked nucleic acid, LNA nucleosides) and 2′-amino-LNA nucleosides derivatives containing the 2-oxa-5-azabicyclo[2.2.1]heptane scaffold were

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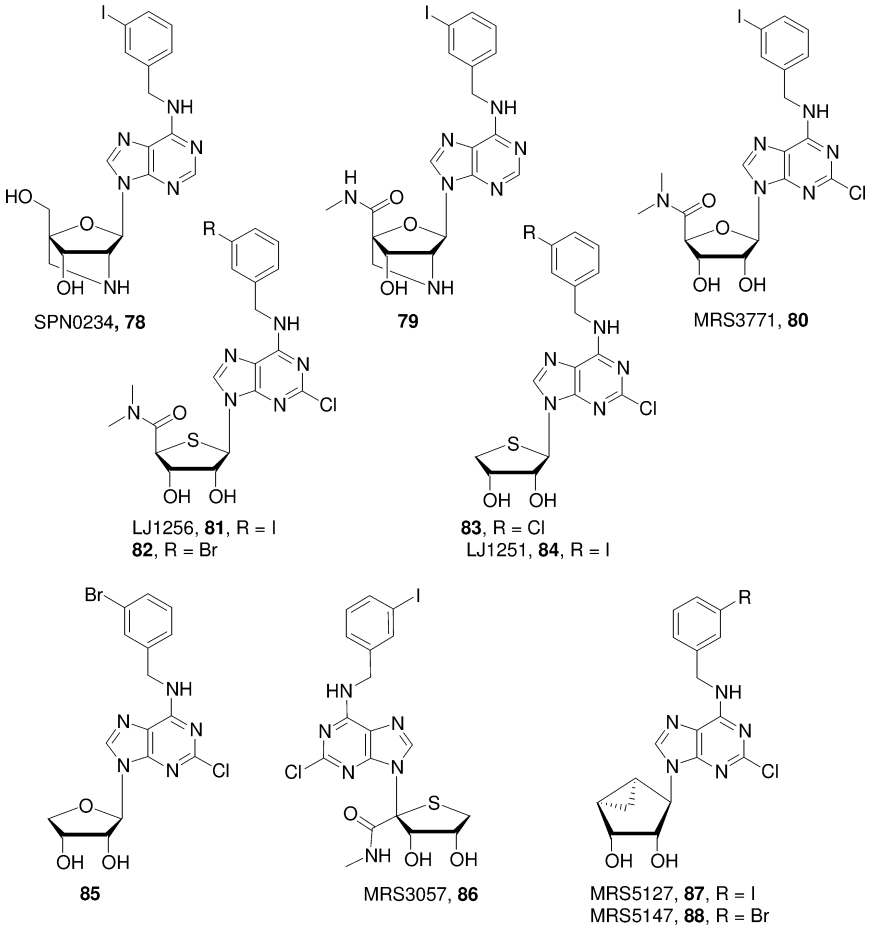

Figure 10. Nucleoside-derived A_3AR antagonists.

distinguished as potent A_3AR antagonists (compounds **78** and **79**, Figure 10).[108]

The fundamental role of the flexibility and H-bonding ability of the 4′-hydroxymethylene or 5′-uronamide moieties of nucleoside derivatives for full activation of the A_3AR has been lately confirmed by both molecular modeling studies[109] and the identification of D-4′-thioadenosine derivatives.[110] The highly selective A_3AR agonists Cl-IB-MECA and its 4′-thio analogue were indeed successfully converted into selective antagonists simply by adding a second *N*-methyl group on the 5′-uronamide position (compounds MRS3771, **80**, and LJ-1256, **81**, respectively).[102] 5′-*N*,*N*-Dimethyluronamide derivatives exhibited higher binding affinity than larger 5′-*N*,*N*-dialkyl or 5′-*N*,*N*-cycloalkylamide derivatives, indicating that steric factors are crucial in binding to the hA_3AR.[111,112] The N^6-(3-Br-benzyl) derivative **82** showed the highest A_3 binding affinity (K_i = 9.32 nM) of this series. The removal of the 4′-substituent of the thionucleoside skeleton led Jacobson and co-workers to the identification of even more potent and selective A_3AR antagonists, the most effective of which have been depicted in Figure 10 (**83** and **84**). Compounds **83** and **84** also demonstrated high affinity at the rA_3AR expressed in CHO cells (K_i of 6.2 and 3.9 nM, respectively), indicating the possibility of evaluation in small animal models for future drug development, and were inactive as agonists or antagonists in a cyclic AMP functional assay at the hA_{2B}AR. The removal of the 2-Cl atom did not affect hA_3R affinity while significantly reducing selectivity versus the remaining AR subtypes.[113,114] The L-enantiomers of 4′-thioadenosines were shown to be totally devoid of AR affinity.[114] A recent series of 4′-oxo bioisosters of **84** were shown to be generally less potent than the corresponding 4′-thio nucleosides although still exerting considerable potency both as human and rat AR antagonists (see compound **85**).[115] Moreover, truncated thioadenosines, substituted at the C^2-position with suitable alkynyl or alkenyl chains, showed a very interesting pharmacological profile as a mixed A_{2A}AR agonist/A_3AR antagonist which might be advantageous for potential antiasthmatic activity.[116] The involvement of the 5′-position of the ribose moiety in receptor activation has been confirmed by recent findings indicating that the shifting of the N^6-(3-iodobenzyl)adenine moiety from the 1′ to the 4′ position of the ribose ring proved to induce A_3AR potent antagonism in the full agonist 4′-thio analogue of Cl-IB-MECA (see compound MRS3057, **86**).[46]

