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REVIEW

Progress in the discovery of selective, high affinity A_{2B} adenosine receptor antagonists as clinical candidates

Rao V. Kalla · Jeff Zablocki

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Abstract The selective, high affinity A_{2B} adenosine receptor (AdoR) antagonists that were synthesized by several research groups should aid in determining the role of the A_{2B} AdoR in inflammatory diseases like asthma or rheumatoid arthritis (RA) and angiogenic diseases like diabetic retinopathy or cancer. CV Therapeutics scientists discovered the selective, high affinity A_{2B} AdoR antagonist **10**, a 8-(4-pyrazolyl)-xanthine derivative [CVT-6883, $K_i(hA_{2B})$=22 nM; $K_i(hA_1)$=1,940 nM; $K_i(hA_{2A})$=3,280; and $K_i(hA_3)$=1,070 nM] that has favorable pharmacokinetic (PK) properties ($t_{1/2}$=4 h and F>35% rat). Compound **10** demonstrated functional antagonism at the A_{2B} AdoR (K_B= 6 nM) and efficacy in a mouse model of asthma. In two phase 1 clinical trials, CVT-6883 was found to be safe, well tolerated, and suitable for once daily dosing. A second compound **20**, 8-(5-pyrazolyl)-xanthine, has been nominated for development from Baraldi's group in conjunction with King Pharmaceuticals that has favorable A_{2B} AdoR affinity and selectivity [$K_i(hA_{2B})$=5.5 nM; $K_i(hA_1)$ > 1,000 nM; $K_i(hA_{2A})$ >1,000; and $K_i(hA_3)$ >1,000 nM], and it has been demonstrated to be a functional antagonist. A third compound **32**, a 2-aminopyrimidine, from the Almirall group has high A_{2B} AdoR affinity and selectivity [$K_i(hA_{2B})$=17 nM; $K_i(hA_1)$ >1,000 nM; $K_i(hA_{2A})$ >2,500; and $K_i(hA_3)$ >1,000 nM], and **32** has been moved into preclinical safety testing. Since three highly selective, high affinity A_{2B} AdoR antagonists have been nominated for development with **10** (CVT-6883) being the furthest along in the development process, the role of the A_{2B} AdoR in various disease states will soon be established.

Keywords A_{2B} · A_{2B} antagonist · A_{2B} receptor · Asthma · CVT-6883 · MRE-2029-F20 · LAS38096 · OSIP339391

Abbreviations

NECA	5′-*N*-ethylcarboxamidoadenosine
HEK	human embryonic kidney cells
cAMP	cyclic adenosine monophosphate
SAR	structure-activity relationship
dAUC	dose-adjusted area under curve
CHO	Chinese hamster ovary
BSMCs	bronchial smooth muscle cells
IL-6	interleukin-6
MCP-1	monocyte chemotactic protein-1
HLFs	human lung fibroblasts
OPN	osteopontin
MMPs	matrix metalloproteases

Introduction

The need for a selective, high affinity A_{2B} adenosine receptor (AdoR) antagonist, to fully establish the therapeutic potential of this class of agents as anti-inflammatory and antiangiogenic agents, has attracted the interest of several medicinal chemistry groups around the world [1–11]. The structural approach taken by these groups can be divided into two classes of compounds, xanthines and non-xanthine derivatives. The xanthine derivatives caffeine and theophylline are considered classic nonselective antagonists for adenosine receptors (Fig. 1). Theophylline **1**, which has 9 μM affinity for the A_{2B} AdoR, displays no selectivity

R. V. Kalla (✉) · J. Zablocki (✉)
Department of Bioorganic Chemistry, CV Therapeutics Inc.,
3172 Porter Drive,
Palo Alto, CA 94304, USA
e-mail: rao.kalla@cvt.com
e-mail: jeff.zablocki@cvt.com

