Blocking Striatal Adenosine A_{2A} Receptors: A New Strategy for Basal Ganglia Disorders

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Abstract: Adenosine A_{2A} receptors are highly concentrated in the striatum, where they play an important modulatory role of glutamatergic transmission to the GABAergic enkephalinergic neuron, which function is particularly compromised in Parkinson's disease and in the early stages of Huntington's disease. An important amount of preclinical data suggested the possible application of A_{2A} receptor antagonists in Parkinson's disease, particularly as adjuvant therapy to the currently used dopaminergic agonists. Several A_{2A} receptor antagonists are currently in clinical trials in patients with Parkinson's disease and initial results have been promising. In recent years, many pharmaceutical companies have started programs to develop A_{2A} antagonists for Parkinson's disease and for other indications, such as neurodegenerative diseases in general, depression and restless legs syndrome. Antagonists with high A_{2A} receptor affinity and selectivity have been developed from various chemical classes of compounds, including xanthines, adenines and other amino-substituted heterocyclic compounds. Novel structures include benzothiazole and thiazolopyridine derivatives. The present review describes properties of standard A_{2A} receptor antagonists including those in clinical development. Furthermore, the different chemical classes of A_{2A} receptor antagonists that have been described in the literature, including recent patent literature, will be presented.

Keywords: Adenosine, xanthines, adenine derivative, adenine analog, Parkinson's disease, adenosine receptor, antagonist, istradefylline, A_{2A} receptor, pharmacophore model.

STRIATAL DYSFUNCTION IN MOVEMENT DISORDERS

The striatum is the main input and information processing structure of the basal ganglia. Cortico-limbic-thalamic glutamatergic and mesencephalic dopaminergic systems converge in the GABAergic medium-sized spiny neurons, which constitute more than 90% of the striatal neuronal population [1]. These are efferent neurons which can be classified into two major classes according to their peptide expression: GABAergic enkephalinergic and GABAergic dynorphinergic neurons [1]. GABAergic enkephalinergic neurons express dopamine and adenosine receptors predominantly of the A_{2A} and D_2 subtype, respectively, while GABAergic dynorphinergic neurons express dopamine and adenosine receptors predominantly of the A_1 and D_1 subtype, respectively [1-3]. In addition, there are different types of GABAergic interneurons (parvalbumin, calretinin or somatostatin internerons) and large cholinergic interneurons [4].

The striatum is functionally subdivided in dorsal and ventral striatum. The dorsal striatum (mostly represented by the nucleus caudate-putamen) is involved in the performance and learning of complex motor acts. The dorsal striatum receives glutamatergic input from sensoriomotor and association cortical areas and dopaminergic input from the substantia nigra pars compacta [1,5] (Fig. **1**). The ventral striatum (mostly represented by the nucleus accumbens) forms part of brain circuits involved in goal-directed behaviours, in the conversion of motivation into action, into the selection of appropriate behavioral responses elicited by specific motivational stimuli. Different from the dorsal striatum, the ventral striatum (mostly represented by the nucleus accumbens) receives glutamatergic input from limbic and paralimbic cortices, as well as from the amygdala and hippocampus, and dopaminergic input from the ventral tegmental area [1,5]. In the dorsal part of the striatum the two subtypes of striatal GABAergic efferent neurons give rise to the two dorsal striatal efferent systems, which connect the dorsal striatum with the output structures of the basal ganglia, the substantia nigra pars reticulata and the internal segment of the globus pallidus (GPi; entopeduncular nucleus in rodents) [1] (Fig. **1**). These are called "direct" and "indirect" pathways. The direct pathway is made of GABAergic dynorphinergic neurons, which directly connect the striatum with the output structures. The indirect pathway consists of GABAergic enkephalinergic neurons, which connect the striatum with the external segment of the globus pallidus (GPe; globus pallidus in rodents), GABAergic neurons which connect the GPe with the subthalamic nucleus (STN) and glutamatergic neurons which connect the STN with the output structures. GPe GABAergic neurons also project directly to the output structures without using the STN relay [1] (Fig. **1**). Stimulation of the direct pathway results in motor activation and stimulation of the indirect pathway produces motor inhibition. Penney and Young [6] suggested that the striato-GPe-STN circuit might be involved in the suppression of unwanted motor responses. Dopamine, or dopamine agonists, will induce motor activation by activating the direct pathway (acting on stimulatory D_1 receptors localized in GABAergic dynorphinergic neurons)

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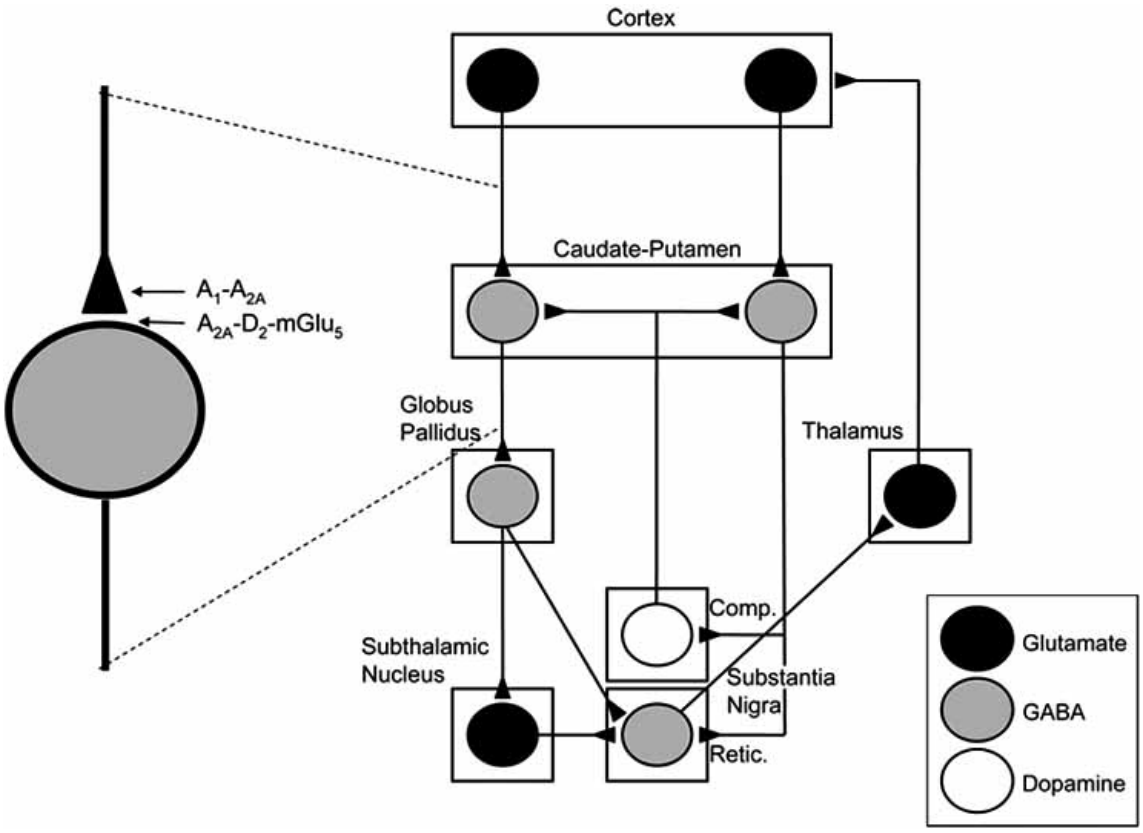


Fig. (1). Scheme of the basal ganglia circuitry (involving the dorsal striatum) with the localization of A_1-A_{2A} receptor heteromers in glutamatergic cortico-striatal terminals and A_{2A}-D_2-$mGlu_5$ receptor heteromers in the GABAergic striatopallidal enkephalinergic neuron (see text).

and by depressing the indirect pathway (acting on inhibitory D_2 receptors localized in GABAergic enkephalinergic neurons) [1,7].

In Parkinson's disease a preferential degeneration of the nigrostriatal dopaminergic system produces striatal dopamine depletion with the consequent impairment of the functioning of these circuits, which is associated with hypokinesia. The main cause of Parkinson's disease is unknown but the recent discovery of mutations in the genes coding for α synuclein, parquin and ubiquitin C-terminal hydrolase L1 in familial Parkinson's disease suggests that the failure of the ubiquitin-proteasome system is a common final neurodegenerative pathway [8]. Hyperactivity of the GABAergic enkephalinergic neurons due to the release from the strong D_2 receptor-mediated tonic inhibitory effects of endogenous dopamine is probably the main patho-physiological mechanism responsible for this hypokinesia [9]. The consequent increased neuronal activity of the STN and the output structures of the basal ganglia (in particular GPi) seem to be the functional hallmark of the Parkinsonian state, giving the basis for surgical treatment (pallidotomy) of this disease [10].

Huntington's disease is a progressive hereditary disorder characterized by dementia and choreoathetosis, which is a motor abnormality with involuntary, purposeless, arrhythmic movements of a forcible, rapid, jerky type [11]. The disease is caused by a mutation in the *huntingtin* (*IT15*) gene, located near the tip of the short arm of chromosome 4 [12]. The mutated gene contains extra copies of CAG repeats, which is translated in the expansion of a glutamine repeat in the huntingtin protein [13]. The pathogenesis of the motor abnormality is a progressive degeneration of the striatal GABAergic efferent neurons with an initial preferential dysfunction of the A_{2A} receptor-expressing GABAergic enkephalinergic neurons in the early stages of the disease [14]. The resultant disinhibition of GPe GABAergic neurons projecting to the STN results in the opposite effect observed in Parkinson's disease, a reduction of the neuronal activity of the STN, thus mimicking the effects of STN lesion and eliciting choreiform movements [14]. With the progression of the disease there is a gradual disappearance of choreiform movements and their replacement by rigidity and hypokinesia, which correlates with the additional progressive degeneration of the GABAergic dynorphinergic neurons [14]. The pathogenesis of the associated dementia is a concomitant cortical neuronal loss [15].

STRIATAL ADENOSINE A_{2A} RECEPTOR FUNCTION

Adenosine plays a very important integrative role in striatal function [2,3]. A_{2A} receptors are more concentrated in the striatum than anywhere else in the brain and they are strategically located, both pre- and post-synaptically to modulate glutamatergic neurotransmission in the GABA-ergic enkephalinergic neurons [16,17]. At the postsynaptic side they are localized at the perisynaptic ring adjacent to the

postsynaptic density, forming heteromeric complexes with $mGlu_5$ and D_2 receptors (Fig. **1**). At the presynaptic side, they are localized in the active zone, intrasynaptic, forming heteromeric complexes mostly with A_1 receptors, but also with $mGlu_5$ and D_2 receptors [3, 17] (Fig. **1**). A_{2A} receptors are Gs-olf protein-coupled receptors whose main signaling pathway is adenylyl-cyclase activation, the cAMP-PKA cascade [2,3]. Important PKA substrates include the dopamine and cyclic adenosine 3´, 5´-monophosphate-regulated phosphoprotein, 32 kDa (DARPP-32) and the nuclear constitutive transcription factor cAMP response element binding protein (CREB) [18]. DARPP-32 acts as an amplificatory mechanism of the cAMP-PKA cascade and it has been suggested to be essential for striatal A_{2A} receptor signaling [19, 20]. The catalytic subunits of PKA can diffuse into the nucleus and induce cellular gene expression by phosphorylating CREB. The immediate-early gene *c-fos* and the *preproenkephalin* gene are very well studied target genes the promoters of which contain consensus sites for pCREB binding [21,22]. Thus, A_{2A} receptor stimulation can potentially activate the cAMP-PKA cascade and increase the expression of immediate-early genes and the *preproenkephalin* gene, which codes for the precursor of enkephalin. Another important target of A_{2A} receptor-mediated PKA activation is the AMPA receptor ($gluR_1$ subunit) [23], which is an initial mechanism contributing to the formation of long-term potentiation (LTP) [24], the most studied electrophysiological correlate of plasticity of excitatory synapses.

