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Progress in the Pursuit of Therapeutic Adenosine Receptor Antagonists

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Abstract

Ever since the discovery of the hypotensive and bradycardiac effects of adenosine, adenosine receptors continue to represent promising drug targets. First, this is due to the fact that the receptors are expressed in a large variety of tissues. In particular, the actions of adenosine (or methylxanthine antagonists) in the central nervous system, in the circulation, on immune cells, and on other tissues can be beneficial in certain disorders. Second, there exists a large number of ligands, which have been generated by introducing several modifications in the structure of the lead compounds (adenosine and methylxanthine), some of them highly specific. Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been cloned and pharmacologically characterized, all of which are G protein-coupled receptors. Adenosine receptors can be distinguished according to their preferred mechanism of signal transduction: A_1 and A_3 receptors interact with pertussis toxin-sensitive G proteins of the G_i and G_o family; the canonical signaling mechanism of the A_{2A} and of the A_{2B} receptors is stimulation of adenylyl cyclase *via* G_s proteins. In addition to the coupling to adenylyl cyclase, all four subtypes may positively couple to phospholipase C *via* different G protein subunits. The development of new ligands, in particular, potent and selective antagonists, for all subtypes of adenosine receptors has so far been directed by traditional medicinal chemistry. The availability of genetic information promises to facilitate understanding of the drug–receptor interaction leading to the rational design of a potentially therapeutically important class of drugs. Moreover, molecular modeling may further rationalize observed interactions between the receptors and their ligands. In this review, we will summarize the most relevant progress in developing new therapeutic adenosine receptor antagonists.

Keywords

G protein-coupled receptor; adenosine receptor; antagonists

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1. INTRODUCTION

The stimulation of cell surface adenosine receptors (ARs) is largely responsible for the broad variety of effects produced by adenosine throughout several organ systems. Based on the widespread and frequently beneficial effects, attributed to the accumulation of endogenously released adenosine, it has long been considered that regulation of ARs has substantial therapeutic potential. Incidentally, much recent focus has been on the cardioprotective[1,2] and neuroprotective[3,4] effects associated with AR activation during periods of cardiac and cerebral ischemia, respectively. On the other hand, it has been proposed recently that antagonists of distinct AR subtypes may be used in the treatment of asthma[5,6] or certain neurological diseases such as Parkinson's disease.[6,7] Comprehensive reviews of the physiological roles of ARs and their potential as clinical targets in a variety of disease states have been published.[6–11]

ARs are members of the superfamily of G protein-coupled receptors (GPCRs), with four subtypes currently recognized, the A_1AR, A_{2A}AR, A_{2B}AR, and A_3AR.[12] With the exception of the A_3AR, the existence of AR subtypes in various tissues had been appreciated prior to their cloning as a result of pharmacological characterization.[12]

The cloning of the four AR subtypes has allowed for significant progresses to be made in the understanding of several facets of AR activity at a molecular level. A schematic representation of AR signaling pathways shown in Figure 1.[11]

Considering the overall protein structure, ARs display the topology typical of GPCRs. Many features of GPCR structure and function have been reviewed recently.[12–15] Here we will highlight some fundamental features that may expand upon the classical view of GPCR structure and function. Sequence comparison between the different GPCRs revealed the existence of different receptor families sharing no sequence similarity even if specific fingerprints exist in all GPCR classes. However, all these receptors have in common a central core domain consisting of seven transmembrane helices (TM1-7), with each TM composed of 20–27 amino acids, connected by three intracellular (IL1, IL2, and IL3) and three extracellular (EL1, EL2, and EL3) loops. Two cysteine residues (one in TM3 and one in EL2), which are conserved in most GPCRs, form a disulfide link which is possibly crucial for the packing and for the stabilization of a restricted number of conformations of these seven TMs. Aside from sequence variations, GPCRs differ in the length and function of their N-terminal extracellular domain, their *C*-terminal intracellular domain, and their intracellular loops. Each of these domains provides very specific properties to these receptor proteins. Particularly, consensus sites for *N*-linked glycosylation exist on the extracellular regions of ARs, although the precise location of the sites for this post-translational modification varies amongst the AR subtypes.[16–19] The carboxyl-terminal tails of the A_{1A}R, A_{2B}AR, and A_3AR, but not A_{2A}AR, possess a conserved cysteine residue that may putatively serve as a site for receptor palmitoylation and permit the formation of a fourth intracellular loop. However, site-directed mutagenesis of this residue has not been performed for any AR subtype, and no role for putative AR palmitoylation has been described.

The A_1AR, A_{2B}AR, and A_3AR are very similar in regard to the number of amino acids composing their primary structure, and in general, these AR subtypes are among the smaller members of the GPCR family. For example, the human homologs of the A_1AR, A_{2B}AR, and A_3AR consist of 326, 328, and 318 amino acid residues, respectively.[20–22] Conversely, the human A_{2A}AR is composed of 409 amino acids.[23] All cloned species homologs of the A_{2A}AR are of similar mass, and this relatively large size is manifested in the carboxyl-terminal tail of the receptor, which is much longer than that of the other AR subtypes. It should be noted that the size of ARs deduced from their primary amino acid structure frequently is not consistent with the mass estimated by polyacrylamide gel electrophoresis of the expressed proteins. The aforementioned post-translational glycosylation of ARs, which may vary in a cell type-dependent fashion, likely accounts for these discrepancies. The human A_1AR and human A_3AR display ca 49% overall sequence identity at the amino acid level, while the human A_{2A}AR and human A_{2B}AR are 45% identical. A general topology of all four receptor subtypes are shown in Figure 2.

Indeed, for all GPCRs, the identification of discrete receptor regions, or even single amino acids that are critical for ligand recognition and are responsible for discerning between agonist and antagonist ligands, has been an area of extensive investigation.[11,24–27] In addition to a basic understanding of receptor activation, it has been hoped that a delineation of ligand–receptor interaction at a molecular level may provide the basis for rational drug design.[11,24–27] As summarized below, both TMs and extracellular regions of ARs have been implicated as playing a role in the formation of the ligand-binding pocket.[11,24–27] Key amino acids identified *via* mutagenesis studies as contributing to the ligand-binding properties of the ARs are briefly summarized in Table I.

Site-directed mutagenesis studies in parallel with different molecular modeling approaches have been recently used as powerful strategy to design potent and selective GPCR ligand.[11,36–39] Of course, the evolution of the field of computer-aided design of ligands (both agonists and antagonists) for GPCRs, including adenosine receptors, has depended on the availability of suitable molecular receptor templates. In fact, due to technical difficulties, which complicate experimental X-ray diffraction and NMR structure determination of GPCRs, the 3D structure of most GPCRs is still unknown. The only known GPCR structure, a 2.8 Å resolution structure of rhodopsin, was published only recently by Palczewski and collaborators.[40] However, a structure-based approach to GPCR drug discovery in the absence, but probably also in the presence, of the real structures requires a multidisciplinary approach, where molecular models represent a structural context to efficiently integrate experimental data and inferences derived from molecular biological, biophysical, bioinformatic, pharmacological and organic chemical methods. Although not always achievable, the success of a synergistic effect among these disciplines is highly dependent on the experimental design. Synergy is best achieved when mutations are structurally interpretable, structural hypotheses are experimentally testable, ligands are well characterized pharmacologically, and the necessary chemical modifications of the ligands are feasible.[11]

In recent decades, numerous medicinal chemistry groups have made intense efforts in searching for ideal ligands for these receptor subtypes.[9–11,41–45] In particular, the search

for selective antagonists held greater appeal than selective agonists, not only for their potential therapeutic applications but also considering the fact that antagonists are preferred molecular probes for pharmacological characterization of receptors. Considering all of these aspects, the search for potent and selective adenosine receptor antagonists has been one of the most highly investigated areas in medicinal chemistry in recent years. It should be emphasized that for all the receptor subtypes the alkylxanthines (e.g., theophylline, caffeine), which are natural antagonists for the adenosine receptors, have represented the starting point for the discovery of potent and selective antagonists.[41–45] Following multiple modifications of the xanthine nucleus, various potent and selective antagonists have been found. Nevertheless, xanthine derivatives have several physico-chemical limitations, including low water solubility. For this reason, several research groups have focused on compounds having a non-xanthine structure for improving the water solubility and consequently bioavailability.[41–45]

The purpose of this review is to summarize the most recent developments made in the field of adenosine receptor antagonists, which for all classes could be subdivided into two large families: (i) xanthine derivatives; (ii) polyheterocyclic derivatives.

2. A_1 ADENOSINE RECEPTOR ANTAGONISTS

The A_1AR could be considered the best-characterized member of the adenosine receptor family. Several antagonists are currently under clinical investigations, and are recently reviewed.[10,41–43]

A. Xanthine Derivatives

A large number of modifications on the xanthine core at the 1, 3, and 8 positions led to the discovery of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, **1**) which was highly potent and selective at the adenosine A_1AR in a rat model,[46] while at the human A_1AR it was 10-fold less potent with a consequent reduction of selectivity versus the other receptor subtypes. Also, DPCPX displays considerable affinity at the human A_{2B}AR.[47] For these reasons the search for a truly selective A_1AR adenosine receptor antagonist in the human model represented a new appealing goal to be achieved (Fig. 3).

In addition to DPCPX, other substituted xanthines have been proposed as A_1AR antagonists, in particular, by introducing chiral substituents to demonstrate the importance of the stereochemistry, or by insertion of polar moieties. The introduction at the 8-position on the 1,3-dipropylxanthine nucleus of a [2-(5,6-epoxy)norbornyl] moiety led to the discovery of BG-9719 (**2**) which was highly potent and selective at the A_1AR in a human model.[48] A small stereochemical effect on the affinity was present with this compound, such that the *R*-isomer was twofold less potent (K_i hA_1 = 0.80 nM) than the *S*-isomer (Fig. 3).