As above-described, the replacement of the flexible ribose scaffold of prototypical A_3AR agonists with a bicyclo[3.1.0]-hexane ring system resulted in (N)-methanocarbaadenosine agonists possessing high potency and selectivity for the A_3AR subtype. A new series of A_3AR partial agonists/antagonists belonging to the (N)-methanocarba family has been recently developed because of the removal of the *N*-methylcarboxamide function (see **87** and **88**).[38] The partial agonist **88** was labeled with ^{76}Br for use as a PET ligand of high affinity.[117] Compound **87** appeared to be an antagonist by Schild analysis of [^{35}S]GTPγS binding to membranes from CHO cells expressing the hA_3AR.[118] However, further analysis determined that it is a high affinity and selective partial agonist, stimulating cAMP production with 45% efficacy compared with NECA. This compound was later radioiodinated and characterized

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pharmacologically.[119] The substitution of the 3′-hydroxyl group of these derivatives with a 3′-amino group proved to drastically compromise hA_3 binding affinity.[120]

A_3 ADENOSINE RECEPTOR ALLOSTERIC MODULATORS

Allosteric ligands interact with receptor domains that are distinct from the orthosteric binding site (the primary binding site of the endogenous ligand on a receptor) of classical ligands. The binding of an allosteric ligand to its site causes conformational change in the receptor protein that is transmitted to the orthosteric site (and vice versa), inducing the formation of a modified GPCR with its own binding and functional properties.[121] The simplest expression of an allosteric interaction arises when the binding of the allosteric modulator either enhances or inhibits the affinity of the orthosteric ligand for the receptor; thus, in the absence of the orthosteric ligand, the modulator is assumed not to mediate any effect on its own. In this case, allosteric ligands have little or no overlap with the orthosteric site. Therefore, allosteric and orthosteric ligands can both bind simultaneously. More complex is the case of a small molecule behaving both as an agonist on its own and as allosteric modulator through the recognition of a domain that is distinct from the orthosteric one (allosteric agonist). Allosteric modulation of GPCRs now represent one of the most exciting areas in modern drug discovery.[122] This kind of approach has been associated with higher receptor subtype selectivity because of the observation that allosteric sites may be less conserved within a receptor class in comparison with orthosteric sites. Moreover, allosteric modulators are thought to address their action only in the anatomical district in which the production of the endogenous ligands is stimulated as a consequence of a pathologic condition. Allosteric agonist enhancers of the A_3AR are of considerable interest as therapeutic agents and as pharmacological tools to explore various signaling pathways. Allosteric modulation of the A_3AR was first observed by Gao et al.[123] with a number of 3-(2-pyridinyl)isoquinoline derivatives, previously reported as A_3AR antagonists (see above). This study described VUF5455[123] (**89**, Figure 11) as a first generation A_3AR allosteric enhancer, still suffering from low water solubility and antagonistic properties. Nevertheless, a clear indication of distinct structural requirements for modulating allosteric enhancement and competitive antagonistic activity at the A_3AR stimulated the same author to examine new chemical entities, among which are 1*H*-imidazo[4,5-*c*]quinolines known as non-xanthine AR antagonists.[124] Of this class, DU124183[124] (**90**) was then claimed as second generation A_3AR enhancer with potency comparable to that of **89** but, in contrast to this, able to potentiate the maximum agonist efficacy by approximately 30%. Compound **90** also displayed some orthosteric activity with moderate affinity (K_i = 820 nM) to hA_3AR. Later, structure−activity optimization led to compound LUF6000[125] (**91**) capable of enhancing the maximum efficacy of Cl-IB-MECA (by 45−50%) and other A_3AR agonists,[126,127] as well as able to convert an A_3AR antagonist into a specific agonist. Compound **92**, bearing an adamantyl moiety at the 2-position, exerts allosteric enhancer activity similar to that of **91**,[128] thus demonstrating a high steric tolerance around this position. The molecular simplification of **91** allowed the identification of a series of 2,4-disubstituted quinolines as allosteric enhancers of the A_3AR.[129] Compound LUF6096[129] (**93**), which can be considered the direct bicyclic analogue of **91**, was the most potent compound of this series with reduced orthosteric effect toward A_1AR and A_3AR subtypes if compared to **91**.

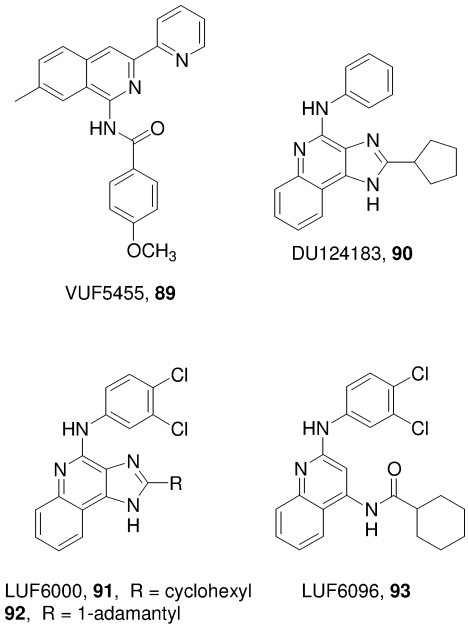

Figure 11. A_3AR allosteric modulators.