A_{2A} and D_2 receptors form heteromeric complexes with reciprocal antagonistic interactions which regulate the function of the GABAergic enkephalinergic neurons [2,3,7, 25-31]. Stimulation of A_{2A} receptors decreases the affinity of D_2 receptors for agonists by means of an intramembrane interaction [25], while stimulation of D_2 receptors inhibits A_{2A} receptor-induced activation of adenylyl-cyclase [2,3, 27]. It must also be pointed out that some studies suggest that A_{2A} and D_2 receptor can interact synergistically, with stimulation of D_2 receptors potentiating the effects of A_{2A} receptor stimulation. These conditions seem to depend on the isoform of adenylyl cyclase involved or on the interruption of a previous long-term exposure to D_2 receptor agonists [32-34] (Yao *et al*., 2002; Kudlacek *et al*., 2003; Vortherms and Watts, 2004). In any case, the main isoform of adenylyl cyclase in the striatum is AC5, and co-stimulation of G_s- and G_i-coupled receptors shows antagonistic interactions at the AC5 level [35,36]. In fact, D_2 receptor stimulation has been shown to counteract cAMP accumulation induced by A_{2A} receptor stimulation in membrane preparations from mouse striatum [37]. By means of the strong antagonistic D_2-A_{2A} receptor interaction at the adenylyl cyclase level, and due to the existence of a tonic effect of dopamine on D_2 receptors, under normal conditions the ability of A_{2A} receptors to activate the cAMP-PKA signaling pathway is impaired [3, 7]. In fact, the systemic administration of A_{2A} receptor antagonists produces either no effect or a modest decrease in the striatal expression of *c-fos* or *preproenkephalin* [38-43]. We have previously shown that concomitant stimulation of $mGlu_5$ receptors, which form heteromeric complexes with A_{2A} receptors, allows A_{2A} receptor stimulation to counteract the inhibitory effects of the tonically stimulated D_2 receptor and to activate gene transcription in the GABAergic enkephalinergic neurons [44]. Most probably, these are conditions that are met during strong cortico-striatal stimulation, where a strong release of glutamate should also be associated with adenosine release (reviewed in ref. 3).

Previous studies have provided evidence for functional antagonistic interactions between A_1 and A_{2A} receptors that modulate glutamate release in the striatum [45]. We have recently demonstrated the existence of A_1-A_{2A} receptor heteromers in the cell surface of co-transfected cells [17]. By means of immunogold detection and co-immunoprecipitation experiments, A_1 and A_{2A} receptors were found to be co-localized in striatal glutamatergic nerve terminals, intrasynaptic, in the active zone. A functional characteristic of A_1-A_{2A} receptor heteromerization was found in both co-transfected cells and rat striatum: an intramembrane A_1-A_{2A} receptor interaction, by which A_{2A} receptor activation reduces the affinity of A_1 receptors for agonists. Furthermore, heteromerization was found to decrease the potency of the non-selective adenosine receptor antagonist caffeine on A_{2A} but not on A_1 receptors [17]. The A_1-A_{2A} receptor heteromeric complex provides a mechanism of fine-tuning neuromodulation, by which low and high extracellular concentrations of adenosine inhibit and facilitate glutamate release, respectively [17]. Under basal conditions, there is a preferential stimulation of A_1 receptors, which would be associated with a decrease in the probability of glutamate release. Under physiological conditions of stronger adenosine release, a sufficiently stronger A_{2A} receptor activation can override the inhibitory effect imposed by A_1 receptors, which are now inhibited by means of the A_1-A_{2A} intramembrane interaction, which is associated with an increase in the probability of glutamate release [3,17]. In summary, activation of pre-synaptic A_1-A_{2A} and post-synaptic A_{2A}-D_2 and A_{2A}-$mGlu_5$ heteromers appears to be particularly suited to modulate the efficacy of glutamatergic neurotransmission in the striatal GABAergic enkephalinergic neurons. In agreement, we have recently found that administration of a selective A_{2A} receptor antagonist or caffeine counteracts both MAPK activation and also PKA-mediated AMPA receptor phosphorylation in GABAergic enkephalinergic induced by cortical electrical stimulation [46]. Since previous studies have shown that cortical stimulation selectively activates ERK1/2 phosphorylation and immediate early gene expression in striatal GABAergic enkephalinergic neurons [47], our results demonstrate that A_{2A} receptors strongly modulate the efficacy of glutamatergic synapses on striatal enkephalinergic neurons.

ADENOSINE A_{2A} RECEPTOR ANTAGONISTS IN BASAL GANGLIA DISORDERS

The most effective and most commonly used symptomatic treatment for Parkinson's disease is still L-3,4-dihydroxyphenylalanine (L-DOPA) associated with a peripheral DOPA decarboxylase inhibitor, such as carbidopa. L-DOPA is then taken up by dopaminergic cell terminals and metabolized to the endogenous neurotransmitter dopamine. However, as the disease advances, the therapeutic index (ratio between therapeutic versus secondary effects) of L-DOPA decreases and its antiparkinsonian effect is very often associated with adverse effects, including progressive decline in symptomatic benefit, end-of-dose "wearing-off",

"on-off" phenomenon and dyskinesia. The term dyskinesia implies excessive and choreiform purposeless movements, which interfere with physiological motor activity. L-DOPA-induced dyskinesia used to affect between 60 and 70% of all patients, although now this proportion has decreased to 20-30% due to the awareness of the dose of L-DOPA being a main factor involved in the appearance of dyskinesia. Another main factor is the degree of striatal dopamine denervation, which lowers the threshold at which the dopamine agonist primes for the appearance of dyskinesia. Thus, L-DOPA does not induce dyskinesia in humans without Parkinson's disease and occurs more prominently in severely affected patients and after prolonged treatment (for reviews, see refs. [48-51]. One important aspect of L-DOPA-induced dyskinesia is that it is very difficult to treat. Once it appears, L-DOPA-induced dyskinesia is persistent or even permanent. The most probable pathogenetic mechanism responsible for L-DOPA-induced dyskinesia is a chronic stimulation of D_1 receptors following dopamine denervation, which induces preferential phenotypic changes in the GABAergic dynorphinergic neurons of the dorsal striatum (the "direct" pathway). Dopamine denervation alters D_1 receptor sensitivity to agonists and also D_1 receptor signaling [47] and the concomitant stimulation of these functionally different D_1 receptors leads to increased dynorphin expression and upregulation of D_3 receptors [52-54].

It follows from the above-mentioned functional role of striatal A_{2A} receptors that A_{2A} receptor antagonists could provide a new therapeutic approach for Parkinson's disease. First, A_{2A} receptor blockade, by means of the strong antagonistic A_{2A}-D_2 receptor interaction, could potentiate the effects of L-DOPA, with a possible increase in its therapeutic index (predominant effect on the indirect versus the direct pathway). Second, A_{2A} antagonists can potentially decrease glutamate-dependent excitation of GABAergic enkephalinergic neurons, which is highly increased with dopamine depletion (see above), by means of both pre- and post-synaptic mechanisms independent of D_2 receptor function.

In the experimental animal the systemic administration of selective A_{2A} receptor antagonists counteracts most of the biochemical as well as the motor depressant and cataleptic effects secondary to the genetic inactivation or pharmacological interruption of D_2 receptor mediated neurotransmission. This has been repeatedly demonstrated in a number of experimental models involving rodents pretreated with D_2 receptor antagonists, reserpine or MPTP or after genetic inactivation of D_2 receptors [55-62] and involving MPTP-treated monkeys [63, 64]. Since reserpinized mice, rats with unilateral 6-OH-dopamine lesions and MPTP-treated monkeys are well-established validated models of Parkinson's disease, the results of these experiments strongly supported the hypothesis, put forward in 1992 [65], that A_{2A} receptor antagonists could be used in Parkinson's disease. However, it is still a matter of debate if they can be useful as monotherapy or if they would be more efficacious when combined with D_2 receptor agonists. An argument against monotherapy is the lack of A_{2A} receptor antagonist-induced contralateral turning in the rat with a unilateral lesion of the nigrostriatal pathway [60, 66, 67], which is a behavior that predicts antiparkinsonian activity. Results from electrophysiological experiments do not either support a possible efficacy of A_{2A} receptor antagonist monotherapy. Thus, the local infusion of an A_{2A} receptor antagonist could not counteract the increased spontaneous activity of striatal neurons induced by the nigrostriatal denervation, although it strongly potentiated the D_2 receptor agonist-induced inhibition of striatal neuronal activity [67]. Nevertheless, in the same animal model, studies with *in vivo* microdialysis showed that oral administration of A_{2A} receptor antagonists counteracts the increased extracellular levels of GABA in the globus pallidus ipsilateral to the nigrostriatal denervation [68]. Thus, it seems that some pharmacological and, maybe, therapeutic effects secondary to A_{2A} receptor blockade can only be observed with the concomitant stimulation of D_2 receptors. This is also in agreement with some results obtained with D_2 receptor knockout mice where the motor effects of A_{2A} receptor antagonists were attenuated [59]. These experiments performed under complete inactivation of D_2 receptors demonstrate that some A_{2A} receptor functions are dependent on the integrity of D_2 receptors. This is shown even more dramatically in the study by Zahniser *et al*. [69], where a very significant functional uncoupling of A_{2A} receptors (lack of A_{2A} receptor agonist induced GABA release in striatal/pallidal slices) was found in D_2 receptor knockout mice. Nevertheless, in all animal models of Parkinson's disease tested so far (reserpinized mice, rats with unilateral 6-OH-dopamine lesions, MPTP-treated monkeys) A_{2A} receptor antagonists strongly potentiate the motor activation induced by L-DOPA or D_2 receptor agonists [60,64,67,70-73]. Importantly, co-treatment with A_{2A}R antagonists and L-DOPA did not increase the non-wanted dyskinetic effects in MPTP-treated monkeys [64, 71].

A_{2A} receptor antagonists are now being evaluated in clinical trials in patients with Parkinson's disease. The closest to its widespread clinical application is istradefylline (KW-6002, with a European patent granted in 1999, EP-0590919), which has completed Phase II and is currently in Phase III (reviewed in ref. 74). The efficacy, safety and tolerability of istradefylline have been investigated in two placebo-controlled, double blind studies in patients treated with L-DOPA [75,76]. The results from these two studies demonstrate that istradefylline is safe and well tolerated and that it improves the therapeutic index of L-DOPA, reducing the "off" and increasing the "on" periods without increasing the severity of dyskinesia. So far, there is no data available supporting a possible beneficial effect of istradefylline monotherapy.

There is some experimental evidence for a neuroprotective effect of A_{2A} receptor antagonists, especially in neurological diseases where excessive neuronal activation by excitatory amino acids is supposed to be a main pathogenetic mechanism ("excitotoxicity"), such as in cerebral ischemia. This is in fact the rationale behind some patents of use of A_{2A} receptor antagonists. However A_{2A} receptor agonists have also been shown to be neuroprotective in different experimental models against a variety of insults. A_{2A} receptor antagonists seem to exert neuroprotection by a presynaptic effect, by inhibiting glutamate release, while A_{2A} receptor agonists seem to exert a direct protective effect in the cells suffering from the insult [77-82]. Furthermore, it

has also been suggested that A_{2A} receptor antagonists can provide a neuroprotective effect in slow, progressive neurodegenerative disorders, including Parkinson's disease and Huntington's disease, where excitotoxicity could also play some pathogenetic role [83-85]. Thus, A_{2A} receptor antagonists protect against the excitotoxic effects of quinolinic acid when injected in the rat striatum, an animal model of the neurodegeneration process in Huntington's disease [80, 81]. Furthermore, A_{2A} receptor antagonists have also shown to protect dopaminergic cells against the toxic effects of MPTP in mice [86, 87] and istradefylline has been reported to protect nigral dopaminergic cells against 6-hydroxydopamine-induced toxicity in rats [87]. However, negative results on the neuroprotective effects of pharmacologic or genetic inactivation of A_{2A} receptors against 6-hydroxydopamine toxicity have also been reported [88, 89]. Finally, recent studies have shown that istradefylline and other A_{2A} receptor antagonists, such as 8-(3-chlorostyryl) caffeine, are inhibitors of MAO-B, a mechanism of action that could contribute to their neuroprotective effects [90].