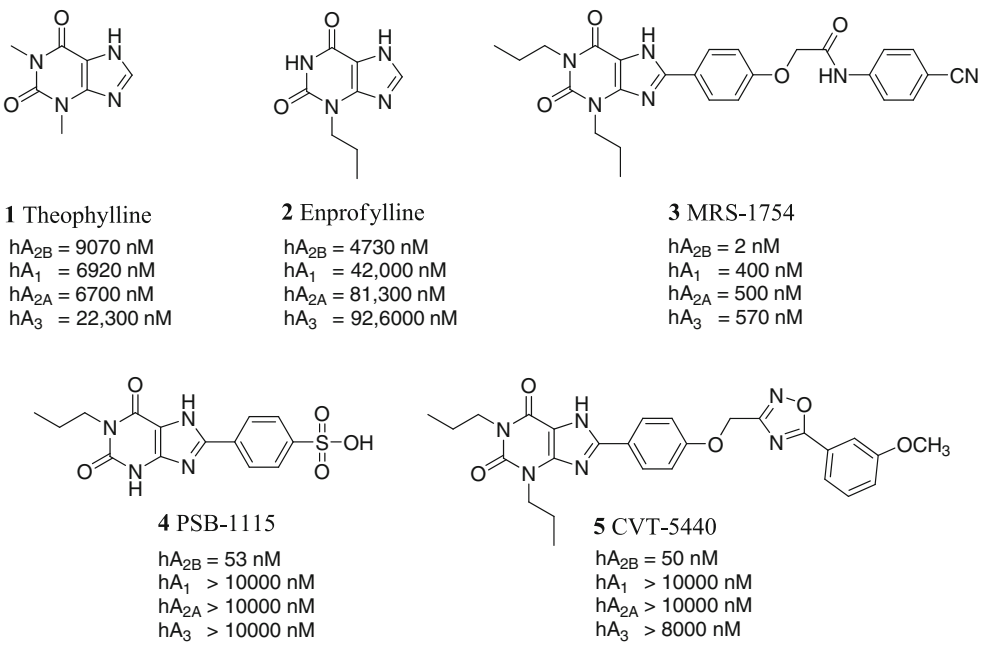

Fig. 1 Classic and prototypical xanthine-derived A_{2B} receptor antagonists

against the other AdoRs [12]. Enprofylline **2**, a 3-propyl xanthine derivative has moderate A_{2B} affinity and low selectivity over the other AdoRs. Following further structural exploration of the xanthine moiety by several groups, the discovery of 8-phenylxanthines as selective A_{2B} AdoR antagonists was made [13–15]. Among these 8-phenylxanthine derivatives, *p*-cyanoanilide **3** (MRS-1754) of Jacobson et al. [16] and a negatively charged compound **4** (PSB-1115) of Muller et al. [17] stand out as selective A_{2B} AdoR antagonists. To address the metabolic stability of compound **3** in human liver microsomal enzymes, Zablocki et al. [18] synthesized compound **5** (CVT-5440) that contains a bioisostere of the metabolically labile amide group present in **3**. Compound **5** demonstrated good affinity for the A_{2B} AdoR and selectivity over the other AdoRs. Improved in vitro metabolic stability was also observed in **5** compared to **3**, but **5** still has a very low systemic exposure in rats when dosed orally, presumably due to low solubility.

Xanthines

CV Therapeutics (CVT) chemists started with these initial leads in their search for the discovery of a selective, high affinity A_{2B} AdoR antagonist with good pharmaceutical properties [19, 20]. Kalla et al. [21] have explored various heterocycles as bioisosteric replacements for the phenyl group at the 8-position of xanthine and discovered that the 8-(pyrazol-4-yl)xanthines display good A_{2B} AdoR affinity (Fig. 2). The prototypical compound 1,3-dipropyl-8-(1*H*-pyrazol-4-yl)xanthine **6** (CVT-5450) has high A_{2B} AdoR affinity (9 nM), but displayed very low selectivity. Following oral dosing in rats, **6** displayed very high levels of systemic exposure; this encouraged CVT chemists to probe the 8-(pyrazol-4-yl)xanthine ligand to increase the selectivity [8]. Benzyl substitution on the pyrazole ring increased the selectivity compared to the phenyl, phenethyl, and phenpropyl derivatives. Optimization of the phenyl ring substitution suggested that the electron withdrawing groups F and CF_3 at the *meta*-position increased selectivity toward the A_{2B} AdoR. Compound **7**, 1,3-dipropyl-8-(1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)xanthine that has 3-CF_3 benzyl substitution on the pyrazole ring, displayed better selectivity compared to the unsubstituted derivative **6**. Replacing the 1,3-dipropyl groups of the xanthine core with various alkyl groups like methyl, ethyl, butyl, and isobutyl groups suggested that smaller alkyl groups relative to propyl increase the A_{2B} AdoR affinity and selectivity compared to the large groups. Compound 1,3-dimethyl-8-(1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-xanthine **8** (CVT-6975) has very high A_{2B} AdoR affinity and selectivity [21]. This observation prompted further investigation of the differential alkyl substitution at N-1 and N-3 positions [22]. Compound **9** displayed better affinity and selectivity compared to the dipropyl derivative **7**, but has weaker affinity and selectivity compared to the dimethyl derivative **8**. The 3-ethyl-1-propyl-8-(1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-xanthine **10** (CVT-6883) has very good A_{2B} AdoR affinity, and also it displayed good selectivity over other AdoR subtypes [22].