Very recently, 2-arachidonylglycerol and other cannabinoid ligands were tested for their potential effect as hA_3AR modulators. Interestingly, 2-arachidonylglycerol proved to increase the rate of [^{125}I]AB-MECA dissociation, behaving as a negative allosteric modulator.[130]

A_3 ADENOSINE RECEPTOR RADIOLIGANDS

In the past, a well-known A_3AR agonist radioligand as [^{125}I]AB-MECA was introduced in saturation and competition binding experiments performed in native tissues and in transfected cells.[131] Other radioligands have been characterized as [^{125}I]MRS5127 (see compound **87**) or [^{125}I]MRS1898 (the iodinated analogue of **20**) which display both r- and hA_3ARs affinity in the nanomolar range.[58] More recently, a selective agonist radioligand [^{3}H]HEMADO ([^{3}H]2-hexyn-1-yl-N^6-methyladenosine) has been studied with high affinity and selectivity versus hA_3ARs.[132] An antagonist radioligand as [^{3}H]MRE3008F20 (see compound **51**) has been used in different human cells or tissues showing a high affinity and selectivity for hA_3ARs.[16,17,133−136] It has also been introduced as an A_3AR positron emission tomography ligand for in vivo imaging named as [^{18}F]FE@SUPPY (5-(2-fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate)[137] that exhibits high affinity and selectivity for A_3ARs.[138] In addition, another antagonist radioligand named as [^{3}H]PSB11 (see compound **66**, Figure 8) has been used to characterize the allosteric binding site for A_3ARs.[97]

A_3 ADENOSINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

A_3ARs are widely distributed in the central nervous system but at low levels and with a reduced affinity. The role of A_3ARs in several pathophysiological conditions is often controversial even if they may contribute to neurotransmission.[139] A proconvulsant effect of A_3ARs has been observed in the immature brain, suggesting the possibility of facilitating seizure-induced neuronal damage.[140] It has been reported that A_3AR agonists

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have depressant effects on locomotor activity, suggesting a possible inhibition of excitatory neurotransmission in cortical neurons.[139] A nociceptive role for A_3ARs involving both central nervous system and proinflammatory effects in peripheral tissues has been highlighted.[141] Major evidence for A_3ARs in neurodegenerative phenomena emerges from studies performed in vivo and in vitro models of hypoxia/ischemia. It has been hypothesized that A_3ARs play a protective role in the first phase of ischemia by decreasing synaptic transmission.[142] Moreover, prolonged A_3AR stimulation is able to transform the effects from protective to injurious, increasing the excitotoxicity.[143] Glial A_3AR activation by high adenosine levels, caused by a brain injury, may be implicated in neuroinflammatory tissue responses.[144] An up-regulation of A_3ARs has been reported in the hippocampus of a transgenic mouse model of Alzheimer's disease where an altered oxidative phosphorylation was detected prior to amyloid deposition.[145] It has been reported that the stimulation of A_3ARs rapidly enhances the activity of antidepressant-sensitive serotonin transporters (SERTs) and that the stimulation of SERT activity is lost in A_3AR knockout mice.[146] A_3AR-stimulated SERT activity is primarily mediated by p38 MAPK-linked pathways, supporting the potential use of agents that block A_3ARs and selectively diminish SERT surface expression and activation and suggesting their use for the treatment of mood disorders characterized by hyposerotonergic states.[146] Recently, the presence of ARs has been evaluated in lymphocytes from Parkinson's disease (PD) patients, revealing that A_1AR, A_{2B}AR, and A_3AR did not change while A_{2A}ARs were significantly up-regulated in PD if compared with healthy subjects.[147] As a consequence, further in vitro and/or in vivo studies aimed at verifying the A_3AR modulation effect of agonists versus antagonists will help to better clarify their role in these neurodegenerative diseases.

A_3 ADENOSINE RECEPTORS IN CARDIOVASCULAR SYSTEM

The different effects of adenosine on electrical and mechanical properties of the heart have been reported.[148] Generally the actions of adenosine are protective and serve to shelter the heart during conditions of inadequate blood flow or during increased cardiac work.[149] Vasoconstriction mediated by A_3ARs may involve indirect signaling through nonvascular cell types such as mast cells, which may reside within the vascular wall, via the release of factors such as histamine and thromboxane.[150] Also the vasoconstriction response is due to A_3ARs being closely dependent on the inhibition of cyclic AMP accumulation in smooth muscle and in cultured aorta.[151] It is also reported that A_3ARs mediate vascular protection and contribute to limitations in infarct size and in postischemic myocardium by a mechanism that involves PKC, K_{ATP} channel activation, phosphorylation of p38MAPKs, and glycogen synthase kinase (GSk-3β).[152] Moreover, A_3ARs enhance cellular antioxidant capacity that contributes to vasoprotection and reduces cardiac myocyte death, suggesting a strong support for an A_3-dependent cardioprotective response including the reduction in infarct size, inhibition of apoptosis, and improvements in postischemic contractile function.[153] It has also been reported that a low expression of A_3ARs confers substantial resistance to ischemic insult, while only a 5-fold increase in expression leads to cardiomyopathy.[154] The activation of the A_3ARs increases the vascular permeability depending on mast cell activity[155] and stimulates vascular growth, acting with A_{2B}ARs to promote angiogenesis via the expression of angiogenic factors in mast cells or stimulate HIF-1α and vascular endothelium growth factor (VEGF) expression.[156]

It was reported that ischemia and reperfusion can cause significant injury of skeletal muscle, which is the most vulnerable tissue in the extremities.[157] Trauma, autogenous skeletal muscle transplantation, surgical incision, and vascular surgery can also induce skeletal muscle damage with deleterious systemic consequences. The protection of skeletal muscle from ischemia and reperfusion injury is an important therapeutic aim to ameliorate muscle and organ injury.[157] It has been observed that A_3ARs induce protection of the skeletal muscle, modulating the expression of different metalloproteinases that are able to degrade collagens, gelatins, and stromelysins and metallothioneins that, reducing reactive oxygen species (ROS), render tissue protection.[152]

Atherosclerosis, a multifactorial disease of the large arteries, is the major cause of heart disease and stroke worldwide. Epidemiological studies have discovered several relevant environmental and genetic risk factors associated with this pathology.[158] Recently, it has been shown that adenosine in hypoxic foam cells stimulates HIF-1α accumulation by activating ERK 1/2 pathway. Further, adenosine through the activation of A_3ARs stimulates VEGF secretion in a HIF-1α dependent way. Adenosine stimulates foam cell formation, and this effect is strongly reduced by A_3AR antagonists and by HIF-1α silencing. So as a consequence, the potential use of A_3AR antagonists could be of interest to block important steps in the atherosclerotic plaque development.[159]

In the cardiovascular system, A_3AR modulation appears to have an important role even if extensive evidence reveals an elevated complexity of signaling pathways causing cardioprotection.