In summary, there are enough experimental and clinical data supporting a role of A_{2A} receptor antagonists in the symptomatic, but not pathogenetic, treatment of Parkinson's disease. The most recent results suggest the main value of A_{2A} receptor antagonists is the increase in the therapeutic index of L-DOPA. Much less clear is the evidence for their possible role in Huntington's disease. First, there is no clear evidence for a neuroprotective effect of A_{2A} receptor antagonists in slow, progressive neurodegenerative diseases. Second, A_{2A} receptor antagonists could only worsen hyperkinetic movements during the first stages of the disease, by inhibiting the function of the dorsal GABAergic enkephalinergic neurons.

ADENOSINE A_{2A} RECEPTOR ANTAGONISTS

In the past 15 years a number of selective A_{2A} receptor antagonists have been developed, and several classes of compounds have been patented. Most A_{2A} receptor antagonists belong to two different chemical classes, (i) xanthine derivatives (and analogs), and (ii) amino-substituted heterocyclic compounds, which are derived from adenine or structurally - more or less - related to adenine. In addition, by screening of compound libraries, novel structures have been identified that do not have any similarity with either xanthine or adenine. Several review articles on A_{2A} receptor antagonists have appeared [91-98]. An excellent and comprehensive recent article by Vu summarizes the progress from 2003-2005 [98]. The present review describes properties of standard A_{2A} receptor antagonists including those in clinical development. Furthermore, the different chemical classes of A_{2A} receptor antagonists that have been described in the literature, including recent patent literature, will be presented.

Xanthines

The alkaloid caffeine (**1**), 1,3,7-trimethylxanthine, mediates its pharmacologic actions by a blockade of A_1, A_{2A}, and A_{2B} receptors; caffeine is virtually nonselective (Fig. **2**). The first "selective" A_{2A} receptor antagonist described in the literature was the caffeine analog 3,7-dimethyl-1-propargylxanthine (DMPX, **2**) [99]. Like caffeine, the compound possesses low A_{2A} receptor affinity and moderate selectivity versus A_1 receptors; it is even more potent at the A_{2B} than at the A_{2A} receptor subtype. The 8-phenyl-1,3-dipropylxanthine derivative XAC (**3**, xanthine amine congener) was the first highly potent A_{2A} receptor antagonist exhibiting low nanomolar affinity [100], but the compound is only moderately selective in humans and even slightly A_1-selective in rat. The observation that N7-methylation in 8-substituted xanthine derivatives was better tolerated by the A_{2A} than the A_1 receptor [101], and that the 8-substituent had to be coplanar for achieving high A_{2A} receptor affinity [102, 103] led to the first highly potent and selective A_{2A} receptor antagonists, the xanthine derivatives CSC (**4**) [104], KW-6002 (istradefylline, **5**) (reviewed in ref. 105), and MSX-2 (**6**) along with its water-soluble phosphate prodrug MSX-3 (**7**) [106-108] (Fig. **2**).

The most common substitutents at N3 in A_{2A} receptor-selective xanthine derivatives have been small alkyl residues, such as methyl, propyl, and 3-hydroxypropyl (reviewed in refs. 92, 94). Recently, the develoment of a new synthetic approach allowed the preparation of a series of xanthine derivatives with more variations in the 3-position [109]. It was found that the A_{2A} receptor tolerated bulky, functionalized substituents in the 3-position. For instance, N3-phenoxypropyl-substituted 8-(methoxystyryl)xanthine derivatives (compounds **8** and **9** Fig. **3**) are potent and selective A_{2A} antagonists [109].

(E)-8-(3-chlorostyryl)caffeine (CSC)

In addition to its A_{2A} receptor blocking activity, CSC (**4**) has been reported to be a potent inhibitor of monoamine-oxidase B (MAO-B, K_i ca. 100 nM), an enzyme which metabolizes dopamine [110,111]. This activity may contribute to the potency of CSC in animal models of Parkinson's disease, as well as its neuroprotective effect (see above). All other styrylxanthine derivatives investigated so far, including KW-6002, are less potent MAO-B inhibitors than CSC [90, 111].

Istradefylline (KW-6002)

Istradefylline has been intensively studied *in vitro* and in a number of animal models. It is currently in Phase III clinical trials for Parkinson's disease. In phase II clinical trials istradefylline reduced motoric dysfunction without producing dyskinesias (reviewed in ref. 74). Application to the FDA for registration in the USA is planned for late 2006 / early 2007.

MSX-3

Like CSC (**4**) and istradefylline (**5**), MSX-2 (**6**) and MSX-3 (**7**) are 8-(*E*)-styrylxanthine derivatives with high affinity and selectivity for the A_{2A} receptor [94, 107]. Care has to be taken when using the (*E*)-configurated styrylxanthines since they easily isomerize in dilute solutions yielding mixtures of (*E*)- and (*Z*)-isomers, the (*Z*) isomers being only weakly active or inactive [106, 112]. In addition, styrylxanthines can undergo light-induced dimerization in the solid state, and therefore have to be stored under exclusion of light [108]. While compounds **4**, **5**, and **6** are lipophilic and possess low solubility in water, MSX-3 is a water-soluble phosphate prodrug of MSX-2, which is

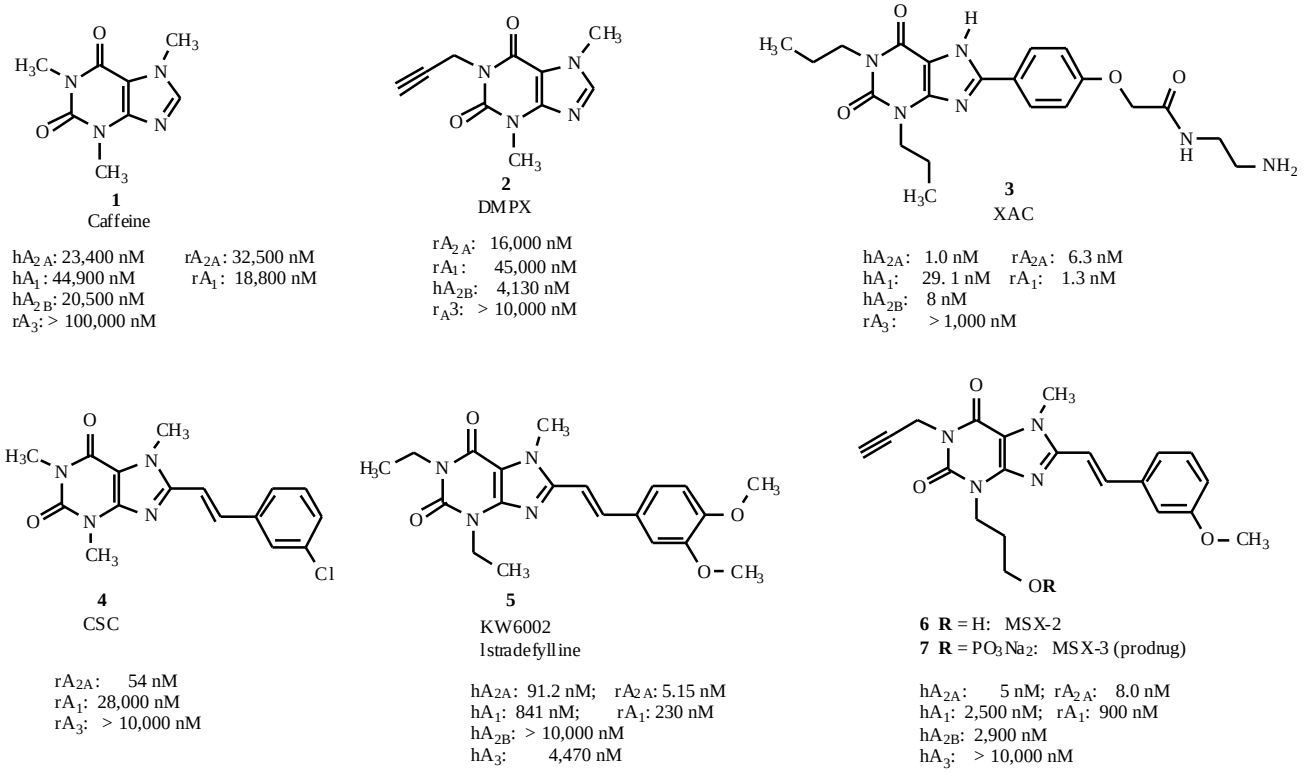


Fig. (2). Standard xanthine derivatives used as A_{2A} antagonists. In all figures, data represent K_i values (h = human, r = rat) and are taken from references indicated in the text.

cleaved *in vivo* by ubiquitous phosphatases to release the A_{2A} receptor antagonist MSX-2 [107]. MSX-3 has proven useful for animal studies and is widely used for studying the *in vivo* effects of A_{2A} antagonists [42, 45, 61, 67, 81, 89, 113-124].

Tricyclic Xanthine Derivatives

Several different types of tricyclic xanthine derivatives have been synthesized [125-127]. Pyrimido[2,1-*f*]purinediones, e.g. compounds **10-12** (Fig. **4**), showed selectivity for A_{2A} receptors [125]. Replacement of the N-methyl groups in the pyrimidinedione ring by propyl residues increased A_1 receptor affinity yielding non-selective compounds, e.g. **12**.

Pyrimdo[2,3-*d*]pyrimidinediones

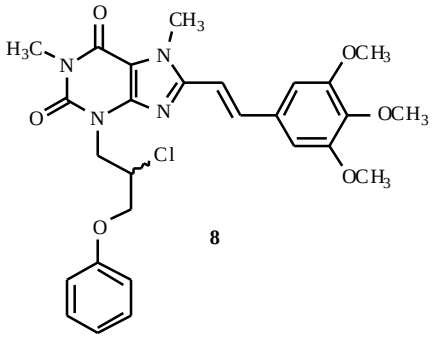
Bicyclic pyrido[2,3-*d*]pyrimidinediones, such as compound **13** (Fig. **5**), can be envisaged as hybrid molecules derived from xanthine and adenine. They contain the pyrimidinedione structure from xanthines and the aminopyridine structure which is analogous to the aminopyrimidine part of adenine. A naphthylamino-substituted derivative (**13**) has been found to be a potent A_{2A} receptor antagonist with low selectivity versus A_1 receptors, but it could serve as a new lead structure for developing more potent and selective compounds [128].

Adenine derivatives

Cristalli, Klotz and colleagues studied the structure-activity relationships of various series of adenine derivatives as adenosine receptor antagonists [129,130]. Some low molecular weight compounds, e.g. 8-ethoxy-9-ethyladenine (ANR-94, **16**), were found to exhibit good A_{2A} receptor affinity and selectivity. Morelli evaluated selected adenine derivatives in rat models of Parkinson's disease. ANR 94 reversed haloperidol-induced catalepsy, potentiated L-DOPA effects on turning behaviour in unilaterally 6-hydroxydopamine-lesioned rats, and induced contralateral turning behaviour in rats sensitized to L-DOPA [131].