Recently, a fluorescent derivative of the xanthine amine functionalized congener (XAC)[49] was shown to be useful for visualizing the A_1AR in small areas of cell membranes using fluorescence correlation spectroscopy.[50]

B. Polyheterocyclic Derivatives

Numerous classes of heterocyclic derivatives were shown to bind to the A_1AR. Among the first such derivatives were the ^{1}H-imidazo[4,5-*c*]quinolin-4-amines, which were synthesized based on a prediction from early ligand modeling.[51] Most of these derivatives could be considered an extension of the xanthine structure. More recently, some synthetic triazolo-purinones (**3,4**), clearly derived as tricyclic extensions of the xanthine nucleus, showed very promising affinity at the A_1AR subtype with significant degree of selectivity versus the A_{2A}AR subtype (Fig. 4).[52]

Another class of A_1AR antagonists is represented by 3-aryl-[1,2,4]triazino[4,3-*a*]-benzimidazol(^{10}H)-4-one derivatives. In particular, compound **5** displayed high affinity at the bovine A_1AR and significant selectivity in comparison to the A_{2A}AR and A_3AR subtypes (Fig. 4).[53] A related A_1AR antagonist could be considered the triazolo-quinoxaline **6** which displays high potency and good selectivity (Fig. 4).[54] Isosteres of **6**, such as pyrazolo-quinolines or imidazo-quinoxalines have been also reported as A_1AR antagonists. Although the affinity at A_1AR was in the nanomolar range, none of the reported compounds were found to be highly selective.[55,56]

A series of non-xanthine heterocycles displaying high potency at A_1AR and selectivity versus all the other subtypes is represented by 7-deaza-adenines. One particular derivative **7** (APEPI) proved to be highly potent and selective (Fig. 4). The A_1AR affinity is prevalently due to the *R*-enantiomer. Many other modifications have been made on this nucleus (e.g., replacement of phenyl ring at the 2 position or structure simplification to an indole nucleus), but none of these variations improved both affinity and selectivity.[56,57]

Very simplified heterocyclic derivatives as adenosine receptor antagonists are represented by thiazole and thiadiazole derivatives (Fig. 5). In particular, thiadiazole **8** (LUF5437) has been considered the starting point for this new class of compounds.[58] In fact, complete hydrogenation of phenyl ring led to derivative **9** (LUF5472), which was less potent at the A_1AR but more selective. Replacement of the thiadiazole nucleus with a thiazole moiety seemed to be well tolerated at the A_1 receptor.[58,59]

Another class of simplified analogs structurally related to the xanthine core consists of derivatives of the pyrazolo[1,5-*a*]pyridine nucleus. Compound **10** (FK453) represents the lead compound of this series showing favorable affinity and selectivity for the A_1AR compared to the A_{2A}AR (Fig. 5). Various modifications have been performed on this nucleus but when acryloyl amide was constrained into a pyridazinone nucleus and nitrogen was substituted with an isobutyryl moiety (compound **11**) a significant increase of potency and selectivity was obtained (Fig. 5).[60–62]

Also, a naphthyridine nucleus has been investigated for A_1AR antagonists. In a naphthyridine series, compound **12** proved to be a promising antagonist displaying affinity in a sub-nanomolar range and high levels of selectivity in a bovine model, while unfortunately in human the compound dramatically lost potency and consequently selectivity.[63,64]

Very recently, IJzerman and coworkers synthesized a large number of pyrimidine derivatives designed with the help of molecular modeling. This study permitted to identify the compound **13** that was potent and selective as a human A_1AR antagonist (Fig. 5).[65]

As clearly described, chemically diverse classes of compounds have been identified as A_1AR antagonists. Nevertheless, considering that many have not yet been examined at all four adenosine receptor subtypes and the species differences are evident, most of these synthetic compounds should be reexamined in a human model for better understanding and for consideration as clinical candidates.

C. Biological Actions of A_1 Adenosine Receptor Antagonists

Peripheral applications envisioned for A_1 receptor antagonists include kidney protection and cardiac anti-arrhythmic agents.[42,43] Since caffeine is best known for its stimulant activity in the central nervous system, adenosine antagonists of the A_1 receptor and other subtypes have been of interest in cognitive disorders. A novel, potent, and selective adenosine A_1 receptor antagonist FR194921 exerts both cognitive-enhancing and anxiolytic activity, suggesting the therapeutic potential of such compounds for dementia and anxiety disorders.[66]

3. A_{2A} ADENOSINE RECEPTOR ANTAGONISTS

Both xanthines and non-xanthines have been developed as selective A_{2A}AR antagonists. A_{2A}AR antagonists proved to be attractive for the treatment of several diseases of the central nervous system, such as motor dysfunctions, due to the clearly demonstrated interaction between A_{2A}AR and D_2 dopamine receptors (both at the protein and second messenger levels) in the basal ganglia.[67] For this reason, A_{2A}AR antagonists could be considered potential drugs for the treatment of neurodegenerative disorders such as Parkinson's disease.

A. Xanthine Derivatives

The first xanthine analog, which displayed good potency at the A_{2A}AR subtype (100 nM) and significant selectivity in comparison to the A_1AR (45-fold) was the 8-unsubstituted 1-propargylxanthine (**14**) (Fig. 6).[68]

Starting from this observation, a program to screen various 1-, 3-, 8-substituted xanthines led to the discovery of the first very potent and selective A_{2A}AR antagonist, 1,3-dipropyl-7-methyl-8-(3, 4-dimethoxystyryl)xanthine (KF17837, **15**), which proved to be potent in the nanomolar range at the A_{2A}AR subtype (1 nM) and significantly selective in comparison to A_1AR (62-fold) (Fig. 3).[69,70] In a detailed SAR study on this class of compounds, the 3-chlorostyrylcaffeine (CSC, **16**) was identified as being less potent than **15** at the A_{2A}AR (54 nM) but with an increased selectivity in comparison to the A_1AR subtype (560-fold).[71]

Two major problems have limited the use of these xanthine derivatives as pharmacological tools for studying the A_{2A}AR subtype: (a) the low water solubility;[72] (b) the rapid photoisomerization which they undergo when exposed to daylight in dilute solution.[73] It should be noted that this isomerization process is not relevant when styrylxanthines are administered orally as solid substances. In an attempt to avoid this problem, the styryl moiety has been replaced with different functional groups (e.g., triple bond, cyclopropyl, or

diazo group) or constrained structure. However, none of this isosteric substitutions has led to an improvement in the pharmacological profile, but rather in many cases to a complete loss of affinity.[68,74]

Instead, the introduction of a propargyl at the 1-position in combination with the 8-styryl group by Müller and coworkers seemed to increase affinity at the A_{2A}AR subtypes with the retention of selectivity. These studies led to the discovery of the BS-DMPX (3,7-dimethyl-1-propargyl-8-(3-bromostyryl)xanthine **17**, which could be considered a lead compound of a new series.[75] However, at the 3 and 7 positions, methyl substitution seemed to be desirable for achieving both affinity and selectivity at the A_{2A}AR subtype (Fig. 6).[76–78] 2A Regarding the substitutions at the 8-position, it has been clearly demonstrated that an aromatic ring attached to an ethenyl group is a fundamental requirement for both affinity and selectivity at the A_{2A}AR.[77,79]

For the improvement of water solubility of styryl xanthines, two different approaches have been utilized: (a) introduction of polar groups on the phenyl ring; (b) preparation of phosphate pro-drugs. The introduction of a sulfonate group on the phenyl ring of styryl moiety produces a significant reduction of affinity (20- to 30-fold) at the A_{2A}AR but with retention of selectivity.[80]

More interesting results have been obtained using phosphate ester pro-drugs. In fact, the pro-drug **18**, which was stable in aqueous solution but readily cleaved by phosphatases to liberate MSX-2 (3-(3-hydroxypropyl)-8-(3-methoxystyryl)-1-propargylxanthine, showed a very high affinity and selectivity for the A_{2A}AR (**19**, Fig. 6).[81]

All these studies, performed by several laboratories, have strongly suggested reconsidering the xanthine family as A_{2A}AR antagonists. In fact, such an antagonist, KW-6002 (1,3-diethyl-8-(3-methoxystyryl)-7-methilxanthine, **20**, is already in phase II clinical trials for the treatment of basal ganglia disorders such as Parkinson's disease.[82]

Unfortunately, very recently, more detailed studies performed on MSX-2 (**19**), in contrast with previous studies, clearly demonstrated that styryl xanthines at the solid state upon light irradiation led to dimmer derivatives which are almost inactive at the A_{2A}AR. This should be considered a further limit of clinical use of styryl xanthine derivatives.[83]

B. Polyheterocyclic Derivatives

The first promising A_{2A}AR antagonist with a non-xanthine structure was CGS 15943 (**21**, 9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine),[84,85] which showed affinity but not selectivity versus A_1AR, A_{2B}AR, and A_3AR (Fig. 7).[86] Nevertheless, it has represented the starting point for developing new non-xanthine structures as A_{2A}AR adenosine antagonists. A few years later, bioisosteric replacement of the phenyl ring of CGS15943 with an N7-substituted pyrazole led to the family of N^8-substituted pyrazolo-triazolo-pyrimidines. Two selected compounds of this family named SCH 58261 (**22**, 5-amino-7-(β-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine) and SCH 63390 (**23**, 5-amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-

c]pyrimidine) proved to be potent and selective A_{2A}AR antagonists both in rat and human models (Fig. 7).[87,88]

However, a major problem of this class of compounds is related to their low water solubility and consequently poor bioavailability. The introduction of a hydroxyl group at the para position on the phenyl ring of compounds **22** and **23** led to derivatives **24** (5-amino-7-[β-(4-hydroxyphenyl)ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine) and **25** (5-amino-7-[3-(4-hydroxyphenyl)-propyl]-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine), which not only displayed greater hydrophilic character, but also a significant increase of both affinity and selectivity at the A_{2A}AR subtype, most probably due to hydrogen bond formation (Fig. 7). Therefore, to understand the nature of the hydrogen bond, compound SCH 442416 (**26**, 5-amino-7-[3-(4-methoxyphenyl)-propyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) was synthesized. This derivative showed a surprising increase of affinity for the A_{2A} adenosine receptor to qualify it as a candidate tool for PET studies in its ^{11}C labeled form. The high affinity was consistent with the compound acting as a hydrogen bond acceptor (Fig. 7).[89]

However, the introduction of oxygenated groups on the phenyl ring of the side chain was not sufficient to confer the necessary water solubility, and the introduction of additional functionality resulted in a compelling need to address this problem. Toward this purpose, carboxylic and sulfonic moieties were introduced, which contributed greatly to the water solubility especially in the case of the sulfonic moiety, but a great loss of affinity was observed.[90]

A partial resolution to this problem was obtained by the former Zeneca group in proposing a compound named ZM 241385 (**27**, 4-[2-[[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-*a*] [1,3,5]triazin-5-yl]amino]ethyl]phenol), which proved to be one of the most potent A_{2A}AR antagonists ever reported and having favorable water solubility (Fig. 8).[91]

However, ZM 241385, which could be considered a simplified analog of the pyrazolo-triazolo-pyrimidine series, was found to bind also with good affinity at the human A_{2B}AR. In fact, its tritiated form is actually used in radioligand binding studies of this receptor subtype as well as at the A_{2A}AR.[92]

Recently, a large series of derivatives bearing various substituents at the 5-position on the triazolo-triazine nucleus and the related triazolo-pyrimidine nucleus have been synthesized. In particular, derivative **28** showed great potency and selectivity for the A_{2A}AR as compared with the A_1AR (Fig. 8). Nevertheless, the lack of binding data at the A_{2B} and A_3 prevents a comparison of the derivatives with other fully characterized derivatives. Some of these derivatives, although not displaying exceptional high potency in binding studies, showed good oral efficacy in a rodent catalepsy model of Parkinson's disease.[93–97]

Over the last few years, other classes of compounds have been investigated with the aim of obtaining new antagonist tools for studying A_{2A}AR. Unfortunately, none of the reported compounds showed a better profile than the above-mentioned derivatives. Only two classes

of compounds, the triazolo-quinoxaline[98] and some pyrazolo-pyrimidines,[99] seem to possess promising requirements as A_{2A} adenosine receptor antagonists (Fig. 8).