A_3 ADENOSINE RECEPTORS IN IMMUNE SYSTEM

A_3ARs are present in immune cells and are involved in the pathophysiological regulation of inflammatory and immune processes. The cells of the immune system express all ARs and are responsive to the adenosine modulatory effects in an inflammatory environment. Different animal models of asthma, ischemia, arthritis, sepsis, inflammatory bowel disease, and wound healing have helped to elucidate the regulatory roles of the various ARs in the development and progression of disease.[1] The increasing knowledge of the control of immune and inflammatory systems by adenosine has generated different hypotheses regarding the potential use of therapies based on ARs in the treatment of infection and/or autoimmunity. Several results from in vitro and in vivo studies suggest that the activation of A_3ARs can be both pro- or anti-inflammatory depending on the cell type examined or on the animal species considered.[4] Binding and functional studies have shown that human neutrophils expressed A_3ARs primarily coupled to the AC inhibition and calcium signaling, mediating the inhibition of oxidative burst representative of anti-inflammatory activity.[135] A_3ARs are also responsible for inhibition of superoxide production and chemiotaxis of mouse bone marrow neutrophils.[160] It has been reported that A_3ARs are present on human eosinophils, coupled to signaling pathways linked to cell activation, and are able to protect eosinophils from apoptosis and to inhibit the chemotaxis process.[161] An overexpression of A_3ARs has also been detected in lymphocytes and in Jurkat cells, a human leukemic cell line, where they are associated with inhibition of AC activity and calcium modulation.[133] The effects produced by A_3AR activation of macrophages seem to indicate an anti-inflammatory effect of this receptor subtype.[17] All the data derived from in vitro and in vivo

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studies suggest that the activation of A_3ARs can cause pro- and anti-inflammatory effects primarily depending on the cell type examined (neutrophil, eosinophil, macrophage, T cell, or dendritic cell) or on the cellular model used as in vitro or ex vivo or the use of transgenic animals. Other differences that could be very important are the species considered (human or animal) or the functional response investigated, as degranulation, oxidative burst, migration, maturation, and cytokine production. In addition, the presence and the opposite functional role of other AR subtypes could hamper the identification of the specific role of A_3ARs in cell damage.

A_3 ADENOSINE RECEPTORS IN THE RESPIRATORY SYSTEM

The role of adenosine in regulating the respiratory system is well-known, and elevated levels of adenosine have been found in bronchoalveolar lavage (BAL), blood and exhaled breath condensate of patients with asthma, and chronic obstructive pulmonary disease (COPD). In the past, A_3ARs have been implicated in inflammatory processes, playing an important role in both pro- or anti-inflammatory responses, strictly depending on different cell type involved.[162] In particular, the strongest evidence of an A_3AR functional role in mast cell activation comes from the use of genetic knockout mice where the mast cell degranulation in the absence or in the presence of allergen appears to be dependent on A_3AR activation. Likewise, adenosine failed to induce histamine release from lung mast cells obtained from A_3AR knockout mice and adenosine-induced airway hyperresponsiveness by both A_3AR dependent and independent mechanisms in rodents.[163] The airway hyperresponsiveness was abolished in A_3AR deficient mice, and the reconstitution in these animals of wild type mast cells restored the hyperresponsiveness.[164] In contrast, mice treated with selective A_3 antagonists showed a marked attenuation of pulmonary inflammation, reduced eosinophil infiltration into the airways, and decreased airway mucus production.[164] The involvement of the A_3ARs in a bleomycin model of pulmonary inflammation and fibrosis was investigated in A_3AR-deficient mice that exhibit enhanced pulmonary inflammation and up-regulation in eosinophils related to an increase of chemokines and cytokines. These results suggest that the A_3ARs supply anti-inflammatory functions in the bleomycin model and regulate the production of mediators involved in fibrosis.[165] There is no evidence of A_3AR protein in human lung mast cells, but a high density of the functionally active form of these receptors is expressed in human eosinophils where the inhibition of the proinflammatory functions by A_3AR agonist has been reported.[1] In addition, the A_3ARs are expressed in human lymphocytes and neutrophils where they are involved in the immunological responses in T cells and in the reduction of superoxide anion production.[17,135] Transcript levels for the A_3ARs are elevated in lung biopsies of patients with asthma or COPD where they mediated the inhibition of eosinophil chemotaxis.[166] In asthmatic inflammation, elevated concentrations are present of activated eosinophils that are widely reduced by the A_3AR agonist.[167] In peripheral lung from COPD patients, a decrease of A_{2B}AR density and an increase of A_{2A}AR and A_3AR if compared to smokers with normal lung function were observed.[168] Moreover, in BAL macrophages from COPD patients was found an increase of A_3ARs closely associated with the presence of high levels of proinflammatory cytokines.[147] In the human leukemic monocyte lymphoma cell line (U937), interleukin 1β (IL-1β) and tumor necrosis factor (TNFα) were significantly increased in both A_{2A}AR and A_3AR density. The addition of hydrogen peroxide, a condition present in the COPD lung, to the proinflammatory stimulus did not affect A_{2A}AR or A_3AR expression, although it induced a significant reduction in A_{2B}AR expression. These data suggest the potential use of A_{2B} and/or A_3AR antagonists in pathophysiological conditions related to pulmonary diseases in which inflammation is an important feature.