Adenosine Therapeutics patented 2-alkynyl-substituted adenine derivatives based on structures initially introduced by Cristalli [132]. The compounds resemble the A_{2A} receptor agonists developed by the same company, e.g. ATL 146e [133]. ATL-2 (**15**, Fig. **6**) was the most potent of the representative A_{2A} receptor antagonists exhibiting high A_{2A} receptor affinity (K_i 0.95 nM), but moderate selectivity, especially versus the A_1 receptor. A propargyl substitutent at N9 - replacing the ribose moiety present in the agonistic adenosine derivatives - appears to be favorable for high A_{2A} receptor affinity. ATL-2 (15 mg/kg) led to stimulation of locomotor activity in wild-type, but not in A_{2A} receptor KO mice.

Adenine derivatives substituted in the 8-position by a 1,2,3-triazole ring were developed as potent A_{2A} receptor antagonists [134]. One of the most potent compounds was 9-methyl-2-phenethyl-8-[1,2,3]triazol-2-yl-adenine (**17**). The corresponding 2-butyl and 2-pentyl derivatives were similarly potent. The N3 nitrogen atom appeared not to be important since 3-deazaadenine derivatives were about as



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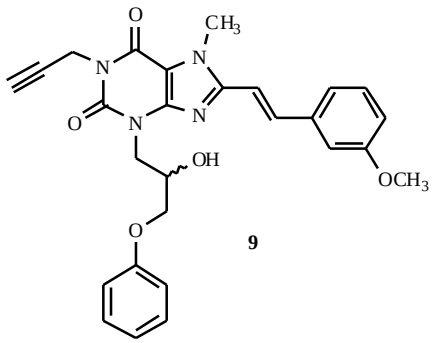
rA_{2A}: 44 nM
rA_1: > 10,000 nM



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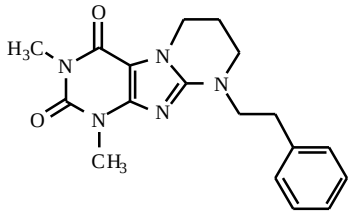
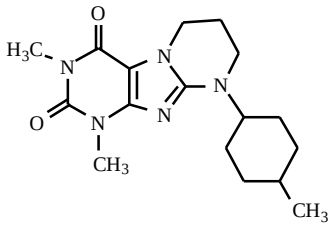
rA_{2A}: 84 nM
rA_1: 5,070 nM

Fig. (3). Styrylxanthine derivatives with bulky 3-substitutents.



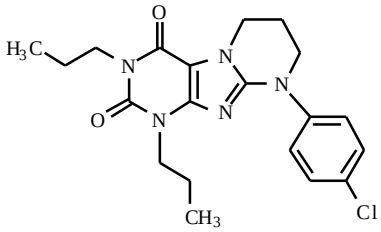
10

rA_{2A}: 320 nM
rA_1: > 25,000 nM



11

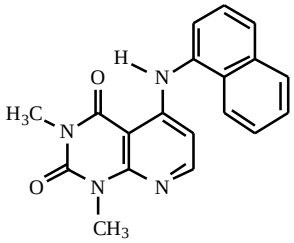
rA_{2A}: 240 nM
rA_{2A}: 620 nM
rA_1: > 25,000 nM



12

rA_{2A}: 380 nM
rA_1: 360 nM

Fig. (4). Tricyclic xanthine derivatives.



13

hA_{2A}: 81.3 nM
hA_1: 153 nM
hA_3: > 10,000 nM

Fig. (5). Aminopyridopyrimidinedione derivative: xanthine-adenine hybrid structure.

potent as the corresponding adenines [134]. Most of the described adenine derivatives that were potent at A_{2A} receptors exhibited only moderate selectivity especially versus the A_1 receptor subtype.

Amino-Substituted Heterocyclic Compounds Related to Adenine

7-Deazaadenines (4-Aminopyrrolo[2,3-d]pyrimidines)

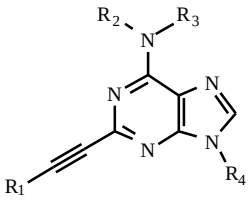
Adenine derivatives lacking a nitrogen atom in the 7-position were first introduced by Eger and Daly [135-138]. OSI Pharmaceuticals patented 7-deazaadenindes of the general formula **18** some of which were reported to be at least 10 times selective for A_{2A} receptors versus the other three adenosine receptor subtypes, e.g. **19** and **20** (Fig. **7**) [139].

Oxazolo[5,4-d]pyrimidines

Holschbach and coworkers synthesized a small series of oxazolo[5,4-*d*]pyrimidines structurally derived from the triazolotriazine derivative ZM-241385 (**53**) [140]. Compounds **21** and **22** belonged to the most A_{2A} receptor-selective derivatives of the series (Fig. **8**). Isomers bearing a 3-furyl instead of a 2-furyl residue were less selective versus A_1 receptors. Compound **21** was obtained in [^{3}H]-labeled form. Radioligand binding studies showed a high degree of non-specific binding rendering the compound unsuitable as a potential ligand for positron emission tomography (PET) after labelling with a neutron-deficient nuclide.

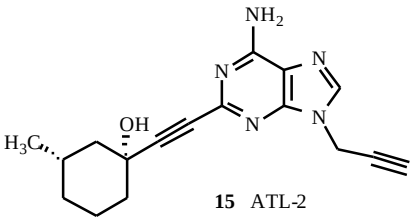
2-Aminopurines and Analogs

2-Aminopurines (general formula of purines **23**, Fig. **9**) are constitutional isomers of adenine (= 6-aminopurine). Particularly 6-(2-furyl)-substituted 2-aminoadenine derivatives (e.g. **26-29**) bearing an aromatic, e.g. (substituted) benzyl residue at N9, were found to be potent A_{2A} receptor antagonists [141,142]. Such compounds were developed by Schering-Plough (**26,27**) as well as by Vernalis (**28,29**).



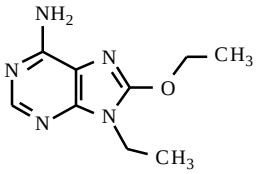
14

2-ethynyladenine derivatives
(Adenosine Therapeutics)



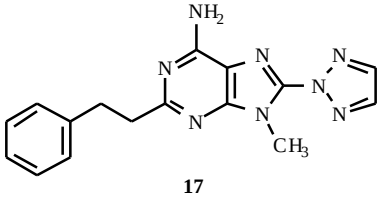
15 ATL-2

hA_{2A} 0.95 nM
hA_1 4.6 nM
hA_{2B} 50 nM
hA_3 599 nM



ANR-94 **16**

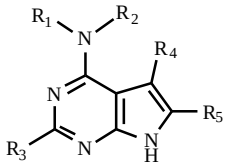
hA_{2A}: 46 nM
hA_1 2400 nM
hA_{2B} >30000 nM
hA_3 2100 nM



17

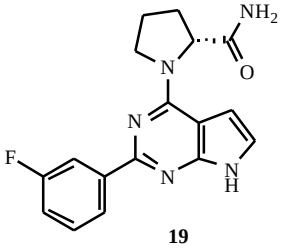
hA_{2A} 4.7 nM
hA_1 80.0 nM
hA_{2B} 2330 nM

Fig. (6). Adenine derivatives.

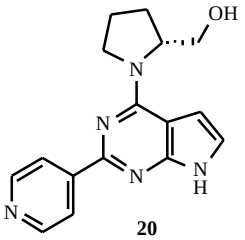


18

OSI Pharmaceticals

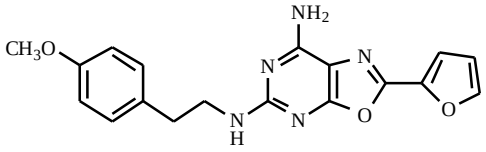


19



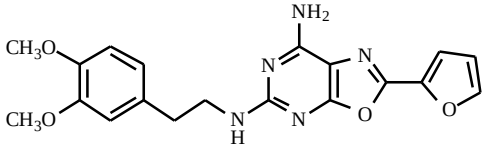
20

Fig. (7). 7-Deazaadenine derivatives (pyrrolo[2,3-d]pyrimidines).



21

pig A_{2A}: 22 nM
pig A_1: 1570 nM



22

pig A_{2A}: 14 nM
pig A_1: 1270 nM

Fig. (8). Oxazolo[5,4-d]pyrimidines.

VER-6947 (**28**) was shown to reverse haloperidol-induced catalepsy in mice in a dose-dependent manner (1-30 mg/kg) [142]. Similarly as in adenine derivatives, the nitrogen atom in the 7-position was not required for high affinity. Corresponding pyrrolo[2,3-*d*]pyrimidines **24** [143] and pyrazolo [3,4-d]pyrimidines **25** [144] were also highly potent, as demonstrated by a recent patent (for example see compound **30**). This latter class of compounds had initially been described by Quinn [145] and recently modified by Briel *et al*. [146].

Thieno- and furopyrimidines

In a further series of A_{2A} receptor antagonists Vernalis replaced the imidazole ring in purines by a thiophene or a furane ring yielding thieno- and furopyrimidines **31** [147]. Selected potent compounds (**32**-**35**) are shown in Fig. **10**.

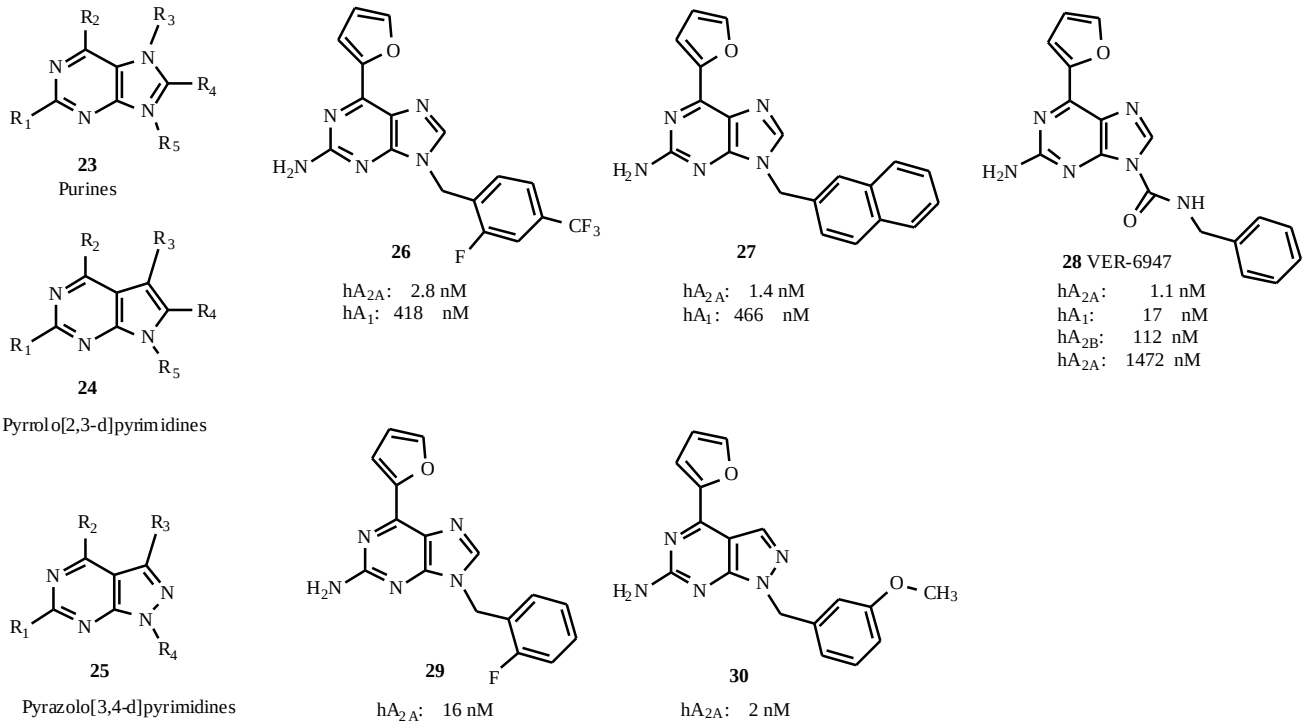


Fig. (9). 2-Aminopurines and related pyrrolo[2,3-*d*]pyrimidines and pyrazolo[3,4-*d*]pyrimidines.