In the triazolo-quinoxaline series, only one compound **29** showed interesting A_{2A}AR affinity. Unfortunately, this nucleus seemed to be very sensitive to any kind of modification. In fact, alkylation of the amino group, or its replacement with a carbonyl group, or substitution of the phenyl ring was detrimental in terms of affinity at the A_{2A}AR. In some cases, the affinity at the human A_3AR was predominant. Instead, in the pyrazolo-pyrimidine series, only one **30** showed a promising binding profile, but was nevertheless of low potency and low selectivity for the A_{2A}AR.

C. Biological Actions of A_{2A} Adenosine Receptor Antagonists

The A_{2A}AR antagonists that is furthest advanced in clinical trials is KW6002, as described above, and other antagonists of this subtype are under development.[82,97,100] The interest in CNS action of A_{2A}AR antagonists also extends to impeding the neurodegenerative process[4,7,8,10] and possibly the treatment of stroke.[101] The peripheral actions of A_{2A}AR antagonists might be complicated by a proinflammatory effect,[102] but might be therapeutically useful for cancer treatment.[103]

4. A_{2B} ADENOSINE RECEPTOR ANTAGONISTS

Most of the high affinity receptor antagonists thus far reported have been xanthine derivatives. Consideration of the potential therapeutic applications of A_{2B}AR antagonists, particularly their possible use an anti-asthmatic agents,[104,105] has stimulated many research groups to search for potent and selective antagonists for this subtype. Recognition of the possibility that the mechanism of action of the anti-asthmatic drugs theophylline (1,3-dimethylxanthine) and enprofylline (3-propylxanthine) might involve the A_{2B} adenosine receptor has spurred this research.[105]

A. Xanthine Derivatives

A large number of substitutions at the 1, 3, and 8 positions of the xanthine core have been performed with the aim of describing an SAR profile for the A_{2B}AR subtype. In particular, it has been observed that 1,3-unsubstituted xanthine derivatives bearing a phenyl ring at the 8-position possesses good selectivity but poor potency at the A_{2B}AR subtype.[106] An optimization of this structure led to the discovery of 1-propyl-8-(4-sulphonyl)phenyl xanthine PSB 1115 (**31**) and some related pro-drugs, such as a 4-nitrophenylester, which were found to be potent and selective A_{2B}AR antagonists (Fig. 9).[107,108]

In the series of 8-phenyl xanthine derivatives, a large number of amides derived from the 8-{4-[(carboxymethyl)oxy]phenyl}-1,3-dipropylxanthine have been prepared and tested as A_{2B}AR antagonists.[109,110] This study led to the discovery of the ([*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-^{1}H-purin-8-yl)phenoxy] acetamide] (**32**, MRS1754) and ([*N*-(4-acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-^{1}H-purin-8-yl)phenoxy] acetamide] (**33**, MRS 1716), which proved to be the most potent and selective human A_{2B}AR antagonists.[111] In fact, the

tritium labeled form of derivative **32** has been prepared and utilized in radioligand binding studies[112] (Fig. 9).

Phenyl replacement with a pyrazole moiety led to compounds which showed a quite similar A_{2B}AR affinity with respect to the phenyl series.[113,114] Recently, Zablocki and coworkers have reported an extended series of 8-aryl xanthines as selective A_{2B}AR antagonists.[115]

In the xanthine family, a new class of deaza-analogs has recently been reported, which displayed affinity at A_{2B}AR in the micromolar range, but poor selectivity versus the A_{2A}AR subtype.[116]

B. Polyheterocyclic Derivatives

Within this category of adenosine antagonists, quite varied structures have been introduced and modified in the search for new A_{2B}AR antagonists.

Starting from the experimental observation that the non-selective A_{2A}AR antagonist CGS15943 (**21**) (Fig. 7) also proved to be an effective A_{2B}AR antagonist both in functional and binding studies, a large number of acyl moieties have been placed at the N5 position (Fig. 10).[117]

The introduction of apolar chains such as the N5-pivaloyl group resulted in compound **34**, which displayed significant selectivity but not high potency at the A_{2B}AR (Fig. 10).[117]

A similar approach has been utilized with the pyrazolo-triazolo-pyrimidine nucleus, whose potency at the A_{2B}AR was obtained while a complete loss of selectivity was observed.[118] Only when bulky substituents at both N5 and N8 positions were present (compound **35**), was a significantly potent and selective A_{2B}AR antagonist obtained (Fig. 10).[119]

Considering that the potent and selective A_{2A}AR antagonist ZM241385, **27** (Fig. 8) proved to be also quite potent at the A_{2B}AR, its tritiated form is usually utilized in radioligand binding studies,[120] several modifications at the 5-position of the triazolo-triazine nucleus have been performed. It has been observed that the hydroxyl group replacement (**36**) enhanced the A_{2B}AR affinity although the selectivity was poor (Fig. 10).[121]

Very promising results at this receptor subtype were obtained upon modification of adenine nucleus.[122] A detailed investigation on this class of compounds permitted the partial optimization of the substitution of adenine nucleus to enhance both potency and selectivity for the A_{2B}AR subtype. In particular, the presence of alkynyl moiety at the 2-position and the presence of a furyl ring at the 7-position led to a very promising potent and selective A_{2B}AR antagonist (**37**).[123] These data suggest that further optimization of the pattern of substitutions at this third position could lead to the discovery of a highly potent and selective A_{2B}AR antagonist (Fig. 10).

Recently, in a screening program focused on the searching of new tools as adenosine receptor antagonists, a quinazoline derivative, named CMB 6446 ((4-methyl-7-methoxyquinazolyl-2-(2′-amino-4′-imidazolinone)) (**38**), proved to be quite potent and selective at the A_{2B}AR subtype with a binding K_i value of 112 nM.[124] (Fig. 10).

However, further efforts to enhance the A_{2B}AR affinity of **38** failed even extensive synthetic modification was made on this class of compounds.

Very recently, a tritiated form of a pyrrolo-pyrimidine derivative, named [^{3}H]OSIP-339391 (**39**) has been proposed as a very promising radioligand for studying A_{2B}AR. In fact, it showed a K_D value of 0.41 nM and selectivities versus the other receptor subtypes higher than 70.[125]

C. Biological Actions of A_{2B} Adenosine Receptor Antagonists

The therapeutic potential-based peripheral actions of A_{2B} adenosine receptor antagonists might include treatment of asthma.[104,105] The anti-asthma drugs, theophylline and enprofylline, are used therapeutically to treat asthma at concentrations to block A_{2B}AR.[104,105] A_{2B}AR antagonists may also serve as novel drugs for type-II diabetes,[126] Alzheimer's disease[127], and cystic fibrosis.[128]

5. A_3 ADENOSINE RECEPTOR ANTAGONISTS

In the last years, many efforts have been made to search for potent and selective human A_3AR antagonists.[11] The interest in blocking this class of receptors arose after the discovery of their involvement in cellular growth.[129]

A. Xanthine Derivatives

Natural xanthines, such as caffeine and theophylline that are considered the natural antagonists for adenosine receptors, show in general very low affinity for the A_3AR subtype (in the high micromolar range).[130] Nevertheless, very recent SAR studies on these compounds indicated that a cyclization between the 7 and 8 positions led to pyridopurine-2,4-dione derivative (**40**) as potent A_3 adenosine receptor antagonists[131] (Fig. 11).

Other positions of the xanthine core have been modified with the aim of improving A_3AR affinity. The discovery of 2-phenylimidazopurin-5-ones as water soluble derivative of xanthines led to PSB-10 (**41**) a highly potent and selective human A_3AR antagonist (Fig. 11).[132] The tritiated form of a related compound named PSB-11 has been used as a high affinity radioligand at this subtype with favorably low non-specific membrane binding.[133]

Following these observations, other structural classes in which the xanthine structure was extended have been reported as A_3AR antagonists. One such class was the triazolo-purines (**42,43**), which proved to be quite potent and selective human A_3AR antagonists (Fig. 11).[134,135]

B. Polyheterocyclic Derivatives

In this class of compounds, different heterocyclic moieties have been identified as potential A_3AR, and extensively reviewed, which can be classified in six families of derivatives: (i) flavonoids; (ii) 1,4-dihydropyridines and pyridines; (iii) triazolo-quinazolines; (iv) isoquinoline and quinazolines; (v) pyrazolo-triazolo-pyrimidines; (vi) various.[11,136] In Figures 12–14, representative members of these family of compounds are presented.

The discovery of flavonoids as human A_3AR antagonists was initiated by a broad screening of phytochemicals, in which it was shown that some flavonoid derivatives possess micromolar affinity at human A_3AR. Optimization of reference compounds, through a classical structure-activity relationship study and with the help of molecular modeling approach, led to MRS 1067 (**44**), which proved to be the most potent (K_i, 561 nM) and selective compound of this series at the human A_3AR subtype (Fig. 12).[137]MRS 1067 was the first reported antagonist suitable also for use with the rat A_3 adenosine receptor,[138] although it has since been superseded by more potent compounds.