A_3 ADENOSINE RECEPTORS IN ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder of unknown etiology that affects approximately 1% of the population worldwide.[169] Recently, data have become available suggesting that the pharmacogenetic characterization may help to predict the efficacy and safety in clinical practice.[169] It is widely accepted that RA must be treated early with effective therapy in order to prevent an unfavorable outcome. No effective and safe pharmacological treatment is available, even though progress has been done in the past years by using biological drugs. Knowledge of the adenosine mechanism has revealed that ARs could represent a useful target of therapy in RA. The stimulation of A_{2A}AR and/or A_3AR mediates a reduction of inflammation via NF-kB signaling pathway and a decrease of proinflammatory cytokines, suggesting their utilization as biomarkers and as predictors of clinical response linked to the available pharmacological therapies. At present, the precise cause of RA remains elusive even if a variety of cells that play a role in RA disease progression, such as macrophages and synoviocytes, could be of particular importance.[170] Clinical evidence in RA patients shows that treatment with an A_3 agonist leads to an improvement in signs and symptoms.[171] Recently it has been shown that A_{2A}AR and A_3AR are up-regulated in untreated RA patients and in methotrexate (MTX) treated RA patients. Treatment with anti-TNF-α normalized A_{2A}AR and A_3AR expression and functionality.[172] The overexpression of A_3ARs in RA has been directly correlated to high levels of proinflammatory cytokines acting via an up-regulation of NF-kB, which is a key player in the pathogenesis of arthritic diseases.[170] In RA patients, adenosine suppressed the elevated levels of proinflammatory cytokines such as TNF-α and IL-1β.[169] It has been reported that A_3AR agonists prevent cartilage damage, osteoclast/osteophyte formation, and bone destruction and markedly reduce pannus formation and lymphocyte formation.[173] A_3ARs were also identified as a novel anti-inflammatory target that was up-regulated in RA and psoriasis, if compared with healthy subjects, and was associated with an altered PI3K-PKB/Akt signaling pathway and NF-kB activation.[174] The presence of cyclic AMP mediates a number of various anti-inflammatory pathways resulting in inhibition of TNF-α and/or IL-1β and ameliorates the symptoms of joint inflammation.[175] Adenosine production and signaling have emerged as an important cell mechanism to regulate inflammation due to an increase of the receptor density and/or functionality. Novel ligands interacting with ARs that are safe and selective in humans allow us to better understand their role in several conditions of inflammation essential for successful drug discovery in RA.[172] In fact, oral treatment with the selective A_3AR agonist IB-MECA (CF101) led to a marked decrease in RA clinical manifestations. IB-MECA treatment reduced inflammation, pannus formation, cartilage destruction, bone resorption, and lysis.[176] The specificity of the response was evident when an A_3AR antagonist was introduced into arthritic animals prior to each treatment to neutralize the anti-inflammatory response.[176]

In a phase I study in healthy subjects, IB-MECA was found to be safe and well tolerated with dose−linear pharmacokinetics.[175] In a phase II study in RA patients, IB-MECA oral administration twice daily for 12 weeks was shown to be safe, well tolerated and able to mediate an improvement of disease signs and symptoms, suggesting the development of these drugs as antirheumatic agents.[171]

The anti-inflammatory effect of A_3ARs was also proven in fibroblast-like synoviocytes derived from synovial fluid of RA patients closely associated with a decrease in NF-kB and TNF-α release.[177] In particular, a novel A_3AR agonist (compound **20**) induced a dose-dependent inhibitory effect on the proliferation of fibroblast-like synoviocytes via deregulation of NF-kB signaling pathway, suppressing the clinical and pathological manifestations of adjuvant induced arthritis in a rat experimental model. Recently, it was reported that A_{2A}AR and A_3AR activation inhibited the NF-kB pathway, diminished inflammatory cytokines such as TNF-α, IL-1-β, IL-6, and mediated a reduction of the release of the metalloproteinase types 1 and 3. A_{2A}AR and A_3AR density inversely correlated with the clinical disease activity score, suggesting a direct role of the endogenous activation of these receptors in the control of RA joint inflammation.[178]

Osteoarthritis (OA) is the most common forms of arthritis and is the most important cause of disability in older adults. At present, the current recommended treatment of OA involves weight loss, physical therapy, and the use of pain relievers. However, these drugs do not reverse the OA degenerative process and show some adverse effects on cartilage metabolism.[179] It is well-known that p38MAPKs are involved in controlling cellular responses such as the release of pro-inflammatory cytokines.[4] The cell signaling pathways initiated by proinflammatory events converge on activation of NF-kB, which drives cytokine transcription and production.[180] Notably, p38 MAPK is one of the kinases implicated in the phosphorylation of NF-kB inhibitors (IkBs). Once phosphorylated, IkBs undergo polyubiquitination and ultimately proteosomal degradation, allowing NF-kB to enter the nucleus and promote the transcription of inflammatory genes, such as TNF-α and IL-8.[180] The role of adenosine and its interaction with ARs in modulating bovine chondrocytes and synoviocytes activity have been documented by using saturation, competition binding experiments, and Western blotting analysis.[181] Functional studies have suggested an anti-inflammatory effect due to A_1AR and A_{2A}AR activation in LPS-induced PGE_2 production mediated by a down-regulation of TNF-α and COX-2 mRNA expression.[182] Furthermore, it has been demonstrated that in different cells or tissues, adenosine is a regulator of NF-kB and MAPK signaling through the interaction with AR subtypes.[175] It has been also reported that the NF-kB signaling pathway is deregulated by the presence of IB-MECA and involved in the OA pathogenesis . In addition, IB-MECA induced apoptosis of inflammatory cells and acted as a cartilage protective agent, suggesting its use as a suitable candidate drug for the OA treatment.[173] More recently, ARs have also been characterized by using binding and functional assays, in human synoviocytes that represent key cells closely associated with articular pathologies.[183]

These results strongly suggest a potential role of ARs in chronic inflammatory arthritis and emphasize the utility to better investigate, from a pharmacological point of view, the modulation of the inflammatory conditions closely associated with these diseases.