Investigation of the monosubstitution at the N-1 position of the 8-pyrazolyl xanthine delivered a very high affinity and selective A_{2B} AdoR antagonists [23]. For example, the 1-propyl-8-(1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-xanthine **11** (CVT-7124) displays high A_{2B} AdoR affinity (6 nM) and very good selectivity. This further

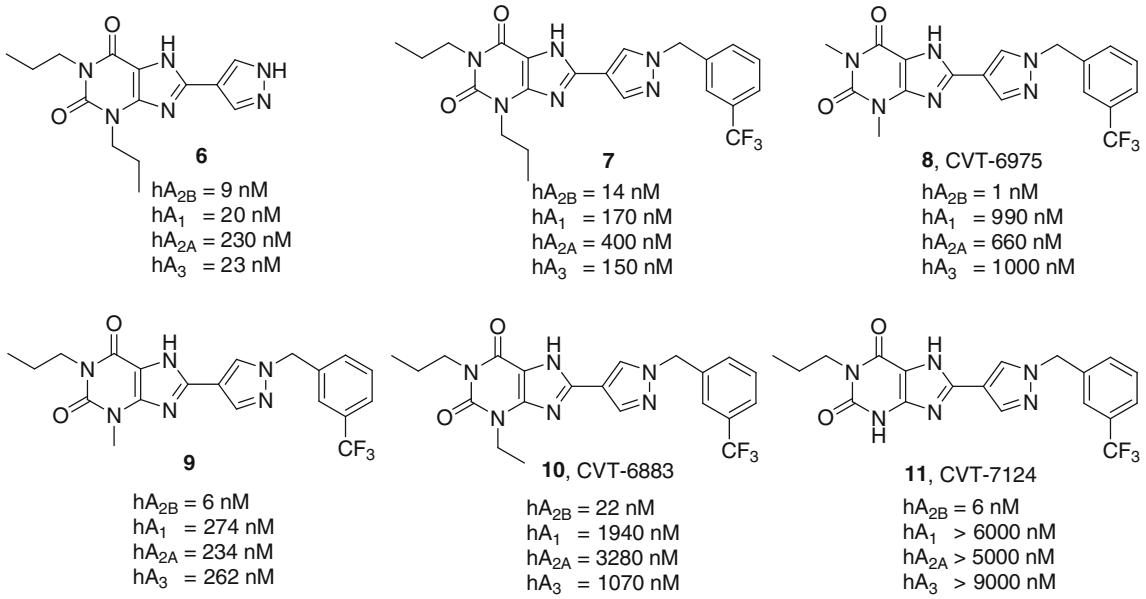

Fig. 2 CVT A_{2B} adenosine receptor antagonists—8-(pyrazol-4-yl) xanthines

supports the Hayallah et al. observation in the 8-phenyl xanthine series of compounds, that the monosubstitution at the N-1 position of the xanthine core enhances the A_{2B} AdoR selectivity [17].

Elzein et al. replaced the phenyl group of **7** with different heterocycles including 3-phenyl-1,2,4-oxadiazoles, 5-phenyl-1,2,4-oxadiazoles and 3-phenyl-isoxazoles as these groups in the 8-phenyl xanthine series [18] improved the selectivity for the A_{2B} AdoR receptor (Fig. 3) [24]. In this series, all the compounds display very good selectivity regardless of the substitutions at the N-1 and N-3 positions of the xanthine core. The 1,3-dipropyl analogue 8-(1-((5-(4-chlorophenyl)-1,2,4-oxadiazol-3-yl) methyl)-1*H*-pyrazol-4-yl)-xanthine **12** and N-1 propyl, N-3 ethyl analogues 3-ethyl-1-propyl-8-(1-((5-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-3-yl)methyl)-xanthine **13** and 3-ethyl-1-propyl-8-(1-((5-(4-(trifluoromethyl)phenyl) isoxazol-3-yl)methyl)-1*H*-pyrazol-4-yl)-xanthine **14** display high affinity and selectivity for the A_{2B} AdoR. Similar to the phenyl series of compounds, the N-1 monosubstituted oxadiazole and isoxazole derivatives of 8-pyrazolyl xanthines displayed high affinity and selectivity for the A_{2B} AdoR. The N-1 propyl derivative **15** (CVT-6694) has a very high A_{2B} affinity (7 nM) and very weak affinity for the A_1, A_{2A}, and A_3 AdoRs [23]. The cyclopropyl methyl analogues **16** and **17** also displayed high affinity and selectivity for the A_{2B} AdoR.