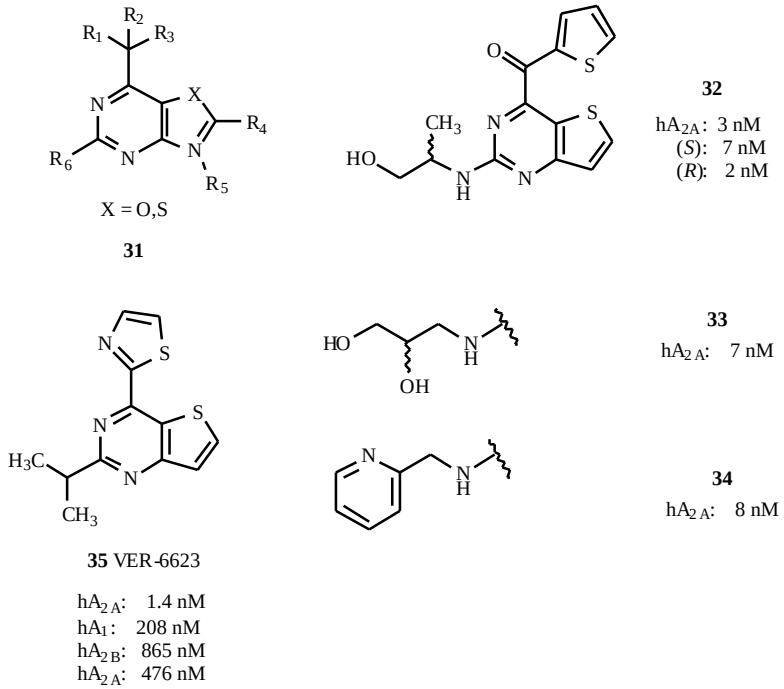


Fig. (10). Thieno- and furopyrimidines.

BIIB014/V2006

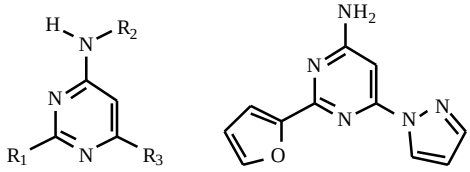
One of the compounds developed by Vernalis, named BIIB014 (previous code: V2006), whose structure has not been disclosed yet, is being clinically developed by Biogen Idec. A phase I clinical study in healthy volunteers was performed with application of single oral doses of up to 100 mg, and repeated doses of up to 50 mg per day for 10 days. It was reported to be well tolerated and suitable for once daily

dosing [148,149]. *In vitro* studies showed that BIIB014/ V2006 was metabolized by various CYP450 enzymes, but showed a low potential for inhibition or induction of the major CYP isoenzymes [150]. BIIB014/V2006 was reported to exhibit a K_i value of 1.3 nM and >50-fold selectivity [150]. In animal models the compound was effective in doses of 0.1-5 mg/kg in various species: mouse and rat haloperidol-induced catalepsy (0.1-1 mg/kg p.o.), rat 6-OHDA test (3 mg/kg p.o.), marmoset MPTP model (< 5 mg/kg p.o.) [150].

Aminouracil Derivatives

4-Aminouracils

Almirall Prodesfarma SA patented a series of 4-aminouracil derivatives **36** (Fig. **11**) which are related to adenine but are lacking the imidazole ring [151]. The substituent in the 2-position (R1) was in many cases a 2-furyl residue. Many examples are given, including **37**, but pharmacological data have not been disclosed.



36 **37**

Almirall Prodesfarma

Fig. (11). 4-Aminopyrimidines.

2-Aminouracils

5-(2-Amino-6-phenylpyrimidine-5-yl)-2H-pyridinone derivatives **38** were developed by Fujisawa for the treatment of Parkinson's disease, anxiety and pain [152]. The selected examples showed high affinity for A_{2A} receptors in the low nanomolar concentration range, but only moderate selectivity. Compound **39** exhibited anticataleptic activity in haloperidol-treated mice after oral application at a dose of 3.2 mg/kg. Similar compounds of the general formula **40** were patented by Eisai Co. [153]. One of the most potent compounds (**41**) contained a 2-furyl substituent and a pyridinone residue in the 5-position. Its A_{2A} receptor affinity and selectivity versus A_1 receptors was high, but **41** also showed high affinity for A_{2B} receptors.

Hoffmann-La Roche identified 2-amino-5-cyano-6-(2-furyl)pyrimidine derivatives **42** by a random screening approach of a proprietary compound library as compounds with affinity for A_{2A} receptors (Fig. **12a**) [154]. A systematic modification and optimization of the structure was undertaken yielding potent A_{2A} receptor antagonists, such as **45-47**. Corresponding deaza analogs, pyridine derivatives **43** and **44**, were also patented [154]. The structure-activity relationships are summarized in Fig. **12b**. The unsubstituted amino function in the neighborhood of a nitrogen ring (N1-pyrimidine) was essential. A 2-(furyl) residue in the 6-position proved to be optimal but other aromatic or heteroaromatic residues were also tolerated. Electron-withdrawing substituents were required in the 5-position, a cyano group giving the best results. A relatively bulky substituent could be accomodated in the 4-position. The best linker was an oxygen bridge. A sulfur or amino linkage gave similar results, but thioethers are not ideal due to metabolic instability, and an NH linker reduced water solubility. A methylene bridge led to a decrease in A_{2A} receptor selectivity. Further details are depicted in Figure **12b**.

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

Based on the lead structure CGS15943 (**59**, Fig. **15**), Baraldi and coworkers developed analogs in which the benzene ring in the triazoloquinoxaline derivative **59** was replaced by a pyrazole ring. SCH-58261 (**48**) from this series has become a standard A_{2A} receptor antagonist [155,156]. An unsubstituted amino group, a 2-(2-furyl) substituent, and a bulky substituent at N7 (e.g phenethyl in **48**) proved to be optimal. Further development by Schering-Plough led to SCH-420814 (**49**), a compound bearing a basic piperazine ring which improves water-solubility without compromising CNS bioavailability [157-160]. SCH-420814 (**49**) was selected for clinical development (see below). Baraldi recently introduced amino-substituted derivatives such as **50**, which exhibited particularly high A_{2A} receptor affinity [156]. Schering-Plough discovered that the metabolically critical 2-furyl residue previously believed to be essential for high A_{2A} receptor affinity and selectivity could be replaced by a propyn-1-yl substituent as in compound **51**, or a 2-pyridyl residue as in **52** (Fig. **13**) [161].

SCH-420814

The potent and selective A_{2A} receptor antagonist SCH-420814 (**49**) is being developed for the treatment of Parkinson's disease. The compound is now in phase II clinical trials. Besides its anticataleptic activity, SCH-420814 showed an antidepressant profile of activity in rodent models of behavioural despair [162]. Further potential indications claimed by Schering-Plough are attention deficit (hyperactivity) disorder (ADHD), neuroleptic-induced extrapyramidal syndrome, and restless legs syndrome [163]. The compound was reported to be active in preclinical models of ADHD. Typical doses that showed activity in *in vivo* models of Parkinson's disease were 0.1-3 mg/kg (p.o.). For example, 0.1-1 mg/kg of SCH-420814 produced a dose-dependent reversal of haloperidol-induced catalepsy in rats and mice. Doses of 1 and 3 mg/kg p.o. produced a dose-dependent improvement in motor function in the MPTP-pretreated cynomolgus monkey without inducing dyskinesia [164]. The clinical phase 1 study of SCH-420814 showed that it was well tolerated in doses of 5-200 mg (single and multiple doses for 10 days). In a phase 2A study in patients it was shown to improve signs and symptoms of Parkinson's disease when given in combination with L-DOPA. In *in vitro* studies SCH-420814 was characterized as a competitive A_{2A} receptor antagonist [162]. SCH-420814 showed decreased dissociation rates from the A_{2A} receptor in comparison with SCH-58261; its calculated off-rate was 30 min.

Derivatives and analogs of ZM241385

5-Amino[1,2,4]triazolo[1,5-a][1,3,5]triazines

The triazolotriazine derivative ZM-241385 (**53**) is a standard A_{2A} receptor antagonist, but it also shows some

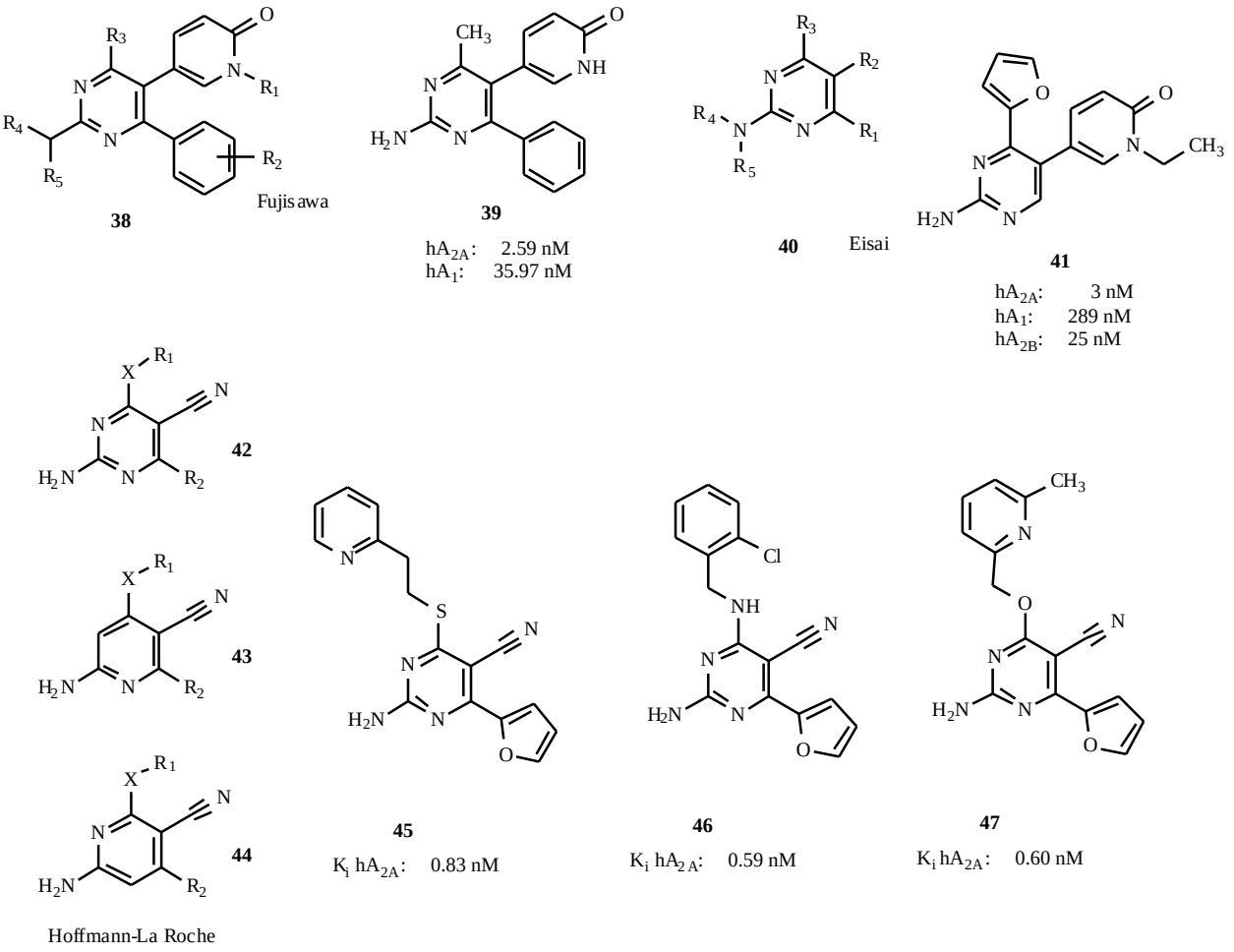


Fig. (12a). 2-Aminopyrimidine derivatives.