A very similar approach was utilized for studying the SAR at the human A_3AR of 1,4-dihydropyridines, which are typically antagonists of the L-type calcium channel. Initially, it was necessary to eliminate binding to these ion channels, which was accomplished through the introduction of extended arylalkynyl groups at the 4-position of the dihydropyridine nucleus in combination with phenyl substituents at the 6-position. These changes not only prevented the recognition at the calcium channel but also significantly improved the affinity at the human A_3AR. In particular, a nitro derivative MRS1334 (**45**) proved to be the most potent analog of this series (Fig. 12).[139]

Simultaneously, the same authors studied the affinity of the pyridines, derived from the oxidation of the corresponding 1,4 dihydropyridines. In this class of compounds, to retain affinity and selectivity at human A_3 adenosine receptor, small groups at the 4-position were found to be essential. This effect could be attributed to the change of the C4-hybridization from sp^3 to sp^2, with a consequent variation of the C5-C4-R4 angle from 68.1° to 0.2°. This study strongly supported by theoretical studies led to the discovery of MRS1523 (**46**), which showed favorable affinity at the human A_3AR (18 nM) and was also the first derivative to possess submicromolar affinity at the rat A_3AR subtype (Fig. 12).[140] For various structural classes, most antagonists described as having high potency at the human A_3AR subtype were consistently found to be weak or ineffective at the rat A_3AR. This pronounced species difference could be correlated with the relatively modest sequence similarity (only 74%) that exists between rat and human A_3AR sequences.[141]

The triazolo-quinazoline derivative CGS 15943 (**21**, Fig. 7), a classic non-selective adenosine receptor antagonist, has been a starting point for searching new potent and selective human A_3AR antagonists. Its acylation led to the discovery of MRS1220 (**47**) a highly potent (0.65 nM) and quite selective human A_3AR antagonist (Fig. 13).[142]

In a program of screening compound libraries by IJzerman and coworkers, it has been found that a series of 3-(2-pyridinyl)-isoquinoline derivatives possessed adenosine A_3AR affinity.[143] The synthesis of related quinazoline derivatives, with a classical bioisosteric substitution of carbon with nitrogen and the substitution of amide spacer with an urea moiety led to a compound, VUF5574 (**48**), which had improved affinity at human A_3AR while being entirely inactive at A_1AR and A_{2A}AR receptor subtypes[144] (Fig. 13).

The discovery of pyrazolo-triazolo-pyrimidines as human A_3AR antagonists, was based on the creation of a hybrid molecule between antagonists and agonists of this subtype. Specifically, the triazolo-pyrazolo-pyrimidine core, typical of classic adenosine receptor

antagonists, was substituted at the N5 position a 4-methoxy phenyl carbamoyl moiety, which resulted to be optimal for having A_3 affinity when introduced at the N8 position of NECA.[130] This combination led to compound **49** which was one of the most potent and selective human A_3AR antagonist ever reported. A classic SAR study combined with molecular modeling simulation permitted the identification of the structural requirements indispensable for receptor recognition. In particular, small substituents (e.g., methyl group) at the N8 position and at the 5-position unsubstituted phenyl ring seemed well tolerated. This resulted in compound **50**, which displayed an increased affinity at the human A_3AR.[145] These results permitted the synthesis of a completely water soluble (15 mM) derivative **51**, in which the phenyl ring was replaced by a pyridinium salt. The introduction of a nitrogen not only improved water solubility but also a significant increased affinity at the human A_3AR. This observation suggested that electrostatic interactions were strongly involved in receptor recognition in this region (Fig. 13).[146] Other derivatives structurally related to this family have been reported, including the triazolo-quinoxalines. Within this structural class, several compounds have been synthesized as antagonists for different adenosine receptor subtypes. These SAR studies permitted the identification of compound **52** as one of the most potent and selective human A_3AR (Fig. 14).[147,148]

Other A_3AR antagonists were the result of library screening in which novel heterocyclic derivatives with high affinity were identified, such as L-249313 (**53**) and L-268605 (**54**) (Fig. 14), however, no detailed SAR has been provided.[149]

Structurally simplified A_3AR antagonists have been reported. The thiadiazole (**55**) and the bioisostere thiazole derivative (**56**) seem to be the promising agents, considering their very straightforward synthetic pathway and their low hydrophobic character (Fig. 14).[150]

Since nearly all of the reported A_3AR antagonists showed significant potency and selectivity at the A_3 adenosine receptor only in the human model, pre-clinical studies *in vitro* and *in vivo* in other species were severely limited. This aspect has been partially avoided by working on the adenosine core, that is, converting a selective A_3AR receptor agonist into an antagonist. This was particularly effective in designing species-independent A_3AR antagonists, since the nucleosides tend to bind well at this subtype across species. In general, adenosine receptor agonism correlates with the presence of a 9-ribose moiety on the adenine structure, while other adenine derivatives (such as 9-methyl or ethyl) are usually adenosine receptor antagonists. A_3AR homology modeling combined with mutagenesis and SAR studies indicated that the ribose moiety also had a requirement of flexibility, particularly in the 5′-region, to fully activate the receptor. Consistent with this finding, a spirolactam analog, that is in which the ribose ring was sterically constrained (MRS1292, **57**)[151] proved to be a potent A_3AR antagonist both in human and rat models (Fig. 14). MRS1292 contained the 5′-amide group, typical of potent agonists such as NECA, however the bicyclic constraint precluded receptor activation. Other modifications of nucleosides, such as the introduction of extended substituents at the 8-position and various substitutions of the N^6 and 2 positions[23,24] tended to convert agonists into antagonists.[152]

C. Biological Actions of A_3 Adenosine Receptor Antagonists

A_3AR antagonists appear to be of use in reducing intraocular pressure, which would be useful in the treatment of glaucoma.[153] The A_3AR promotes flow into the aqueous humor by coupling in a positive fashion to chloride inflow in non-pigmented ciliary epithelial cells.[153] A_3AR antagonists have also been of interest in possibly treating allergic conditions and inflammation.[6,9–11]

6. CONCLUSIONS

Potent and selective antagonists have been developed for the four subtypes of adenosine receptors. These advances have been based on both empirical methods and semi-rational design approaches, such as QSAR and receptor homology modeling. Both xanthines and non-xanthines have filled this need. The first non-xanthine heterocycles to attain nanomolar affinity were designed for the A_{2A} receptor, by a series of studies in various laboratories in which the xanthine nucleus was elaborated and ring modified. The screening of chemically diverse libraries has resulted in novel chemical classes of A_3 receptor antagonists, and also an intensive SAR studies on xanthines led to potent and selective A_3AR antagonists. Now at all four subtypes, non-xanthine classes have been introduced as antagonists and optimized through substitution of functional groups and pendant moieties. The low aqueous solubility seen with many of these optimized compounds has been partially overcome with the introduction of polar or charged groups. Thus, the introduction of selective adenosine antagonists for the therapeutic treatment of a variety of diseases remains hopeful.

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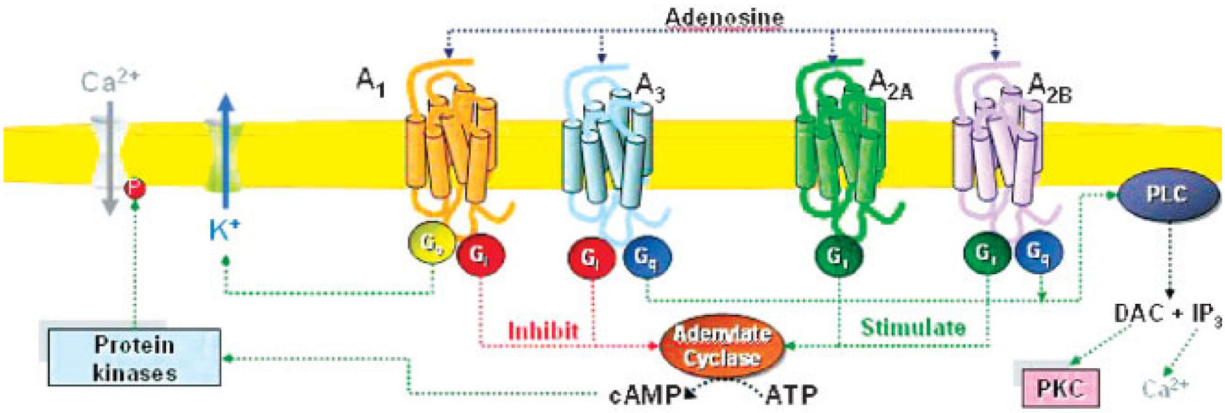


Figure 1.

Signal transduction pathways associated with the activation of the human adenosine receptors. Abbreviations: α, α-subunit of G protein; βγ, βγ-subunits of G protein; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; G_i, G_i family of G proteins; G_s, G_s family of G proteins; G_o, G_o family of G proteins; G_q, G_q family of G proteins; IP3, inositol (1,4,5)-trisphosphate; P, phosphate moiety; PKC, proteinkinase C; PLC, phospholipase C.

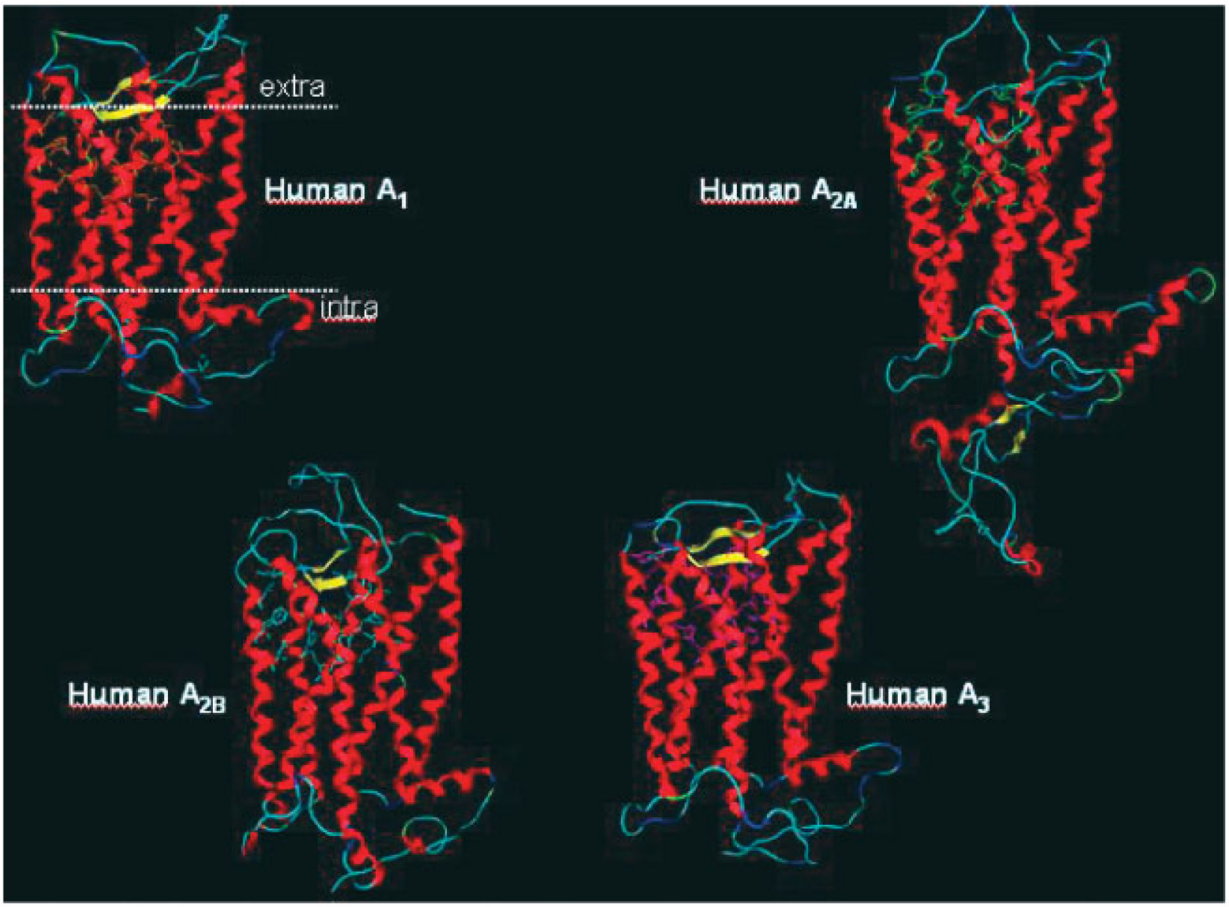
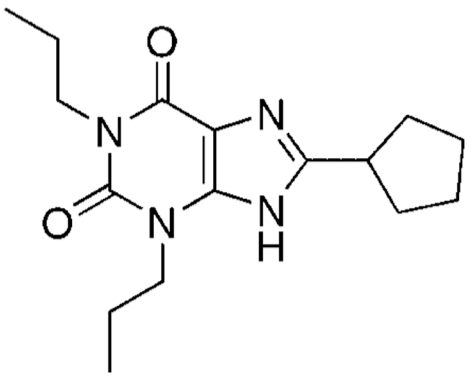
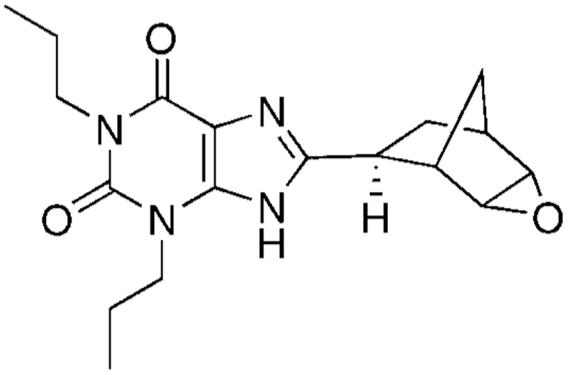