In summary, CVT chemists discovered several high affinity and selective A_{2B} AdoR antagonists. The pharmacophore, 8-(pyrazol-4-yl)xanthine, identified by the CVT chemists can provide selective A_{2B} AdoR antagonists depending on the substitution pattern. From the above compounds, two selective antagonists **10** (CVT-6883) and **15** (CVT-6694) were chosen for further evaluation of the pharmacological and pharmaceutical properties. Compound **10** antagonized the 5′-*N*-ethylcarboxamidoadenosine (NECA)-induced cyclic adenosine monophosphate (cAMP) accumulation in human embryonic kidney (HEK)-A_{2B} cells and NIH 3T3 cells, and compound **15** completely abolished the NECA-induced cAMP accumulation in bronchial smooth muscle cells (BSMCs) [25] proving that these compounds are functioning as antagonists for the hA_{2B} AdoR. Compound **10**, when dosed orally in rats at 2 mg/kg, displayed excellent systemic exposure with a C_{max} 1,100 ng/ml and dose-adjusted area under curve (dAUC) 6,500 ng.h/ml [22] with a long half-life of 4 h (IV dosing, rat). When dosed orally in rats compound **15** exhibited very low systemic exposure. Therefore, compound **10** was selected as a lead molecule and moved into CVT's development program.

Baraldi's group evaluated a series of 8-heterocyclic substituted xanthines as antagonists for the A_{2B} AdoR [26]. Of these derivatives, 8-(pyrazol-5-yl)xanthine derivatives displayed high affinity and selectivity for the A_{2B} AdoR (Fig. 4). These 5-pyrazolyl derivatives **18** and **19** showed good affinity for the A_{2B} AdoR and selectivity over other AdoR subtypes [27]. Both compounds block NECA-induced cAMP accumulation with IC_{50} values in the nanomolar range. Further exploration of the 5-pyrazolyl class resulted in a lead compound **20** (MRE-2029-F20) that has high affinity and selectivity for the A_{2B} AdoR. The tritium-labeled derivative **21** ([^{3}H]MRE-2029-F20) displayed a K_D value of 1.65±0.10 nM in Chinese hamster ovary (CHO) cells expressing hA_{2B} receptors, and

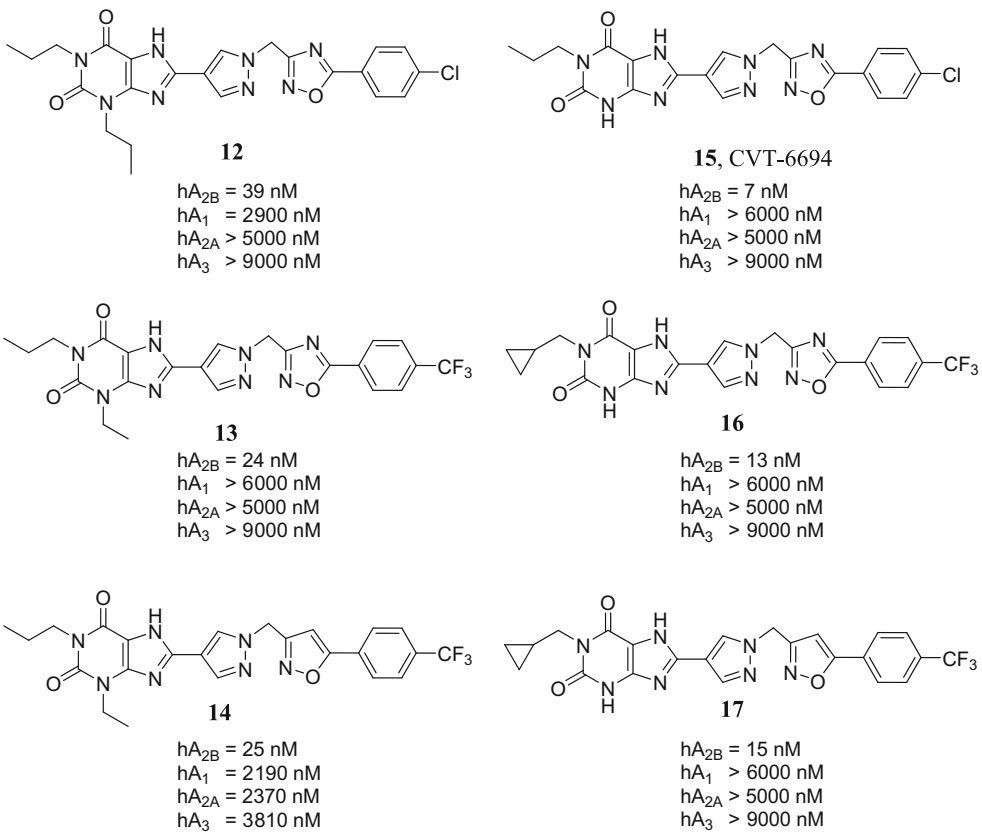

Fig. 3 CVT A_{2B} adenosine receptor antagonists—8-(pyrazol-4-yl)xanthines

it can be useful as a pharmacological tool in binding studies [28].