A_3ARS IN GASTROINTESTINAL AND RENAL DISORDERS

Ulcerative colitis and Crohn's disease, collectively known as inflammatory bowel disease, are severe and debilitating disorders with a growing incidence in both developing and advanced countries.[184] Both diseases are characterized by serious inflammation of the enteric mucosa at different levels of the gastrointestinal tract associated with significant alterations of gastrointestinal motor, secretory, and sensory functions.[185] It has been observed that activation of T lymphocytes and macrophages is followed by a massive release of several proinflammatory cytokines including IL-1, IL-6, and TNF-α which stimulate the secretion of chemotactic cytokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1) responsible for the recruitment of leukocytes into inflamed mucosa.[4] A_3ARs are also emerging for treatment of bowel inflammation; the well-known A_3AR agonist IB-MECA was shown in mice to ameliorate intestinal inflammation and spontaneous colitis. In addition, A_3AR stimulation was able to markedly reduce colonic levels of proinflammatory cytokines such as IL-1, IL-6, and IL-12 and to reduce the local production of macrophage inflammatory proteins such as MIP-1α or MIP-2 with a powerful down-regulation of leukocytes in bowel inflammation.[186] Different studies have evaluated the effects of A_3AR agonists on gene dysregulation and tissue injury in a rat model of colitis. It has been demonstrated that A_3AR agonists prevented the induction of various cytokine/chemokine/inflammatory genes and promoted a marked suppression of ROS production with a significant amelioration of intestinal injury. The effect of adenosine on pain transmission has also been described in an animal model of visceral pain induced by intraperitoneal injection of acetic acid, while the stimulation of ARs induced an inhibitory or facilitatory effect on pain perception.[187] It has been reported that in the stomach, jejunum, colon ileum, cecum, and liver, an up-regulation of A_3ARs was observed during colitis that modulate clear anti-inflammatory processes in intestine and liver.[186,188] More recently, it has been observed that A_3ARs are overexpressed in different autoimmune pathologies such as Crohn's disease and psoriasis. The up-regulation observed in these pathologies could be attributed to adenosine, which accumulates in the extracellular environment under stressed conditions. Most transcription factors such as NFkB and CREB that are identified to promote inflammation were inversely associated with A_3AR up-regulation.[174] Acute renal failure is a major contributor to perioperative mortality and morbidity. Ischemic−reperfusion injury, toxic nephropathy, and myoglobinuria all can lead to the renal failure, which is frequently complicated by many other life-threatening complications including sepsis and multiorgan failure.[189] The preischemic administration of an A_3AR antagonist or the model of A_3AR knockout mice showed a high level of protection in the rat kidney against ischemia−reperfusion injury.[189] In addition, A_3AR activation degranulates mast cells, increases plasma histamine in rodents, and decreases the mortality and the renal and/or hepatic injury in murine septic peritonitis. The administration of A_3AR agonists significantly reduced mortality in mice lacking A_1AR and A_{2A}AR but not A_3AR, demonstrating the specificity of the A_3ARs to mediate protection against sepsis-induced mortality.[190]

A_3ARS AND EYE DISORDERS

It was reported that A_3ARs have been implicated in many ocular diseases such as dry eye, glaucoma, or uveitis. In the past,

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the A_3AR knock out mouse showed lower intracellular pressure, suggesting a role for A_3AR antagonists in the therapy of glaucoma.[106] In addition, the use of A_3AR antagonists may be an alternative approach for treating ocular hypertension in patients affected by the pseudoexfoliation syndrome in open angle glaucoma, which is typically associated with anterior chamber hypoxia and elevated intraocular pressure.[191] On the other hand, A_3AR mRNA and protein have been found to be consistently increased in the nonpigmented ciliary epithelium of the eye in pseudoexfoliation syndrome with glaucoma, compared to normal eye.[192] A_3AR overexpression has also been reported in retinal ganglion cells, which upon agonist treatment showed reduced calcium levels and cell rescue from apoptosis.[193] The anti-inflammatory and the protective effects mediated via A_3AR prompted us to examine the effect of IB-MECA in a model of experimental autoimmune uveitis that represents human uveitis with an autoimmune etiology. IB-MECA inhibited the clinical and pathological manifestations of interphotoreceptor retinoid-binding protein-induced uveitis.[194]