Figure 2.
General topology of all adenosine receptors obtained using a rhodopsin-based homology modeling (modified from Moto et al.[11]).



DPCPX, **1**
hA_1 = 3.9 nM
hA_{2A} = 129 nM
hA_{2B} = 56 nM
hA_3 = 3,980 nM



BG-9719, **2**
hA_1 = 0.43 nM
hA_{2A} = 1051 nM
hA_{2B} = 172 nM
hA_3 = 3,870 nM

Figure 3.
Structure and binding affinities of xanthines as A_1AR antagonists.

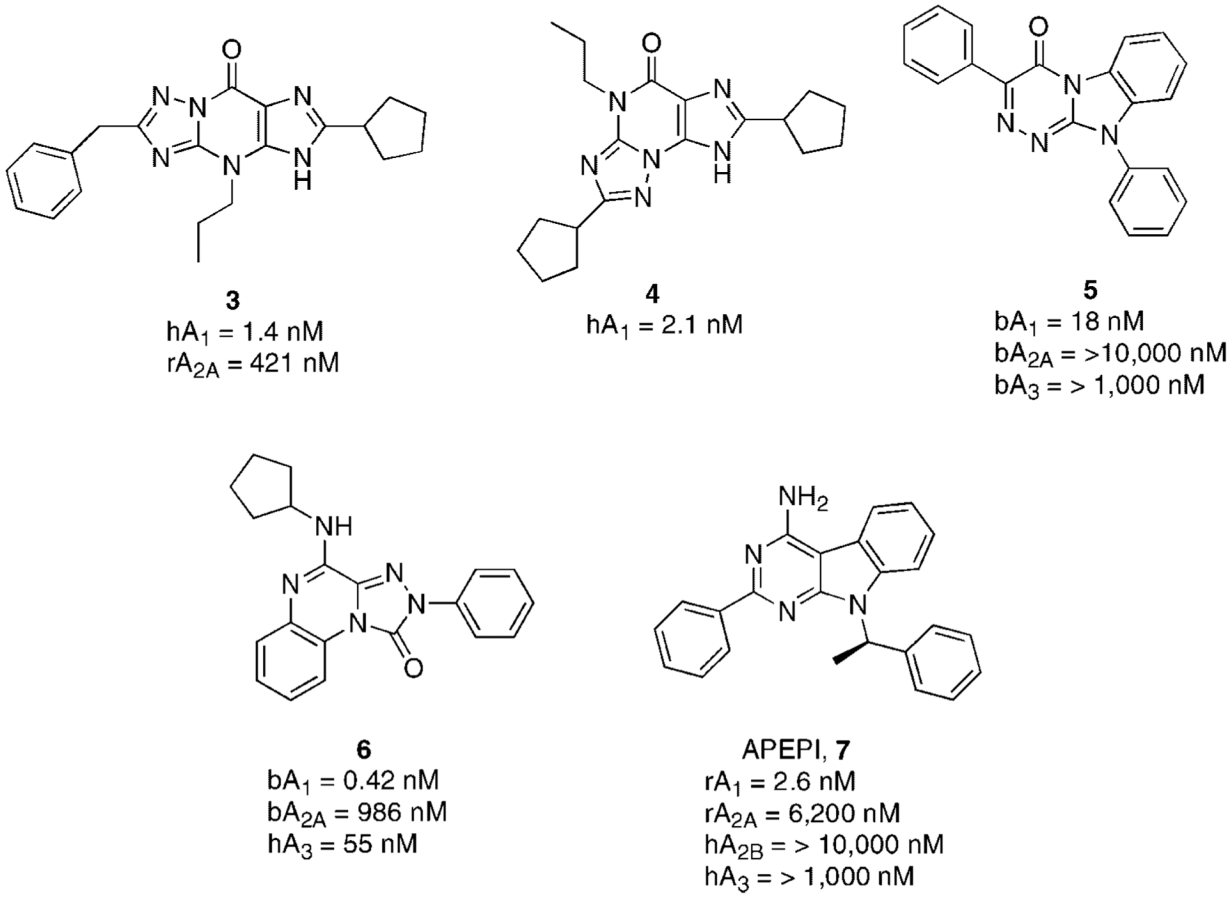


Figure 4.
Structures and binding affinities of non-xanthine adenosine A_1AR antagonists.

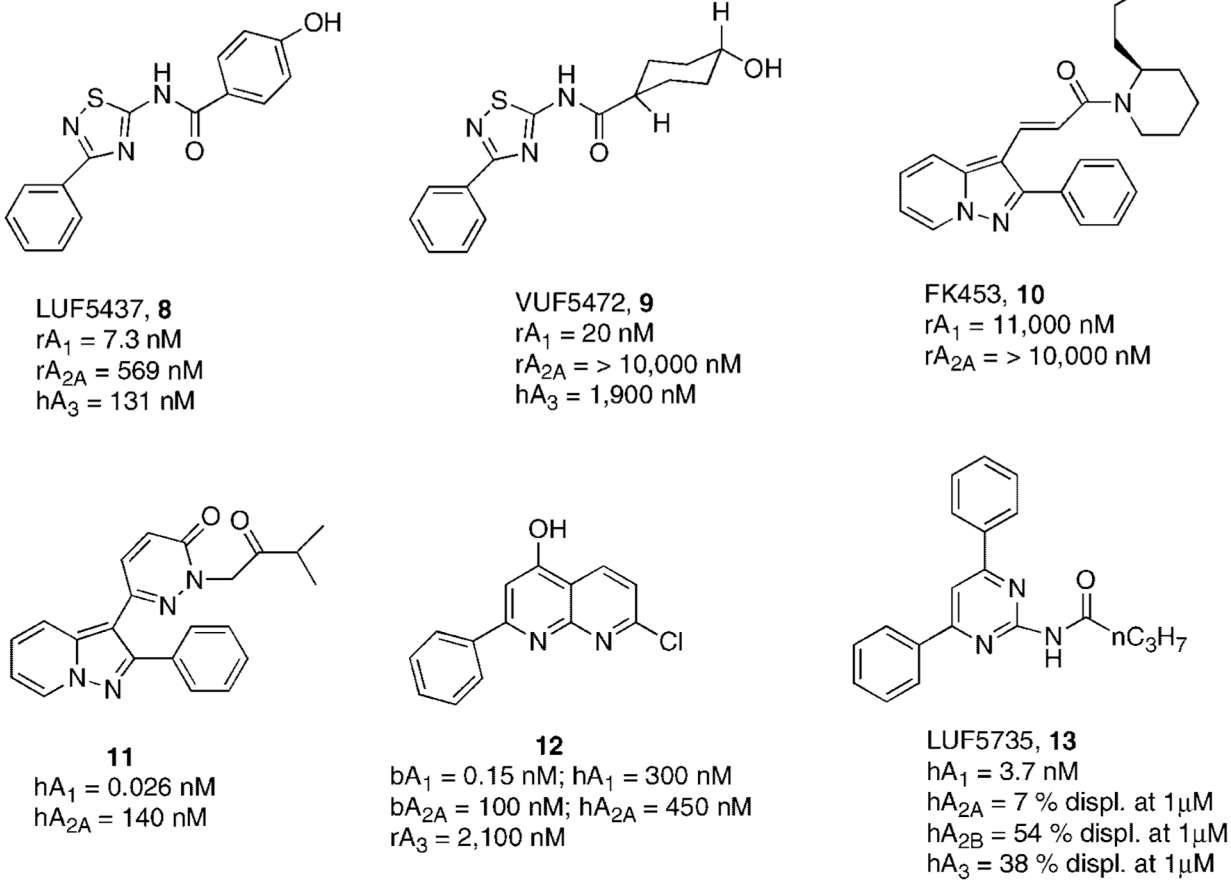


Figure 5.
Structures and binding affinities of simplified A_1AR antagonists.