In recent patent applications, Adenosine Therapeutics described a series of 8-pyridyl substituted xanthines as A_{2B} AdoR antagonists (Fig. 5) [29]. The 8-pyridyl was further extended by substitution with heteroaryl (**23** and **25**), heterocyclyl (**22**), or alaninol (**24**) groups. According to the patent applications, some of these derivatives (**22**–**25**) have an A_{2B} AdoR affinity of <100 nM, but no selectivity data were given, so it is hard to completely evaluate the series.

9-Deazaxanthines

9-Deazaxanthines (pyrrolo[2,3-*d*]pyrimidinones) were initially explored by Grahner et al. as antagonists for the A_1 and A_2 AdoRs (Fig. 6) [30]. In most cases, the authors observed that the structure-activity relationships (SAR) of 9-deazaxanthines are parallel to those of xanthine derivatives and also noticed an increased selectivity over A_1 AdoR. The authors concluded that the xanthines and 9-deazaxanthines bind in the same mode to the adenosine receptors, and thus, the similar SAR. Hayallah et al. have investigated the N-1 monosubstituted 9-deazaxanthines, because the corresponding xanthines generally exhibit high A_{2B} AdoR selectivity [17]. The compound 6-phenyl-3-propyl-1*H*-pyrrolo[3,2-*d*]pyrimidine-2,4(3*H*,5*H*)-dione **26** has displayed good A_{2B} AdoR affinity, but it did not exhibit good selectivity over the A_1 AdoR as expected. Vidal et al. have synthesized 8-phenyl-9-deazaxanthines that have a sulfonamide linker at the *para*-position of the phenyl group, and many compounds exhibited good A_{2B} AdoR affinity [31]. For instance, **27** of the above series displayed 6 nM affinity for the A_{2B} AdoR and displayed good selectivity. In a recent publication, Carotti et al. presented several 9-deazaxanthines that have piperidine amides and piperazine amide substitution at the *para*-position of the 8-phenyl group [32]. Representatives from these classes, compounds **28** and **29** (Fig. 6), respectively, displayed both high affinity and selectivity for the A_{2B} AdoR. CVT chemists have explored the 8-pyrazolyl-9-deazaxanthines as A_{2B} AdoR antagonists [33]. The *m*-F benzyl derivative **30**, 6-(1-(3-fluorobenzyl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-pyrrolo[3,2-*d*]pyrimidine-2,4(3*H*,5*H*)-dione, has good A_{2B} AdoR affinity, but it did not offer good selectivity over other the AdoR subtypes. The corresponding *m*-CF_3 benzyl derivative **31** displayed lower affinity for the A_{2B} receptor than **30**, but it exhibited good selectivity for the A_{2B} AdoR. Overall the 9-deazaxanthines

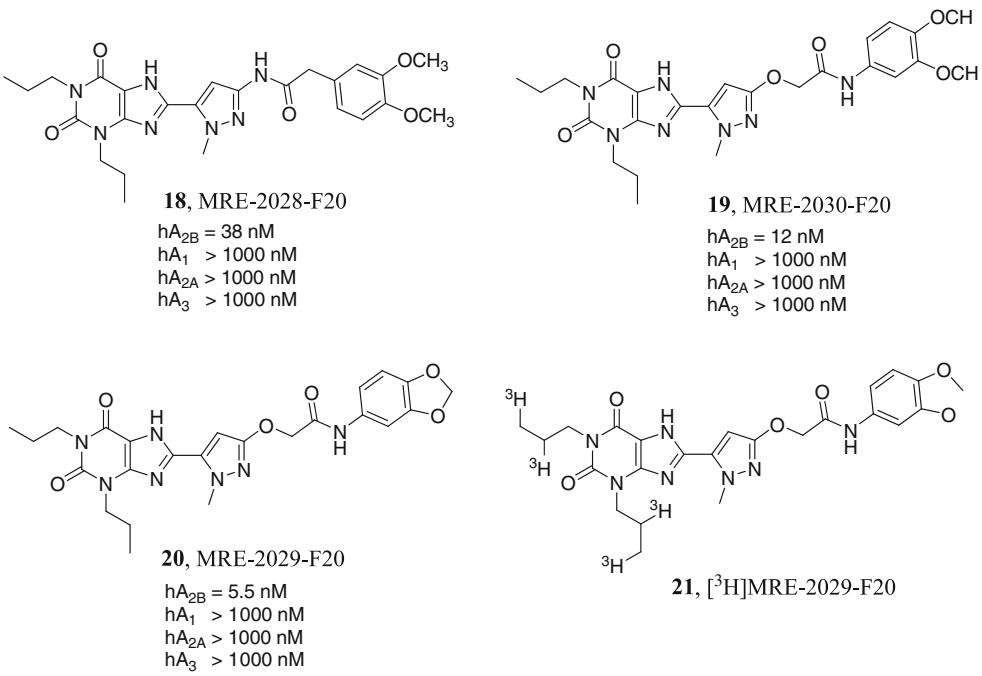

Fig. 4 Baraldi's A_{2B} adenosine receptor antagonists

afforded similar SAR to the parent xanthines with respect to A_{2B} AdoR affinity and, in most cases, higher selectivity.