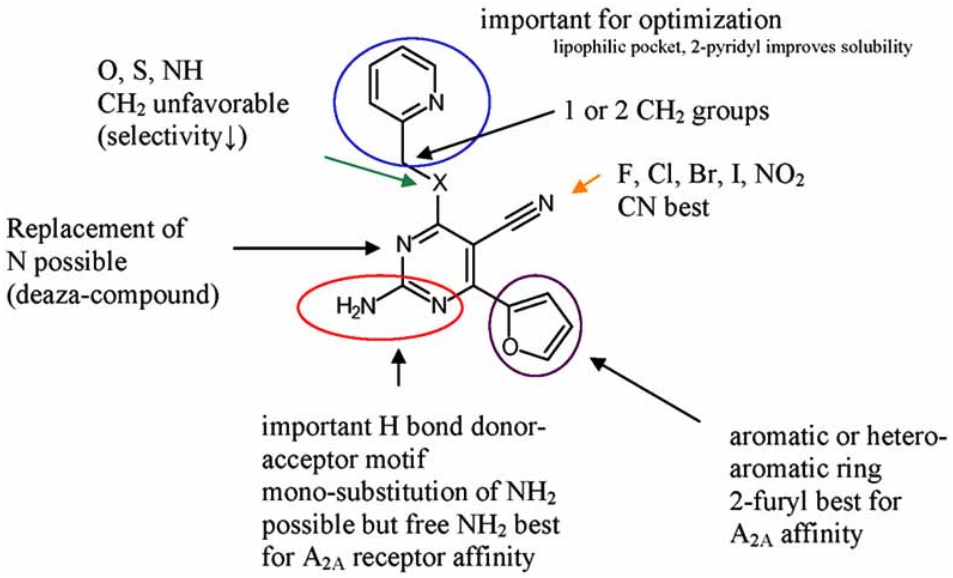


Fig. (12b). Structure-activity relationships of 2-aminopyrimidine derivatives developed by Hoffmann-La Roche.

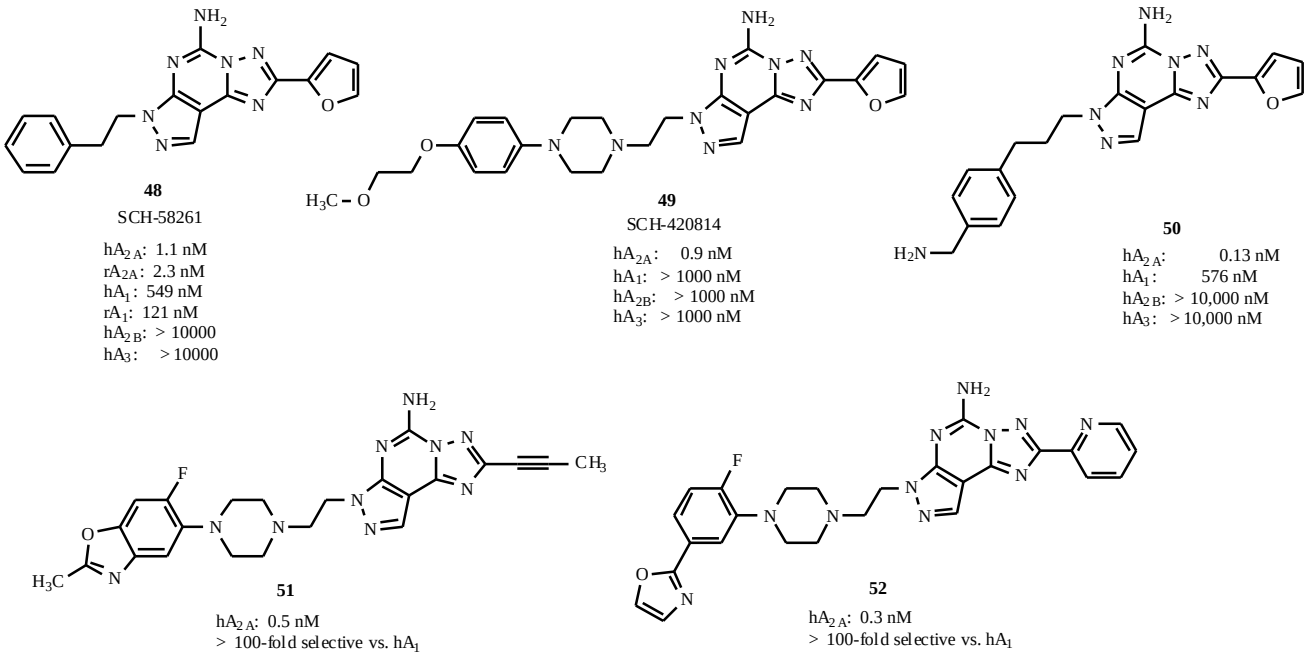


Fig. (13). Pyrazolotriazolopyrimidines.

affinity for A_{2B} receptors (see Fig. **14**) (reviewed in ref. 94). Biogen-Idec successfully developed derivatives that are bearing a basic substituent (e.g. pyrrolidine, piperazine) in the side chain. Some of the most potent examples (**54-58**) are depicted in Fig. **14**. In compounds with bicyclic substitution (e.g. **55**) *cis*-configurated isomers were much more potent than *trans*-configurated isomers. In 7-amino-substituted derivatives (**57,58**) N-methylation (**58**) was well tolerated and led to increased oral bioavailability [165-171].

Triazolopyrimidines

Related compounds with one nitrogen atom less in the 6-ring heterocycle yielding triazolopyrimidines were investigated by Kyowa-Hakko Kogyo, Biogen-Idec and Schering-Plough (Fig. **15**) [168,171,174-176]. The compounds are structurally related to the standard non-selective adenosine receptor antagonist CGS 15943 (**59**) (reviewed in ref. 94). Matasi *et al*. reported that piperazine derivative **66** exhibited anticataleptic activity *in vivo* in rat (haloperidol-induced catalepsy) at an oral dose of 3 mg/kg, while the corresponding piperidine analog lacking the nitrogen atom connected to the terminal phenyl ring, that exhibited similar A_{2A} affinity and selectivity *in vitro*, was inactive *in vivo* [175]. However, piperazine derivative **66** turned out to be a potent inhibitor of hERG potassium channels, which may result in severe cardiac side-effects [176]. Affinity to hERG K^+ channels may be correlated with increased basicity (protonation) of compounds but also depends on steric factors. In a further series of derivatives morpholino-piperidine **67** was identified as a potent and selective A_{2A} antagonist that did not interact with hERG channels; it showed promising *in vivo* activity in rat models of haloperidol-induced catalepsy (3 mg/kg p.o., 1 and 4 h after administration), and A_{2A}-agonist-induced hypolocomotion at 1 and 3 mg/kg p.o. [176].

Triazolopyrazines and Triazolopyridines

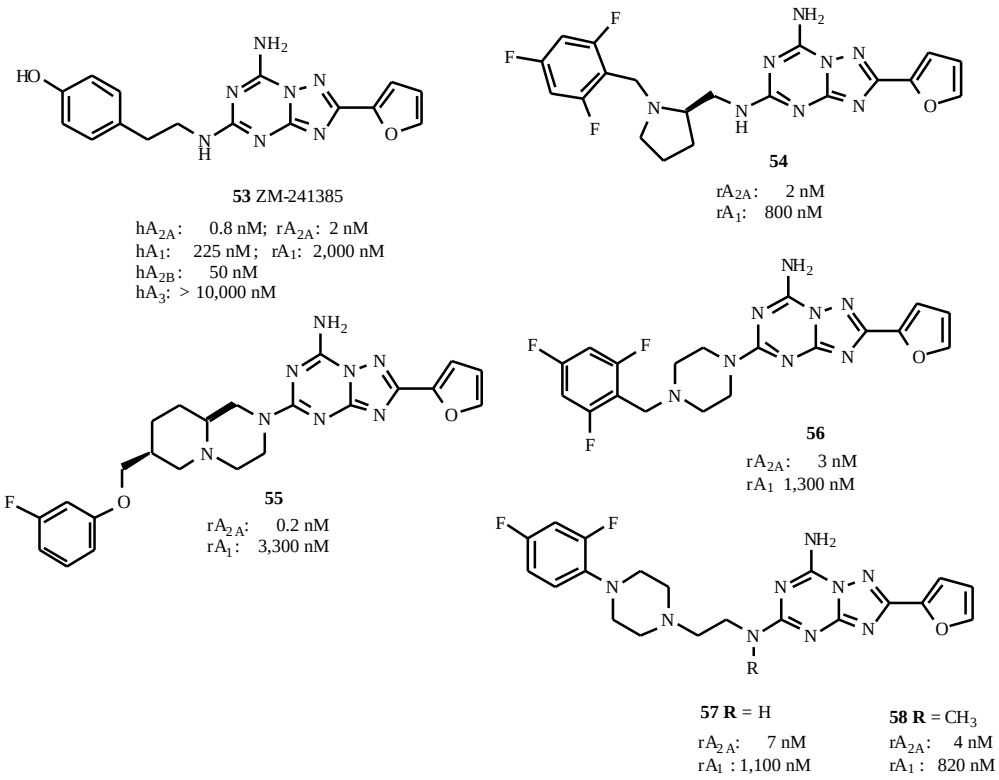
Further variations of essentially the same scaffold include triazolopyrazine derivatives, such as **68**, in which one of the triazole ring nitrogen atoms was shifted to another position in comparison with the triazolopyrimidine derivatives, and the triazolopyridines, such as **69**, which have one nitrogen atom less in the 6-ring heterocycle (Fig. **16**) [171, 174, 177, 178]. High affinity and selectivity could be achieved in both classes of compounds. Some derivatives were shown to have anticataleptic activity in animal models [177].

4-Amino-1,2,4-triazolo[4,3-a]quinoxalines

Colotta and coworkers evaluated the structure-activity relationships of 4-amino-1,2,4-triazolo[4,3-*a*]quinoxalines as A_{2A} receptor antagonists [179]. The compounds are structurally related to CGS15349 (**59**). Some derivatives, e.g. **70**, were potent at and selective for bovine A_{2A} receptors. However, in a comparison of rat A_{2A} and A_1 receptor affinities, selectivity appeared to be much lower [180].

Arylindenopyridines and Arylindenopyrimidines

Ortho-McNeil Pharmaceutical Inc. developed tricyclic indeno-pyrimidine (**71**) and -pyridine derivatives (**72**) with high affinity for A_{2A} receptors [181]. Some derivatives were also potent A_1 antagonists. Selected compounds are shown in Fig. **18**: they exhibit a primary amino group in the 2-position of the heterocyclic ring and a 2-furyl substituent. A structural resemblance to the previously discussed "adenine analogs" is obvious (see Fig. **22**). Compounds **73** and **74** were active in a mouse model of haloperidol-induced catalepsy after peroral application. At a dose of 10 mg/kg the compounds inhibited catalepsy by 90 % (**73**) and 87 % (**74**). Schering-Plough identified the same class of compounds as A_{2A} receptor antagonists by a high throughput screening approach [182].