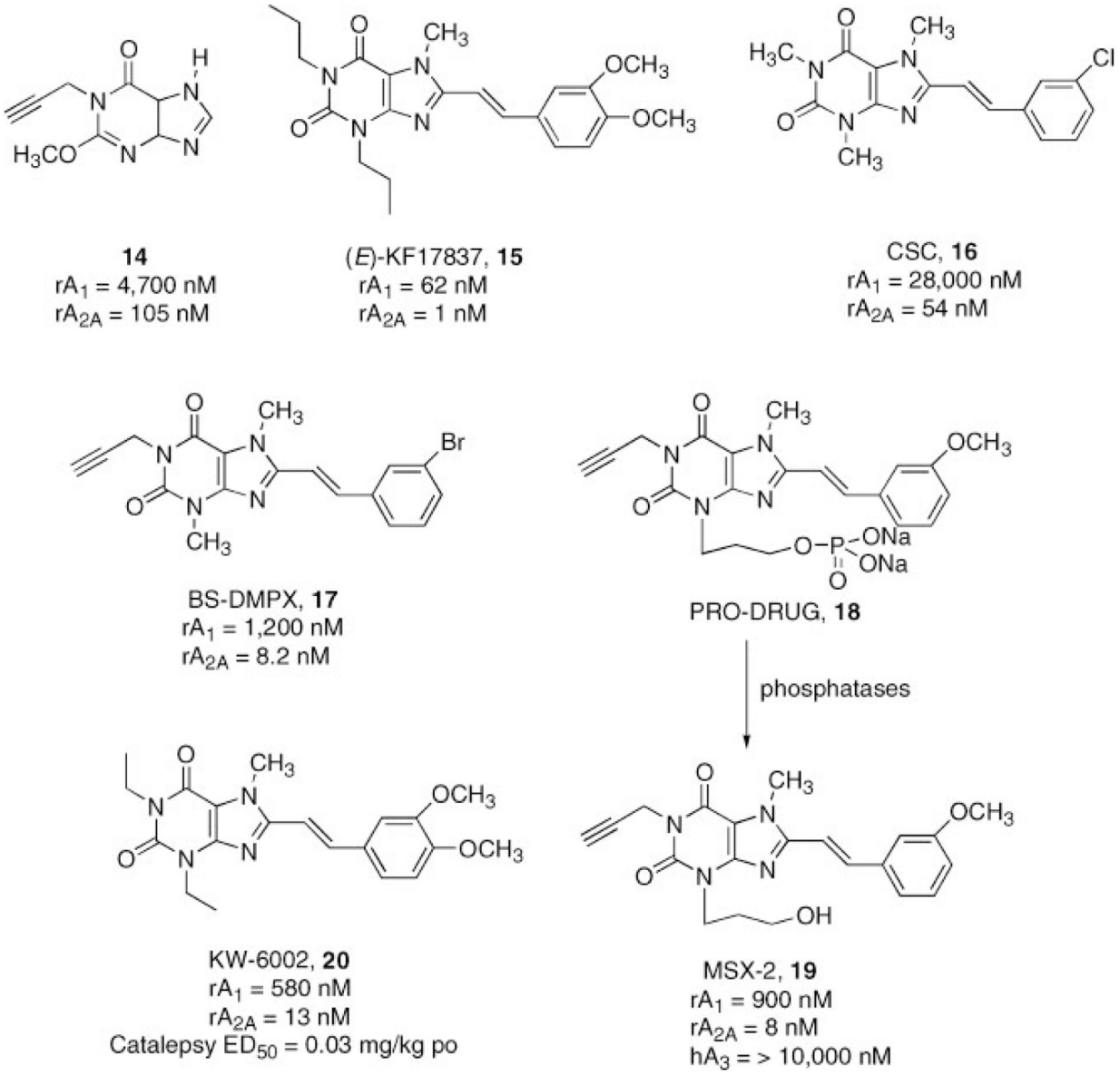


Figure 6.
Structures and binding affinities of xanthines as A_{2A}AR antagonists.

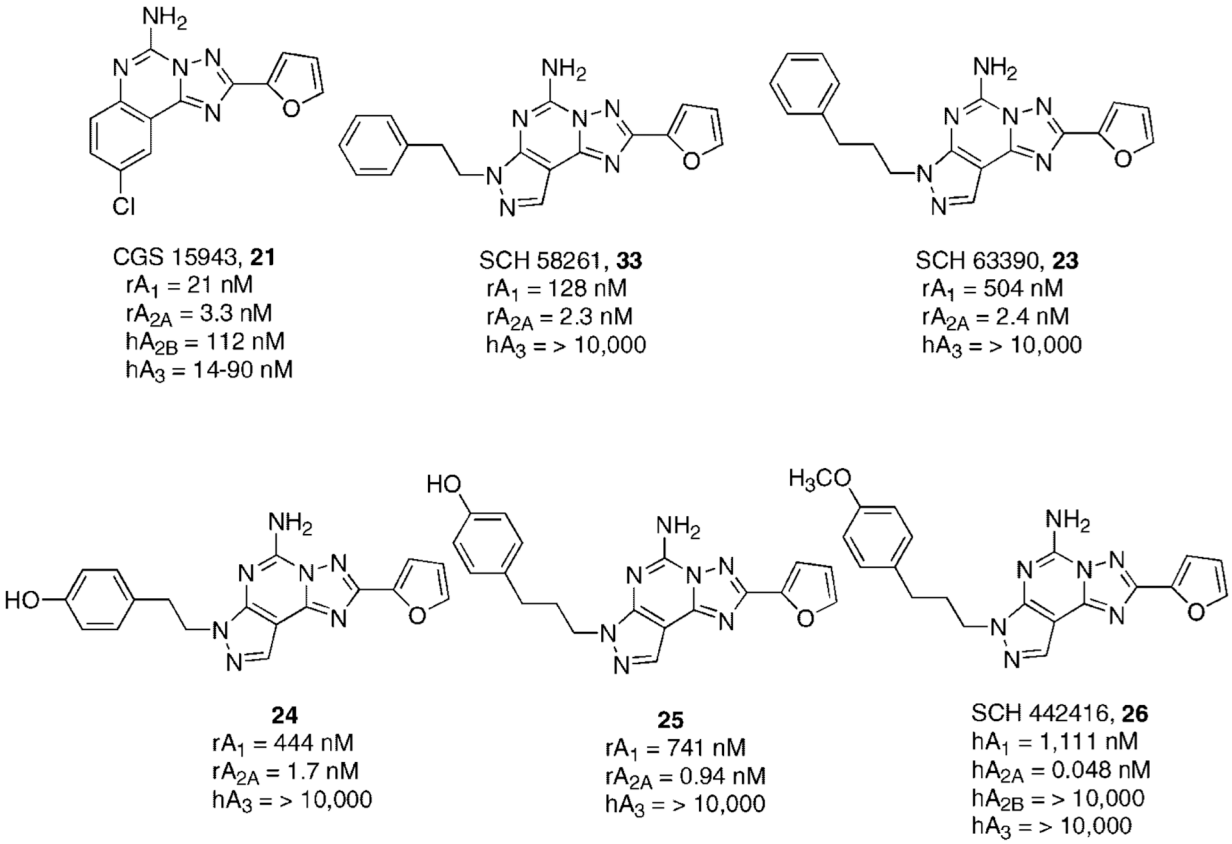


Figure 7.

Structures and binding affinities of triazolo-quinazoline and pyrazolo-triazolo-pyrimidines as A_{2A}AR antagonists.

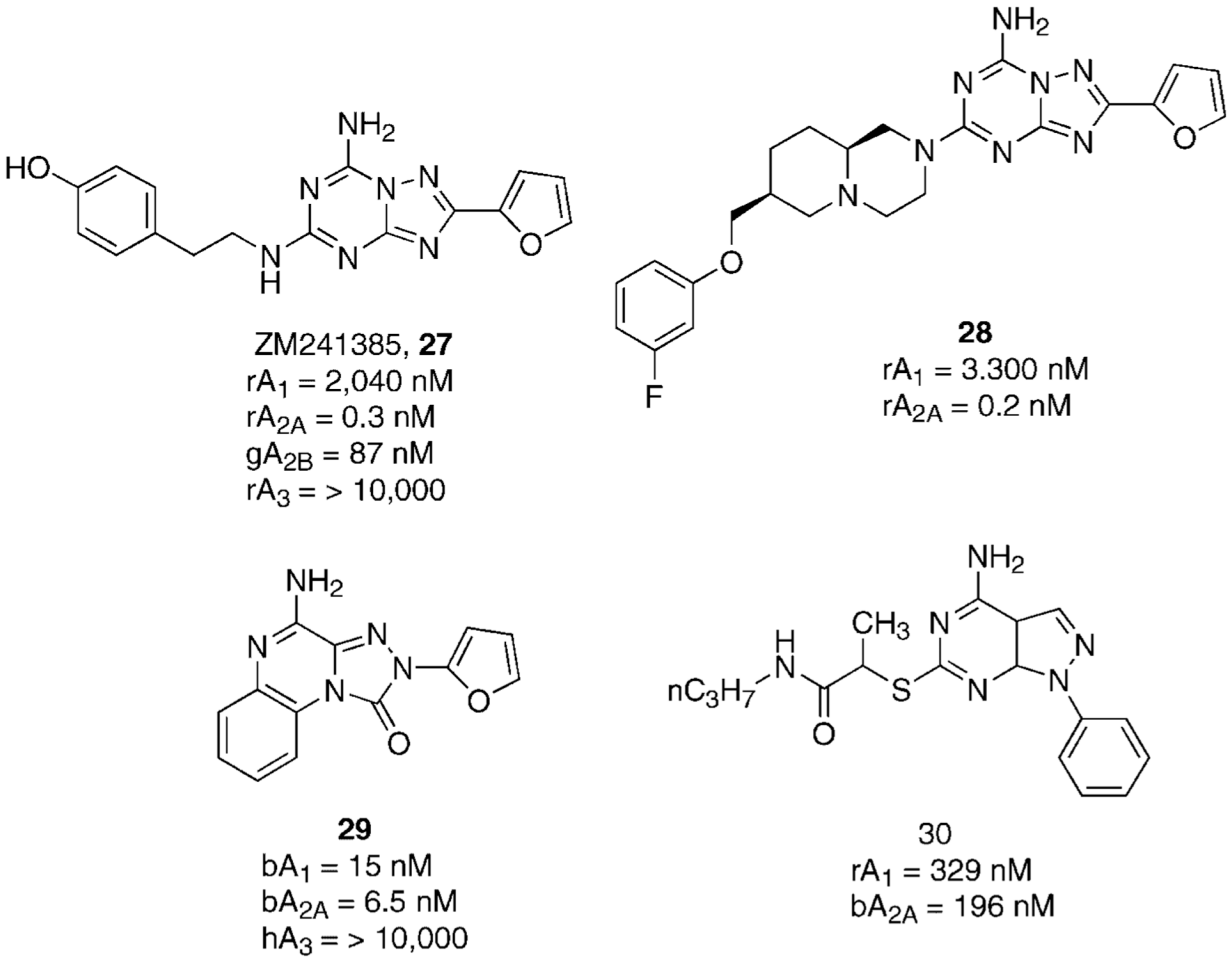


Figure 8.
Structures and binding affinities of triazolo-triazine and new tools as A_{2A}AR antagonists.