Non-xanthine analogues

Two series of compounds, 2-aminopyridines and 2-aminopyrimidines, were published as A_{2B} AdoR antagonists in patent applications from Almirall Prodesfarma (Fig. 7) [34, 35]. From these series of compounds, Vidal et al. recently published on the common core and substituents, namely, *N*-heteroaryl 4′-furyl-4,5′-bipyrimidin-2′-amines, as high affinity and selective A_{2B} AdoR antagonists [36]. For example, the 2′-amino(3-pyridyl) derivative **32** (LAS38096) has a A_{2B} affinity of 17 nM and has very good selectivity. Similar analogues, 2′-amino(5-pyrimidinyl) derivative **33** and 2′-amino(6-oxo-1,6-dihydropyridin-3-yl) derivative **34**, displayed good A_{2B} affinity of 24 and 16 nM, respectively, and both compounds have very good A_{2B} AdoR selectivity as well. Compound **32** inhibited the NECA-induced cAMP levels in HEK-293 expressing human A_{2B} AdoR and CHO cells transfected with mouse A_{2B} AdoR with IC_{50}s of 321 nM and 349 nM, respectively. Following oral dosing in rats (10 mg/kg), compound **32** displayed good systemic exposure with a C_{max} of 11 μM and an AUC of 16 μM/h. It also displayed good exposure following oral dosing in mouse and dogs. Based on its in vitro pharmacology and pharmacokinetic profile, **32** was moved into preclinical development.

Adenine derivatives have been explored as adenosine receptor antagonists by several research groups (Fig. 8) [37, 38]. Cristalli and coworkers reported a series of 2-substituted 9-alkyl derivatives as selective A_{2B} receptor antagonists (not shown) [39]. Harada et al. at Eisai explored