■ A_3ARS AND CANCERS

Adenosine is present at high concentrations in cancer tissues and in the interstitial fluid of several tumors, at concentrations sufficient to interact with ARs.[7] In particular, A_3ARs are present in different types of tumor cells, such as HL60 and K562 human leukemia,[135] Jurkat lymphoma,[133] U937 monocytic−macrophagic human cell line,[159] Nb2 rat lymphoma,[195] A375 human melanoma,[134] PGT-β mouse pineal gland tumor cells,[196] human glioblastoma,[197,198] and human prostatic cells.[199] A_3ARs are involved in the tumor growth and in the regulation of cell cycle and mediate both pro- and antiapoptotic effects closely associated with the level of receptor activation.[161,200] A_3ARs are able to mediate the inhibition of telomerase activity and arrest at the G0/Gi phase of the cell cycle leading to a cytostatic effect in Nb2-11C lymphoma cells.[195] In addition, it was demonstrated that A_3ARs inhibit tumor growth by regulation of the WNT pathway, which mediates cell cycle progression and cell proliferation.[1] N^6-(3-Iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA) treatment induced down-regulation of the expression of NF-kB, known to regulate the transcription of cyclin D1 and c-Myc.[201] The A_3ARs reduced the ability of prostate cancer cells to migrate in vitro and metastasize in vivo. In particular, it has been reported that activation of the A_3ARs in prostate cancer cells reduced PKA-mediated stimulation of ERK1/2, leading to lower NADPH oxidase activity and cancer cell invasiveness.[199] In prostate cancer cells, IB-MECA inhibited cell proliferation and induced G1 cell cycle arrest and/or apoptosis via a mitochondrial signaling pathway.[202] It has been found that inhibition of cell proliferation or induction of apoptosis with A_3AR agonists was only obtained at micromolar doses.[9,52,203] In fact, IB-MECA, at micromolar doses in breast cancer cells, inhibited cell proliferation through interaction with receptors such as the estrogen α subtype.[204] Moreover, Cl-IB-MECA at micromolar doses interacts with all ARs, causing different complications to clarify functional cell response associated with a specific receptor subtype. The involvement of the A_3ARs in hypoxic conditions, in an in vitro model reproducing the microenvironment of solid tumors in vivo, has suggested that the signaling pathway generated by this receptor stimulation involves the MAPK activity that is required for HIF-1α expression. Opposite effects are mediated by A_3AR activation that in glioblastoma and colon cancer cells stimulate VEGF expression, while in pheochromocytoma cells promote VEGF down regulation.[205] The HIF-1α regulation by A_3AR activation induced an increase in angiopoietin-2 and/or VEGF, depending on the cell model investigated.[156] Clinical investigations have clearly shown that the prevalence of areas of hypoxic tissue is a specific property of solid tumors.[4] Furthermore, hypoxia appears to induce an increase of intracellular adenosine levels and to stabilize the most important factors involved in hypoxia such as HIF-1α.[198] The actions of adenosine are primarily linked to an increase of angiogenesis and to the release of VEGF and HIF-1α.[205] So the pharmacologic inhibition of HIF-1α may be useful to improve cancer treatment based on the cooperation between hypoxic and adenosine signaling.[15,156,198] A_3AR density was up-regulated in colon carcinoma tissues and closely correlated to the disease severity. In addition, the A_3AR alteration reflected a similar behavior shown in lymphocytes or neutrophils derived from colon cancer patients, suggesting that these receptors may represent an interesting biological marker.[206] Moreover, Cl-IB-MECA enhanced apoptosis via the modulation of NF-kB signaling pathway in thyroid cancer cells and reduced the ability of prostate cancer cells to migrate in vitro and metastasize in vivo.[207] Recently, it has been reported that A_3AR selective agonists induce an anti-inflammatory and anticancer effect in a xenograft animal model utilizing Hep-3B hepatocellular carcinoma cells.[208] In this model, the A_3AR up-regulation was present in inflammatory liver tissues similar to those previously found in other inflammatory conditions.[173] It has also been reported that in malignant mesothelioma pleura (MMP), mRNA and protein expression of A_3ARs was statistically increased with respect to healthy mesothelial pleura (HMP). In particular, A_3AR density in MMP was increased by 2.5-fold in comparison with HMP. A_3ARs were also up-regulated in malignant mesothelioma cells (MMC) if compared with human healthy mesothelial cells (HMC). Stimulation of A_3ARs decreased proliferation and exerted a cytotoxic and proapoptotic effect on MMC and on HMC, exposed to asbestos and TNF-α, but not on HMC with an involvement of the deregulation of Akt/NF-κB cell survival pathway.[209] These data suggest that A_3ARs could represent a biological marker and that A_3AR modulation could be used in cancer treatment.

■ CONCLUSIONS AND PERSPECTIVES

It is well-known that the clinical use of exogenous adenosine is limited because it does not possess AR specificity and is rapidly metabolized by adenosine deaminase. Thus, instead of using adenosine, the synthetic chemical approaches have been concentrated toward the development of receptor-specific adenosine agonists or antagonists. All the pharmacological evidence found in the adenosine research field led to the design of thousands of compounds, few of which are now in clinical trials for treating different pathologies, with the aim to identify new leads potentially useful as novel diagnostic and therapeutic agents. It has been indeed well described that A_3ARs play an important role in different human pathologies, suggesting the potential use of selective agonists and/or antagonists acting through AR modulation. The chemical development of several classes of hA_3AR selective agonists and/or antagonists has permitted the identification of clinical candidates with an important impact on the drug discovery process (see Table 3). Some A_3AR antagonists are available in preclinical studies as **42** (National Institutes of Health, Figure 7) for stroke,[210] **60** (Otsuka Pharmaceutical, Figure 7) and **77** (National Institutes of Health, Figure 9) as antiglaucoma agents.[25] Other A_3AR

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Table 3. A_3AR Ligands under Preclinical or Clinical Investigation (Data from Thomson Reuters Integrity)

compd	therapeutic group	highest phase	organization
	Agonist		
6a, IB-MECA	glaucoma	phase II/III	Can-Fite Biopharma
	antineoplastic enhancing agent		
	psoriasis		
	colorectal cancer		
	dry eye syndrome		
	RA		
	solid tumors		
	osteoarthritis		
	oncolytic drugs		
	keratoconjunctivitis	phase II	
	uveitis	phaseI	
6b, Cl-IB-MECA	liver cancer	phase I/II	Can-Fite Biopharma
	hepatitis C		
	liver and biliary tract disorders		
16	angina pectoris	preclinical	Pfizer
18	oncolytic drug	preclinical	U.S. Department of Health & Human Services
20	acute myocardial infarction	preclinical	Can-Fite Biopharma
	arthritis		National Institutes of Health
	oncolytic drug		
	stroke		
	Antagonist		
30b	arthritis and COPD	preclinical	Novartis
42	stroke	preclinical	National Institutes of Health
60	glaucoma	preclinical	Otsuka Pharmaceutical
SAR-137272	asthma and COPD	preclinical	Sanofi
77	glaucoma	preclinical	National Institutes of Health
	Allosteric Modulator		
91	osteoarthritis	preclinical	National Institutes of Health