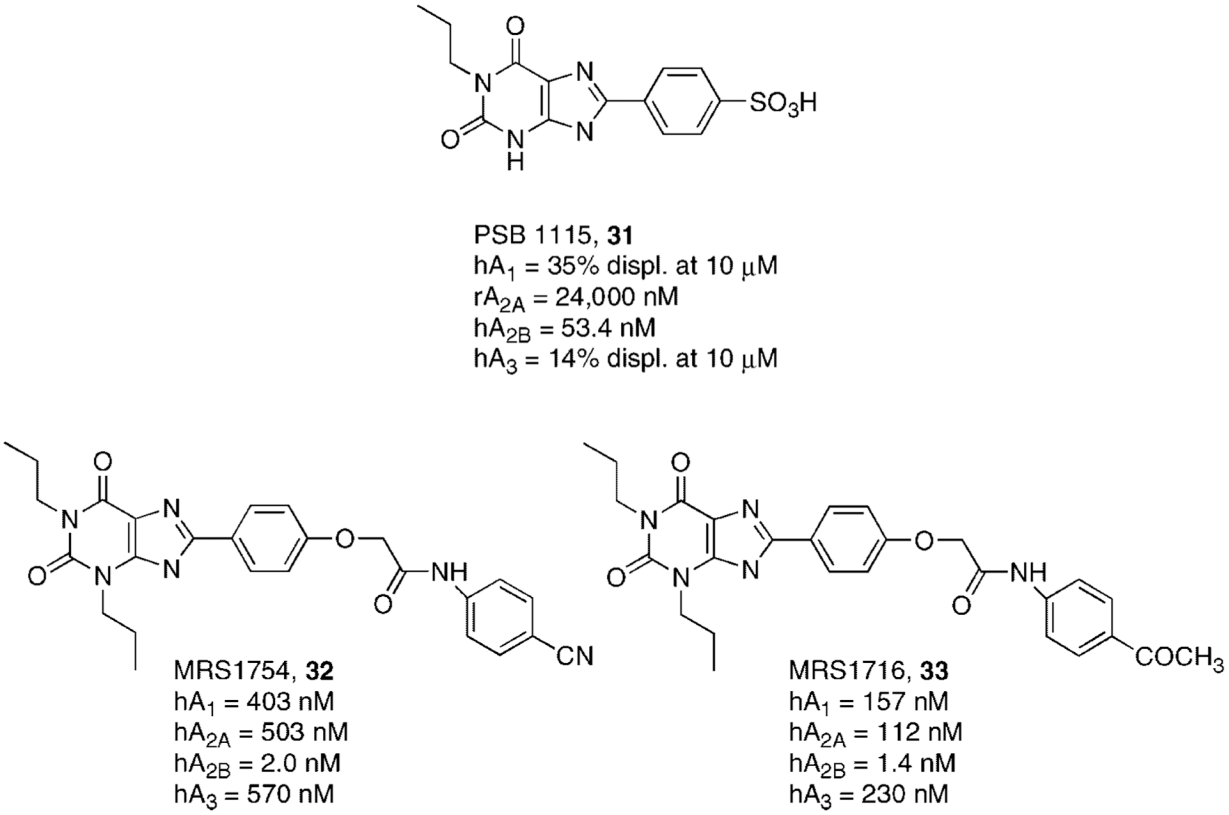


Figure 9.
Structures and binding affinities of xanthine derivatives as A_{2B}AR antagonists.

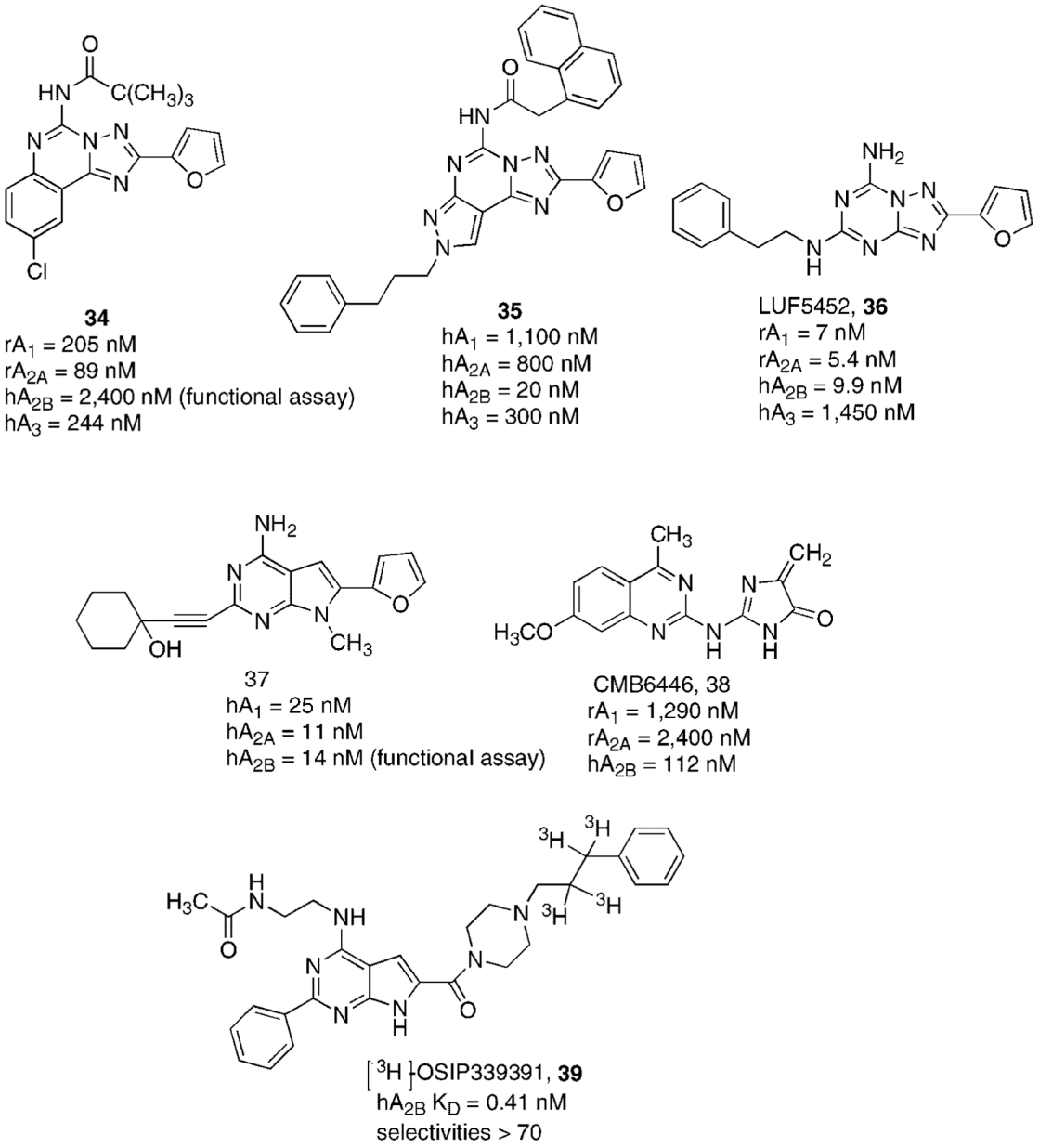
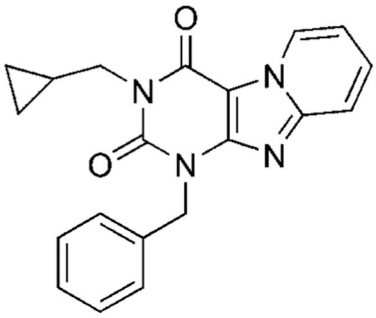


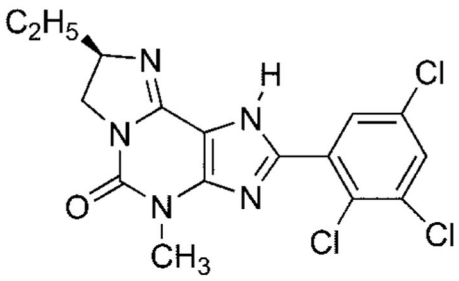
Figure 10.

Structures and binding affinities of polyheterocyclic derivatives as A_{2B}AR antagonists.



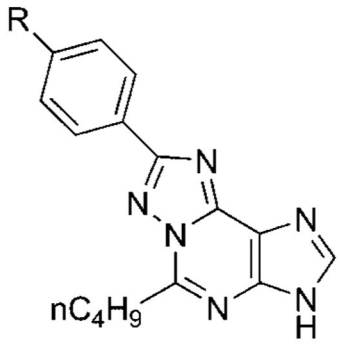
40

hA_1 = 40 % (displ at 10 μM)
hA_{2A} = 242 nM
hA_3 = 4.2 nM



PSB-10, **41**

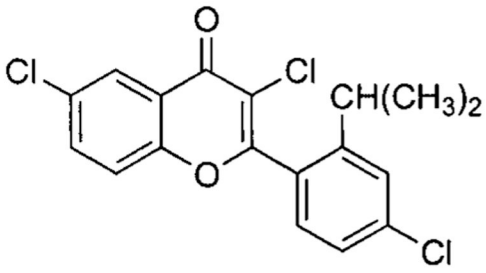
hA_1 = 1,700 nM
hA_{2A} = 2,700 nM
hA_3 = 0.43 nM



R = OCH_3, **42**
hA_1 = 398 nM
hA_{2A} = 822 nM
hA_{2B} = 1,030 nM
hA_3 = 0.18 nM

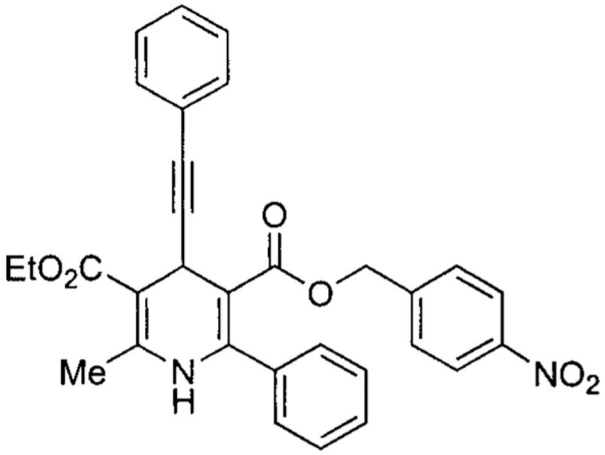
R = CF_3, **43**
hA_1 = 4 % (displ at 10 μM)
hA_{2A} = 31 % (displ at 10 μM)
hA_{2B} = 6 % (displ at 10 μM)
hA_3 = 0.95 nM

Figure 11.
Structures and binding affinities of xanthine derivatives as human A_3AR antagonists.