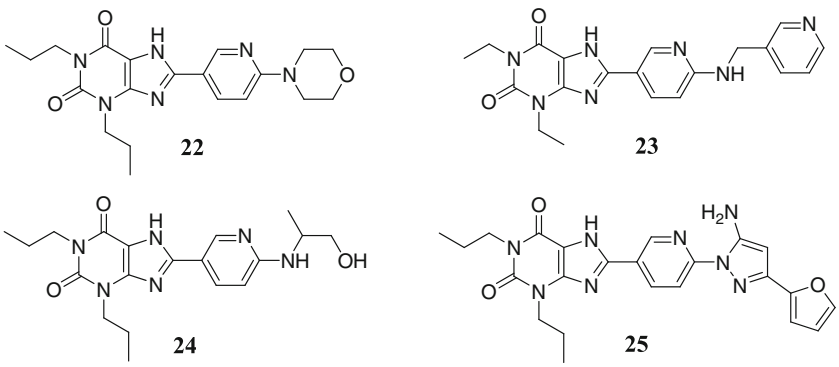

Fig. 5 Adenosine Therapeutics A_{2B} adenosine receptor antagonists

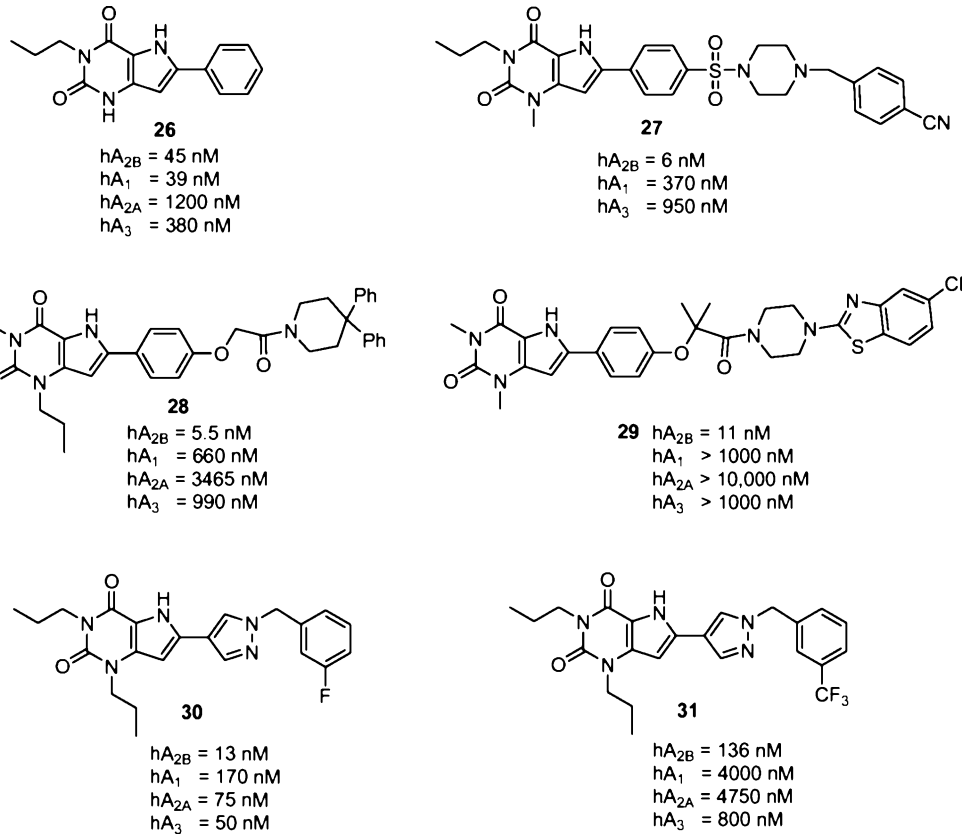

Fig. 6 Deazaxanthines as A_{2B} antagonists

the 2-alkynyl-8-aryl-9-methyl adenine derivatives as A_{2B} AdoR antagonists [40]. Of these derivatives, compound **35** with a 3-F phenyl substitution at the 8-position displayed good A_{2B} affinity, but no binding selectivity over other AdoR subtypes (Fig. 8). Substituting the 3-F phenyl of **35** with a 2-furyl group provided compound **36** with good A_{2B} affinity, but again with no selectivity. Further optimization of the 9-position of the adenine derivative **35** led to the 3-benzamide derivative **37** with excellent A_{2B} affinity [41]. Compound **37** displayed good selectivity over the A_1 AdoR subtype only. These analogues inhibited NECA-induced cAMP production in CHO K1 cells expressing the human A_{2B} AdoR demonstrating that these compounds are antagonists. Further optimization of the SAR may lead to selective A_{2B} antagonists in the adenine series.

In recent publications, scientists at OSI Pharmaceuticals have shown that 2-phenyl-7-deazaadenines (pyrrolopyrimidines) display good A_{2B} AdoR affinity (Fig. 8) [42]. A lead compound **38** in the pyrrolopyrimidine series demonstrated excellent A_{2B} AdoR affinity and promising selectivity. A tritium-labeled analogue **39** ([^{3}H]OSIP-339391) of **38** was synthesized, which displayed a K_D value of 0.41±0.06 nM for binding to human A_{2B} AdoR expressed in HEK-293 cells. This represents a selective and high affinity radioligand that can be a useful tool in further characterization of the pharmacology of the A_{2B} AdoR.