53 ZM-241385

hA_{2A}: 0.8 nM; rA_{2A}: 2 nM
hA_1: 225 nM; rA_1: 2,000 nM
hA_{2B}: 50 nM
hA_3: > 10,000 nM

54

rA_{2A}: 2 nM
rA_1: 800 nM

55

rA_{2A}: 0.2 nM
rA_1: 3,300 nM

56

rA_{2A}: 3 nM
rA_1 1,300 nM

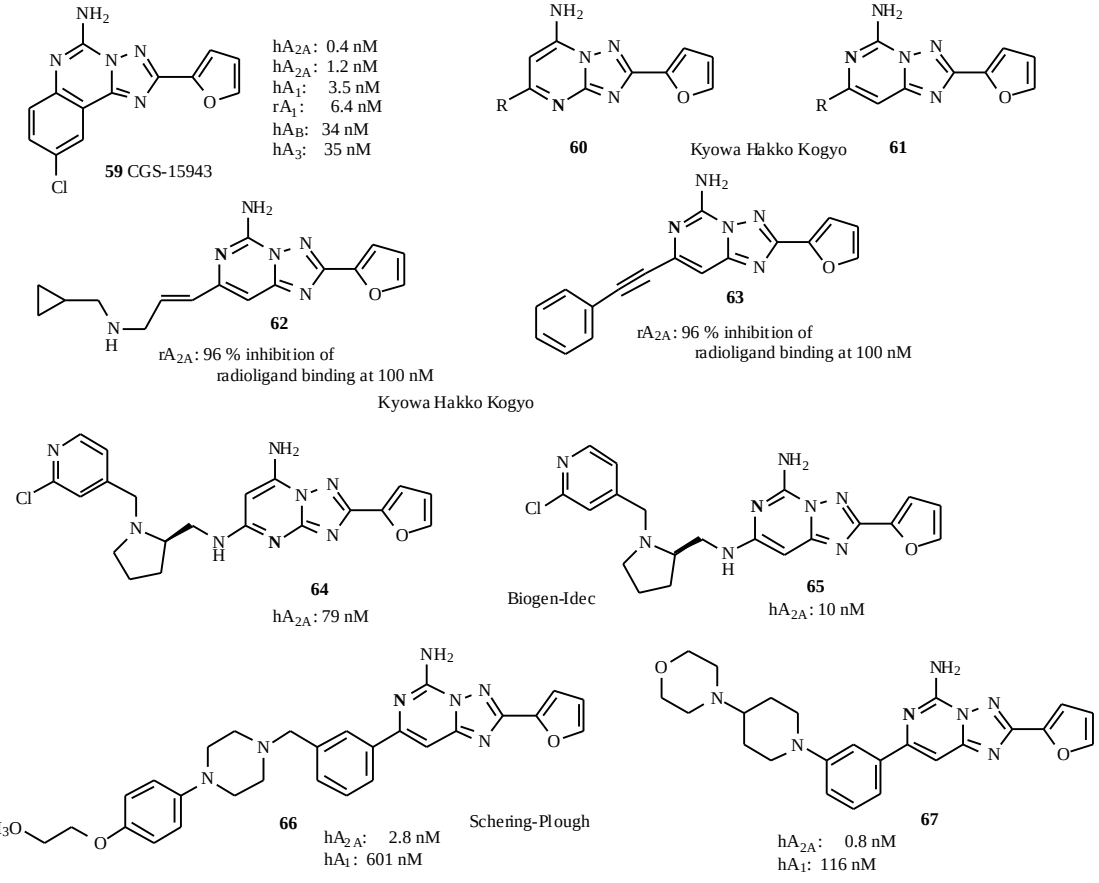
57 R = H

rA_{2A}: 7 nM
rA_1 : 1,100 nM

58 R = CH_3

rA_{2A}: 4 nM
rA_1 : 820 nM

Fig. (14). Triazolotriazines.



59 CGS-15943

hA_{2A}: 0.4 nM
hA_{2A}: 1.2 nM
hA_1: 3.5 nM
rA_1: 6.4 nM
hA_B: 34 nM
hA_3: 35 nM

60

Kyowa Hakko Kogyo

61

62

rA_{2A}: 96 % inhibition of radioligand binding at 100 nM

63

rA_{2A}: 96 % inhibition of radioligand binding at 100 nM

Kyowa Hakko Kogyo

64

hA_{2A}: 79 nM

Biogen-Idec

65

hA_{2A}: 10 nM

66

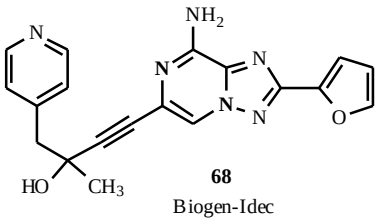
hA_{2A}: 2.8 nM
hA_1: 601 nM

Schering-Plough

67

hA_{2A}: 0.8 nM
hA_1: 116 nM

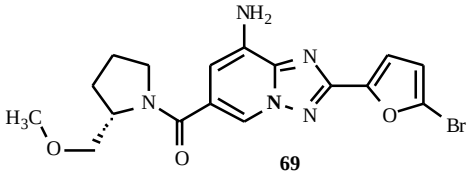
Fig. (15). Triazolopyrimidines.



68

Biogen-Idec

rA_{2A}: 1.1 nM
rA_1: 100 nM

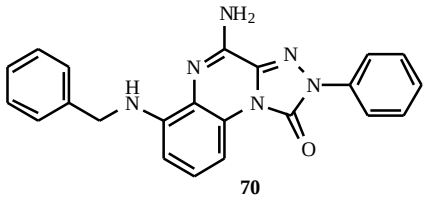


69

Hoffmann-La Roche

hA_{2A}: 16 nM
hA_1: 1053 nM

Fig. (16). Triazolopyrazine and Triazolopyridine derivatives.



70

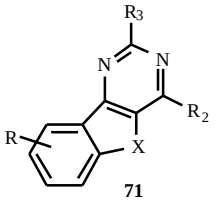
rA_{2A}: 17 nM; bA_{2A}: 6.5 nM
rA_1: 20 nM; bA_1: 730 nM
hA_3: > 1000 nM

Fig. (17). Triazoloquinazolinone derivative.

The unsubstituted amino function was essential. 2-Furanyl substitution in the 4-position enhanced selectivity for A_{2A} versus A_1. Substituents in the 8-position increased A_{2A} affinity (see compounds **76** and **77**, Fig. **18**).

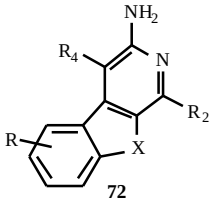
Benzothiazoles and thiazolopyridines

Hoffmann-La Roche discovered a novel scaffold for adenosine receptor antagonists - 4-methoxy-substituted benzothiazole derivatives **78** - and developed potent and selective antagonists, e.g. **79-81** (Fig. **19**) [183,184]. A related series of thiazolo[5,4-*c*]pyridines **82** containing a nitrogen atom in the benzene ring (Fig. **19**) was also patented. A

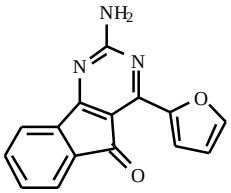


71

Ortho-McNeil

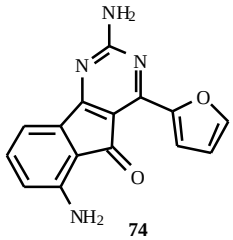


72



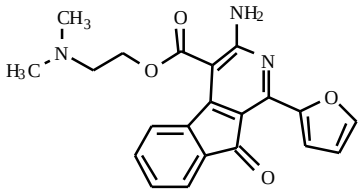
73

hA_{2A}: 0.26 nM
(44-fold selective vs. A_1, functional assays)



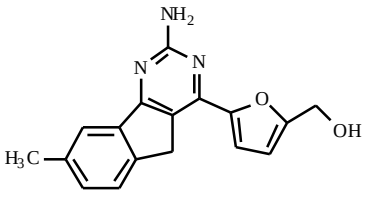
74

hA_{2A}: 0.05 nM
(7-fold selective vs. A_1, functional assays)



75

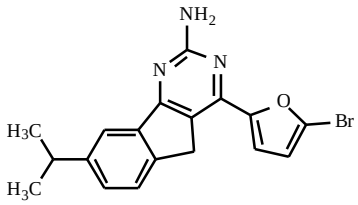
hA_{2A}: 9.89 nM
(46-fold selective vs. A_1, functional assays)



76

hA_{2A}: 2.6 nM
hA_1: 245 nM

Schering-Plough



77

hA_{2A}: 0.8 nM
hA_1: 82.4 nM

Fig. (18). Arylindenopyrimidines and Arylindenopyridines.

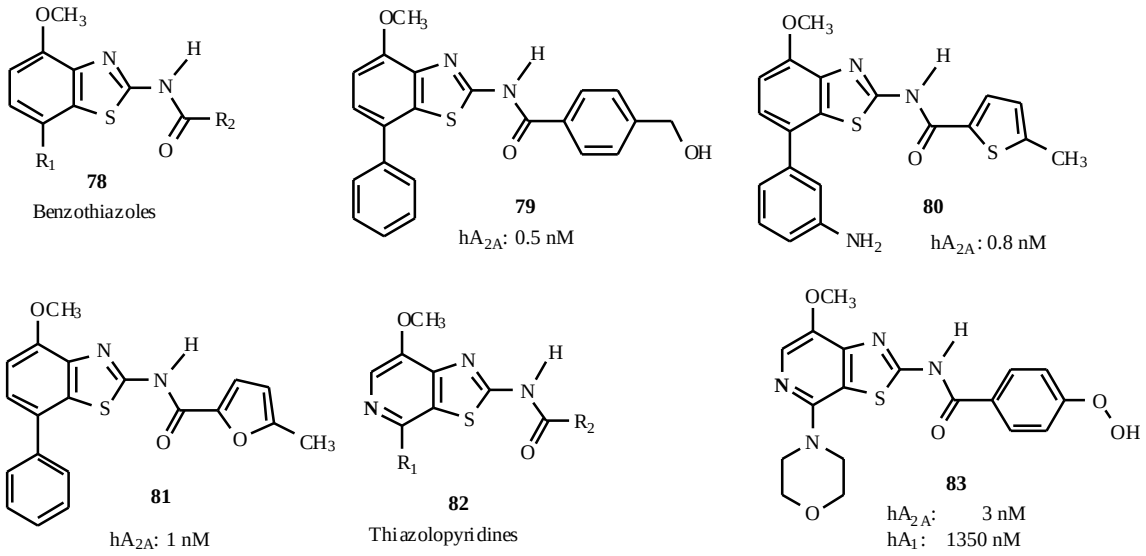


Fig. (19). Benzothiazole and thiazolopyridine derivatives.

potent, A_{2A}-selective example from this class of compounds is derivative **83** [185].

Triazoles

Another new structural class discovered by a high throughput screening approach by Hoffmann-La Roche is simple, phenyl- and benzyl-substituted triazoles (**84** and **85**, Fig. **20**) [186]. The *m*-methoxy substitution of the phenyl ring proved to be important. Various lipophilic substituents at the benzyl group were tolerated. The compounds showed satisfactory solubility (2-20 μg/mL) and compound **84** exhibited good permeability tested with artificial membranes [186].

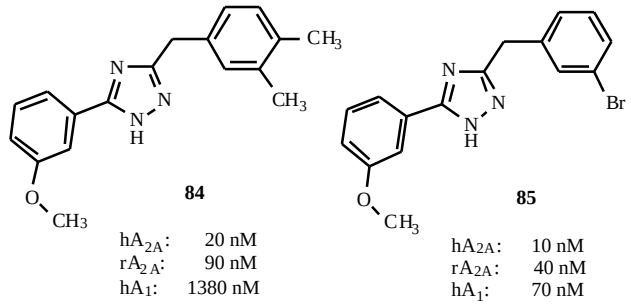


Fig. (20). Triazole derivatives.