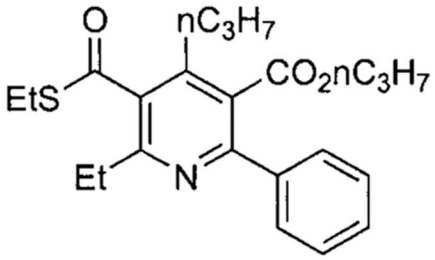
MRS 1067, **44**

rA_1 = 36% (% displ. at 10 μM)
rA_{2A} = 19% (% displ. at 10 μM)
hA_3 = 561 nM



MRS 1334, **45**

rA_1 = > 100 μM
rA_{2A} = > 100 μM
hA_3 = 2.69 nM



MRS 1523, **46**

rA_1 = 15,600 nM
rA_{2A} = 2,050 nM
rA_3 = 113 nM
hA_3 = 18.9 nM

Figure 12.
Structures and binding affinities of flavonoid, dihydropyridine, and pyridine derivatives as human A_3AR antagonists.

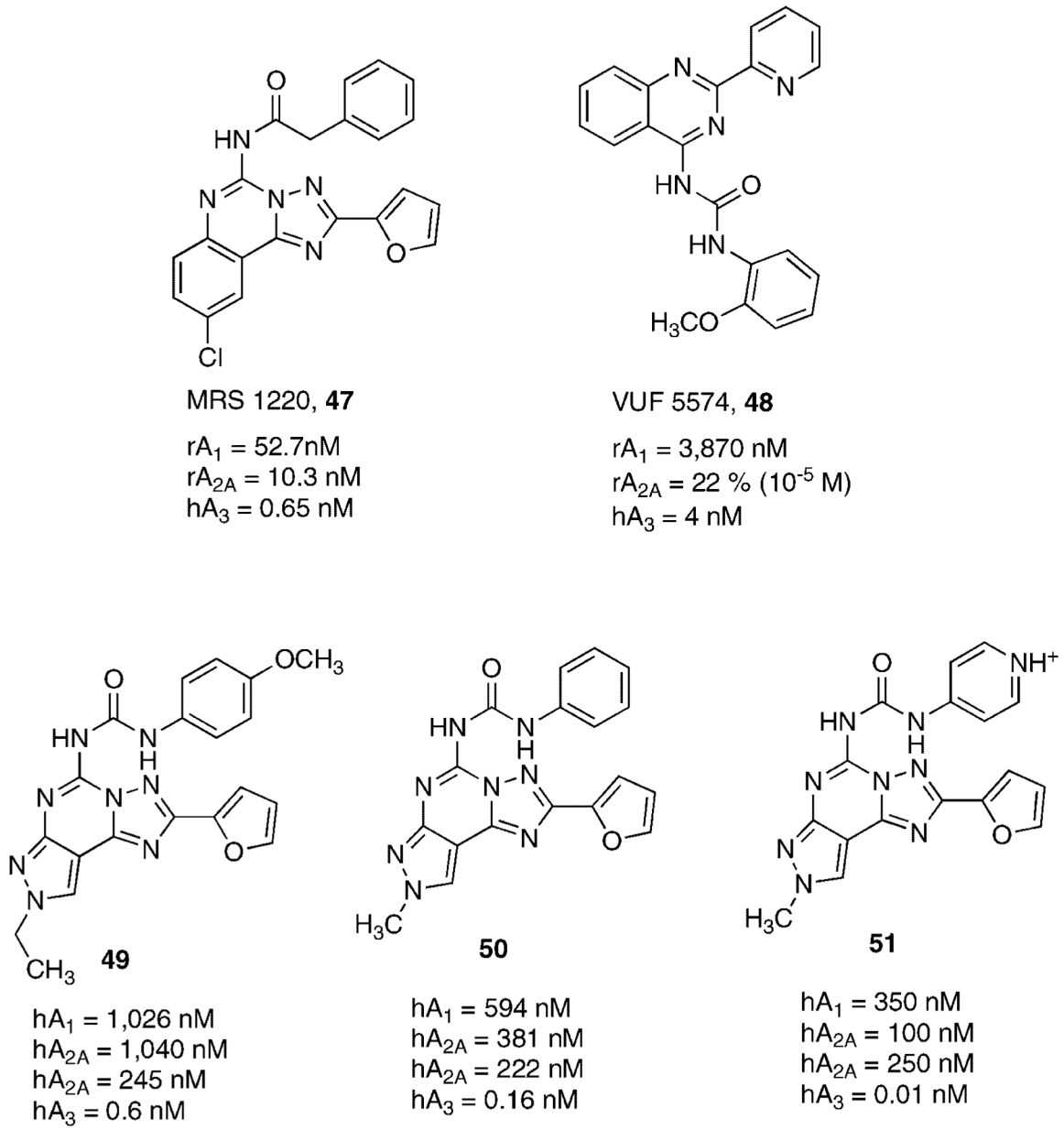
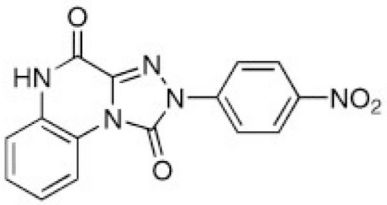
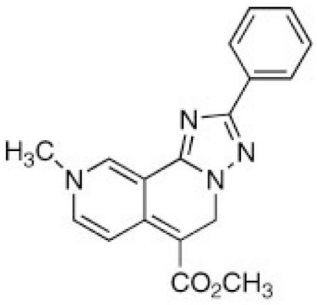


Figure 13.
Structures and binding affinities of polyheterocyclic systems as human A_3AR antagonists.



52

hA_1 = 19 % (% displ. at 20 μM)
hA_{2A} = 21 % (% displ. at 20 μM)
hA_3 = 0.6 nM

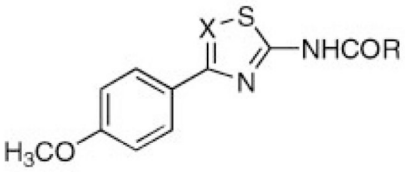


L-249313, **53**

hA_1 = 4,000 nM
hA_{2A} = 19,000 nM
hA_3 = 13 nM

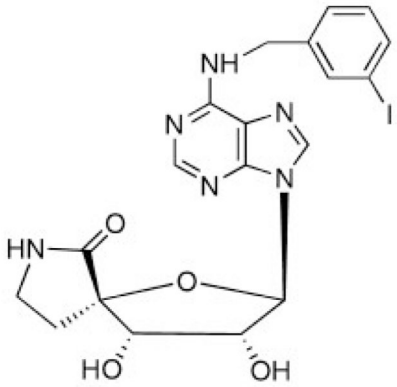
L-268605, **54**

hA_1 = > 10,000 nM
hA_{2A} = > 10,000 nM
hA_3 = 18 nM



X = N, R = CH_3, **55**
hA_1 = 24 % (% displ. at 10 μM)
hA_{2A} = 28 % (% displ. at 10 μM)
hA_3 = 0.79

X = CH, R = CH_2CH_3, **55**
hA_1 = 22 % (% displ. at 10 μM)
hA_{2A} = 47 % (% displ. at 10 μM)
hA_3 = 2.4



MRS1292, **57**

hA_3 = 29.3 nM
rA_3 = 51 nM

Figure 14.

Structures and binding affinities of other heterocyclic derivatives as human A_3AR antagonists.

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Table I

Amino Acids of ARs Implicated in Ligand Binding

A_1AR[1]		
H278L (bovine)	Nearly abolished both agonist and antagonist binding	Olah et al., 1992 [29]
H251L (bovine)	Decreased antagonist affinity 4-fold. No change in agonist affinity	Olah et al., 1992 [29]
T277A (human)	>400-fold decrease in affinity of NECA with slight changes in affinity for *R*-PIA and *S*-PIA. Substitution with serine significantly restored NECA affinity. Nature of residue in position 277 also involved in canine/bovine A_1AR binding specificity Amino acid in position 270 contributes to canine/bovine A_1AR binding specificity	Townsend-Nicholson and Schofield, 1994 [30] Tucker et al., 1994 [31]
I270M (bovine) M270I (canine)	Amino acid in position 270 contributes to canine/bovine A_1AR binding specificity	Tucker et al., 1994 [31]
E16A (human)	Agonist affinity reduced 4- to 40-fold with little change in antagonist affinity	Barbhaiya et al., 1996 [32]
D55A (human)	Increase in agonist affinity with no change in antagonist affinity. Disrupted regulation of agonist binding by Na^+	Barbhaiya et al., 1996 [32]
S94A (human)	Loss of detectable agonist or antagonist binding. Restoration of binding by substitution with threonine	Barbhaiya et al., 1996 [32]
$A_{2A}AR$[1,2]		
S281A	Loss of detectable agonist and antagonist radioligand binding; no agonist activity in functional assays; substitution with threonine enhanced affinity for most agonists.	Kim et al., 1995 [33]
H278A	Substantial loss of agonist and antagonist radioligand binding; functional assays indicated ~300-fold decrease in agonist potency	Kim et al., 1995 [33]
S277A	Substantial decrease in agonist affinity, but antagonist radioligand binding not altered; substitution with threonine restored agonist binding	Kim et al., 1995 [33]
I274A	Substantial decrease in agonist and antagonist radioligand binding; functional assays indicated ~30-fold decrease in agonist potency	Kim et al., 1995 [33]
N253A	Loss of detectable agonist and antagonist radioligand binding; radioligand binding not restored by replacement with glutamine	Kim et al., 1995 [33]
H250A	Loss of detectable agonist and antagonist radioligand binding; no agonist activity in functional assays; substitution with phenylalanine substantially restored binding	Kim et al., 1995 [33]
F182A	Loss of agonist and antagonist radioligand binding; replacement with tryptophan significantly restored binding of ligands	Kim et al., 1995 [33]
E151A	Loss of agonist and antagonist radioligand binding; functional assays indicated 1000-fold decrease in agonist potency; substitution with aspartic acid did not restore radioligand binding	Kim et al., 1996 [34]
T88A	Substantial decrease in agonist, but not antagonist, affinity; partially restored by substitution with threonine	Jiang et al., 1996 [35]
A_3AR[1,2]		
H95A	Substantial decrease in agonist and antagonist affinity	Gao et al., 2002 [36]
K152A	Insignificantly affect the agonist binding but slightly decreased antagonist affinity	Gao et al., 2002 [36]

W243A W243F	Insignificantly affect the agonist binding but slightly decreased antagonist affinity. Trp243 is critical for receptor activation	Gao et al., 2002 [36]
N250A	Loss of agonist and antagonist radioligand binding	Gao et al., 2002 [36]

[1] Amino acids represented in single-letter code with position number shown. The first amino acid is that of the wild-type receptor, with the second residue that used for substitution. References are collected inside square brackets.

[2] All studies referring A_{2A}AR and A_3AR examined the human cloned receptors.