antagonists, named CGH 2466 (thiazole analogue of **30**, Novartis)[211] and SSR 161421 and SAR 137272 (Sanofi, structures not disclosed), have been investigated in the treatment of asthma or COPD.[62] While none of the examined A_3AR antagonists have until now gone beyond the preclinical investigation, a few examples of A_3AR agonists are currently available in specific clinical trials, suggesting their possible using for novel therapeutic treatments. Among dermatological disorders, a phase II/III study in patients with psoriasis has demonstrated that the treatment with IB-MECA was safe, well tolerated, and effective in improving psoriatic area and severity index score.[212] Studies from a phase II/III clinical trial revealed that IB-MECA, given orally, induced statistically significant improvements in patients with dry eye syndrome or with glaucoma.[213] In these diseases, IB-MECA was well tolerated and exhibited an excellent safety profile with no serious adverse events.[214] At present, IB-MECA has also been investigated in phase I clinical trial for uveitis and in phase II clinical trials for keratoconjunctivitis.[194] In addition, a phase II clinical study has demonstrated that oral IB-MECA treatment showed an excellent safety profile and induced a statistically significant improvement in the corneal staining, tear breakup time, and tear meniscus with a decrease in intraocular pressure patients with dry eye syndrome.[213]

In musculoskeletal and connective tissue disorders, such as RA, IB-MECA is available in a phase IIb clinical study where it has been reported that patients with high A_3AR expression at baseline responded positively to the drug.[171] The compound was well tolerated and effective, with a maximum clinical response observed at a dose of 1 mg in RA patients. IB-MECA has been also evaluated in terms of safety and efficacy in a phase II clinical study with patients suffering from osteoarthritis of the knee.[173] These studies have suggested that A_3AR expression levels could have an important predictive value in determining treatment response. In the past, it has been demonstrated that the small orally bioavailable molecule IB-MECA exerts systemic anticancer, antimetastatic, and myeloprotective effects in colon carcinoma-bearing mice and may serve as an adjuvant treatment to enhance the chemotherapeutic index and prevent myelotoxicity.[215] In particular, in colon cancer cell lines the stimulation of A_3ARs mediates a tonic proliferative effect, suggesting that these receptors could be potentially used as a diagnostic marker or a therapeutic target.[200,206] The inhibition of primary colon carcinoma growth and liver metastasis by IB-MECA has been accurately described showing in a colon carcinoma murine model an increase of the chemotherapic effect of 5-fluorouracil in the presence of the A_3AR agonist.[216] IB-MECA has been in phase II clinical trials for colorectal cancer, given that the anticancer effect of the A_3AR agonist has been convincingly demonstrated in vitro and in vivo.[201] Another A_3AR agonist, Cl-IB-MECA, which is a targeted drug with high affinity and selectivity, has been developed in early phase I/II clinical studies for the treatment of liver disease including hepatocellular carcinoma and hepatitits as well as for liver regeneration.[213] Cl-IB-MECA has been also studied in a phase I/II trial for the treatment of chronic hepatitis C and in liver disease to promote liver regeneration.[208] Recently, an interim analysis of a phase I/II trial of Cl-IB-MECA has been reported from Can-Fite BioPharma. This study included 18 patients with hepatocellular carcinoma who were treated with three different doses of Cl-IB-MECA, determining the pharmacokinetic behavior and the safety profile of the long-term administration.[217]

Taken together, these findings suggest that A_3ARs could represent a possible target for pharmacological intervention to prevent different pathologies in both the central nervous system and the cardiovascular system, in inflammatory diseases, and in cancer.

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Notes

The authors declare no competing financial interest.

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Delia Preti received her B.S. in Medicinal Chemistry (2001) and her Ph.D. in Pharmaceutical Sciences (2005) from Ferrara University, Italy. In the following years (2005−2009) she has worked at the Department of Pharmaceutical Sciences of Ferrara University in a postdoctoral research position, focusing her interests in the design and synthesis of adenosine receptors ligands and antitumor compounds inhibiting tubulin polymerization, in collaboration with the company King Pharmaceuticals (North Carolina, U.S.), now part of Pfizer. Since 2009, she has joined a research project based on the design of TRPA1 channel antagonists for the treatment of pain and inflammation at the Department of Preclinical and Clinical Pharmacology of the University of Florence, Italy, in collaboration with the Italian Institute of Technology (Genova, Italy).

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■ ABBREVIATIONS USED

AB-MECA, N^6-(4-aminobenzyl)adenosine-5′-*N*-methylcarboxamide; AC, adenylate cyclase; AR, adenosine receptor; BAL, bronchoalveolar lavage; CHO, Chinese hamster ovary cell; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide; GRK2, G-protein-coupled receptor kinase 2; HEK293, human embryonic kidney 293 cell; HIF-1α, hypoxia inducible factor 1α; HMC, human healthy mesothelial cells; HMP, healthy mesothelial pleura; IB-MECA, N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide; IL-1β, interleukin 1β; JNK, c-Jun N-terminal kinase; LNA, locked nucleic acid; MCP-1, monocyte chemoattractant protein 1; MECA, 5′-(*N*-methylcarboxamido)adenosine; MMP, malignant mesothelioma pleura; NECA, 5′-(*N*-ethylcarboxamido)adenosine; PD, Parkinson's disease; PLC, phospholypase C; PTP, pyrazolotriazolopyrimidine; RA, rheumatoid arthritis

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