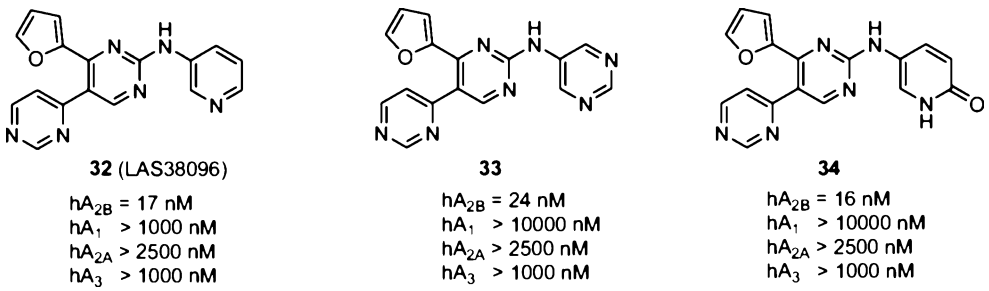

Fig. 7 Almirall A_{2B} antagonists

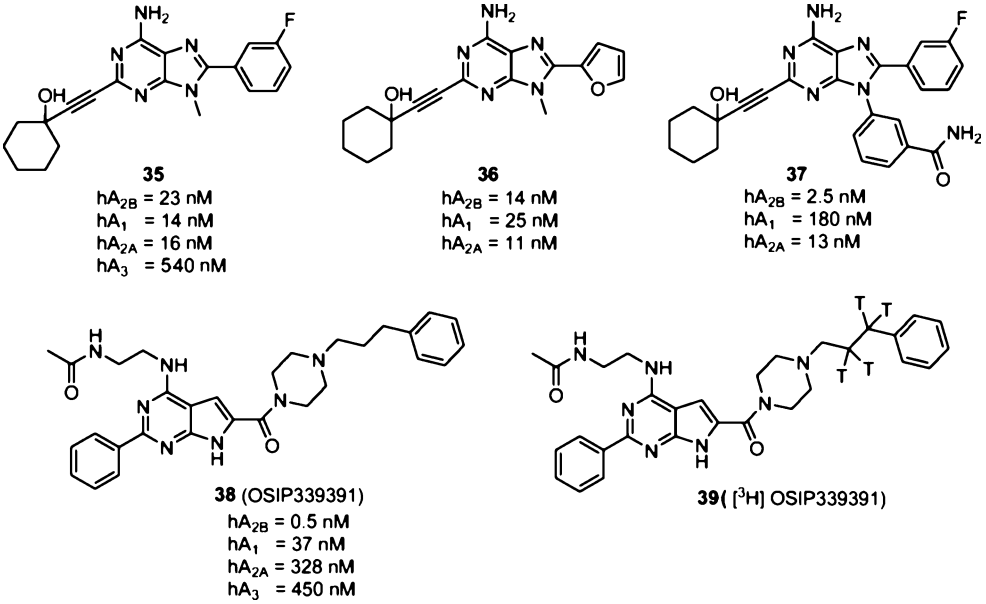

Fig. 8 Purines and 7-deazapurines as A_{2B} antagonists

Pharmacology discussion

Since the goal of obtaining a high affinity and selective A_{2B} antagonist has been achieved by several research groups, the agents obtained have been used to establish the anti-inflammatory properties in both in vitro cellular studies and in asthma models. CVT chemists have synthesized several A_{2B}-selective antagonists including **15** (CVT-6694) and **10** (CVT-6883). Following stimulation with a nonselective agonist NECA, compound **15** attenuated the increased production of both interleukin (IL)-6 and monocyte chemotactic protein-1 (MCP-1) in bronchoalveolar lavage smooth muscle cells [25]. These experiments suggest a novel mechanism whereby adenosine acts as a proinflammatory mediator in the bronchiole airways. Similarly, A_{2B} AdoR subtype is the predominant AdoR expressed in human lung fibroblasts (HLFs), which on activation by NECA increases the release of IL-6 in a concentration-dependent manner and induces the differentiation of fibroblast into myofibroblasts [43]. Synergy exists between hypoxia and NECA activation of the A_{2B} AdoR in HLFs, thus resulting in a pronounced increase in the release of IL-6. The A_{2B} antagonist **15** completely abolished the augmented effect of NECA on the IL-6 release; however, it as expected did not affect the hypoxia-induced release of IL-6 [44]. In a mouse asthma model (ragweed challenge), compound **10** (dose: 1 mg/kg IP, 14-day treatment) was as effective as montelukast in reducing AMP-induced airway reactivity [48]. Compound **10** reduced significantly bleomycin (3.0 U/kg)-induced pulmonary fibrosis and inflammation in mice [47]. Furthermore, **10** (dose: 1 mg/kg IP b.i.d.) relative to vehicle controls reduced lung fibrosis and levels of macrophage-derived mediators of lung remodeling [IL-6, osteopontin (OPN), transforming growth factor (TGF)-β1, and matrix metalloproteases (MMPs)] in adenosine deaminase-deficient (ADA -/-) mice [47].

The selective A_{2B} AdoR antagonist, MRE-2029-F20 synthesized by Baraldi's group, shows the inhibition of cAMP levels in neutrophils, lymphocytes, and HMC1 cells that naturally express the A_{2B} AdoR that may play a role in inflammatory diseases [45]. The selective A_{2B} AdoR antagonist **32** (LAS38096) synthesized by Almirall has been shown to inhibit the NECA-induced production of IL-6 in a dose-dependent manner in both human and mouse fibroblasts [36]. This further confirms the anti-inflammatory properties of A_{2B} AdoR antagonists.

Conclusion

Compound **10** (CVT-6883), a potent selective, orally available, and potentially first in class A_{2B} AdoR antagonist, has been entered into clinical trials by CV Therapeutics [46]. The data from two phase 1 clinical trials, a single ascending dose study in 24 healthy volunteers and a multiple ascending dose study in 30 volunteers, demonstrated that CVT-6883 was safe and well tolerated with no serious adverse events reported. Furthermore, the pharmacokinetic results indicated the suitability of CVT-6883 for once daily chronic dosing. The potential utility of CVT-6883 is in several disease areas including asthma, chronic obstructive pulmonary disease, and pulmonary fibrosis [47, 48].

The discovery of three selective, high affinity A_{2B} AdoR antagonists (**10**, **20**, and **32**) should aid in determining the pharmacological role of the A_{2B} AdoR in various disease states in animal models and in clinical trials.

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