Various structures

Two patents are claiming A_{2A} receptor antagonists with selectivity for G_{olf}-coupled A_{2A} receptors in the striatum versus G_s-coupled receptors found in the periphery [187, 188]. Two of the best compounds exhibiting novel structures for A_{2A} receptor antagonists, the triazoloquinazoline **86** and the alloxazine derivative **87** are shown in Fig. **21**. The selectivity for pig striatal A_{2A} receptors versus A_{2A} receptors

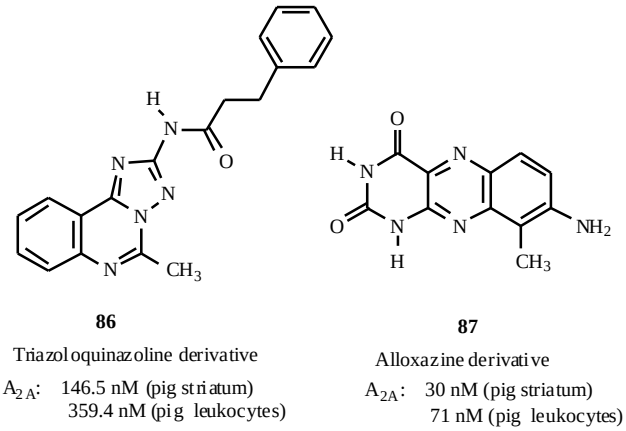


Fig. (21). Various patented structures.

on pig blood leukocytes was moderate. It is not clear whether it is really due to allosteric effects by the coupling G protein or perhaps because of the presence of different receptor homomers or heteromers in striatum (A_{2A}-D_2 or A_{2A}-A_1 receptor heteromers) and leukocytes (A_{2A} homodimer?), which has recently been postulated to result in differences in affinity for caffeine [17].

Comparison of structures and receptor binding model

Many of the potent, A_{2A}-selective adenosine receptor antagonists resemble the physiological agonist adenosine [133]. The N^6-amino group and the N7 nitrogen atom in adenosine derivatives form an important hydrogen bond donor - acceptor motif. The ribose is important for agonistic activity. 5´-Carboxyaminoethyl modification, as in CGS21680 (Fig. **22**), enhances A_{2A} receptor affinity and selectivity. Most A_{2A} receptor-selective agonists are adenosine derivatives bearing a bulky substituent in the 2-position, e.g. CGS21680 (Fig. **22**). Related A_{2A} receptor-selective antagonists similarly feature a hydrogen bond

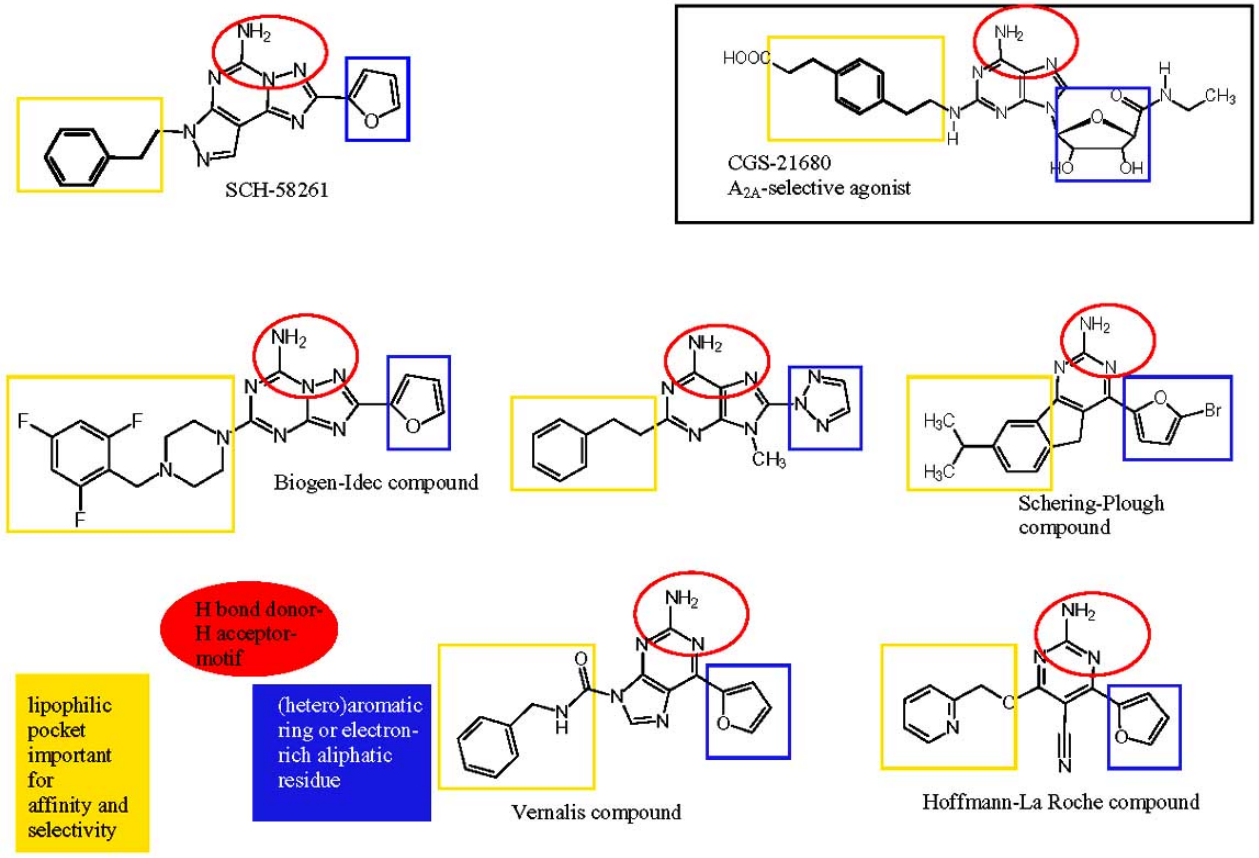


Fig. (22). Pharmacophore model for adenosine A_{2A} receptor antagonists structurally related to adenine.

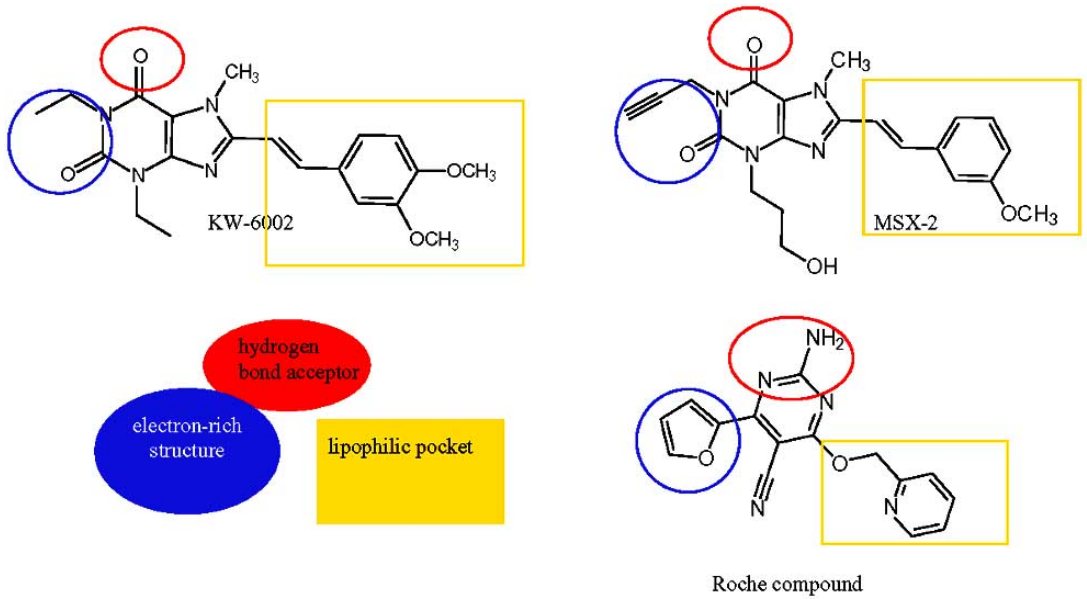


Fig. (23). Pharmacophore model for A_{2A} receptor-selective xanthine derivatives and comparison to pharmacophore model of amino-substituted nitrogen-containing heterocyclic compounds.

donor-acceptor motif (Fig. **22**) and a bulky substituent pointing in the same direction as the 2-substituent in adenosine derivatives such as CGS21680 (Fig. **22**). Instead of the ribose moiety of agonists, antagonists are substituted by a (hetero)aromatic ring or an electron-rich acyclic structure. A 2-furyl residue has been found to be optimal in most of the adenine-like compounds. However, the furan derivatives may be toxic to the liver since they can be oxidized via epoxides to the corresponding aldehydes by cytochrome P450 enzymes. The aldehydes may further react non-enzymatically with glutathion and thiol groups of proteins [189].

A_{2A} receptor selective xanthine derivatives are lacking the typical hydrogen bond donor - acceptor motif, they have only a hydrogen bond acceptor, but no donor. Fig. **23** shows a comparison of the pharmacophores of xanthines and of typical adenine-based antagonists. The styryl residue in xanthines will fill the lipophilic pocket corresponding to the 2-substituent of adenosine derivatives, e.g. CGS-21680, and related antagonists. The 2-oxo group and the N1-substituent in xanthines are close to the ribose moiety in adenosine derivatives and to the 2-furyl residue found in many antagonists. Electron-rich and hydrogen bond accepting groups are required here. Kim *et al*. [190] proposed a binding site for agonists and antagonists at A_{2A} receptors by homology modeling using the structure of bovine rhodopsin as a template and by evaluation of available mutation data of the A_{2A} receptor. They confirmed that there is considerable overlap in the binding sites of adenosine derivatives and antagonists.

CURRENT & FUTURE DEVELOPMENTS

Numerous *in vitro* and *in vivo* studies in various animal models have demonstrated the potential usefulness of A_{2A} receptor antagonists for the treatment of Parkinson's disease. The first clinical studies have confirmed this at least for combination therapy with L-DOPA. Antagonists with high A_{2A} receptor affinity and selectivity have been developed from various chemical classes of compounds, including xanthines, adenines and other amino-substituted heterocyclic compounds. Novel structures include benzothiazole and thiazolopyridine derivatives. Many A_{2A} receptor antagonists that showed high affinity and selectivity *in vitro* were inactive *in vivo*, e.g. due to lacking brain penetration or other inappropriate pharmacokinetic properties. A large number of the developed compounds feature potentially problematic structures (e.g. (*E*)-styryl residue in many xanthines including istradefylline, which may undergo light-induced or enzymatic (*E/Z*)-isomerization in solution, or light-induced solid-state dimerization; furan ring in many non-xanthine antagonists including SCH-420814, which may be metabolized to a reactive epoxide).

Potent A_{2A} antagonists are generally lacking good water-solubility; therefore they are not well suitable for parenteral application. A water-soluble prodrug (MSX-3) of a potent A_{2A}-selective adenosine receptor antagonist (MSX-2, a styrylxanthine derivative) has become a very useful pharmacological tool for studying A_{2A} receptors and their role *in vivo*, e.g. in animal models of Parkinson's disease, allowing systemic (e.g. i.p.) as well as local (e.g. intra-striatal) application.

The first drug that has been used in humans, the styryl-xanthine istradefylline, is expected to be marketed within the next couple of years. Further A_{2A}-selective antagonists, the non-xanthines SCH-420814 and BIIB014/V2006, are currently in clinical trials in patients with Parkinson's disease and initial results have been promising. In recent years, many pharmaceutical companies have started programs to develop A_{2A} antagonists for the therapy of Parkinson's disease and for other indications, such as neurodegenerative diseases in general, depression, and restless legs syndrome. Adenosine A_{2A} receptor antagonists are promising novel drugs.

ACKNOWLEDGEMENTS

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