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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 302–306

Novel 1,3-dipropyl-8-(1-heteroarylmethyl-1*H*-pyrazol-4-yl)-xanthine derivatives as high affinity and selective A_{2B} adenosine receptor antagonists

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Received 9 September 2005; revised 29 September 2005; accepted 3 October 2005
Available online 4 November 2005

Abstract—A series of new 1,3-dipropyl-8-(1-heteroarylmethyl-1*H*-pyrazol-4-yl)-xanthine derivatives as A_{2B}-AdoR antagonists have been synthesized and evaluated for their binding affinities for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs. 8-(1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)-1*H*-pyrazol-4-yl)-1,3-dipropyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**4**) displayed high affinity (K_i = 1 nM) and selectivity for the A_{2B}-AdoR versus A_1, A_{2A}, and A_3-AdoRs (A_1/A_{2B}, A_{2A}/A_{2B}, and A_3/A_{2B} selectivity ratios of 370, 1100, and 480, respectively). The synthesis and SAR of this novel class of compounds are presented herein.

The adenosine receptors are G protein-coupled receptors consisting of four subtypes, A_1, A_{2A}, A_{2B}, and A_3-adenosine receptors.[1] Interaction of adenosine with its receptors initiates signal transduction pathways, including the adenylate cyclase effector system, which utilizes cAMP as a second messenger. While A_1 and A_3 adenosine receptors (A_1-AdoR, A_3-AdoR) coupled with Gi proteins inhibit adenylate cyclase and lead to a decrease in intracellular levels of cAMP, the A_{2A}/A_{2B} adenosine receptor-coupled Gs proteins stimulate adenylate cyclase and hence increase cAMP levels.[2] Studies have demonstrated the synergy between adenosine receptor activation and allergens in inducing mast cell degranulation.[3,4] Studies have also shown that in activated human mast cells the A_{2B}-AdoR plays a major role in the facilitation of allergen-induced release of preformed mediators and cytokines.[5–7] In addition, the selective A_{2B}-AdoR antagonist MRS1754 (**1**) inhibited activation of human mast cells induced by the non-selective AdoR agonist NECA.[3,4,8] Therefore, antagonists at the A_{2B}-AdoR would be expected to provide a novel approach to the management and treatment of asthma by reducing the responsiveness of the airway mast cells to allergen and hence lead to a reduction in airway inflammation and bronchial hyper-responsiveness.

Even though a number of high affinity A_{2B}-AdoR antagonists have been reported, only a few have shown high selectivity for the A_{2B}-AdoR relative to the A_1, A_{2A}, and A_3-AdoRs.[9,10] Our initial efforts to identify high affinity and selective A_{2B}-AdoR antagonists had led to the discovery of compound **2** that possesses a novel N-substituted pyrazole moiety in the 8-position of the xanthine ring (Fig. 1).[11,12] Even though compound **2** showed good affinity and selectivity for the A_{2B}-AdoR (vs. A_{2A} and A_3-AdoRs) and modest selectivity versus the A_1-AdoR, it contains an amide bond that constitutes a metabolic liability.[13] Therefore, we directed our efforts to the optimization of compound **2**, with the goal of improving its A_{2B}-AdoR binding affinity and selectivity as well as enhancing its metabolic stability.

Amide bonds have been replaced with a wide variety of structural moieties in attempts to achieve metabolic

Keywords: Adenosine; Antagonists; Asthma.
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doi:10.1016/j.bmcl.2005.10.002

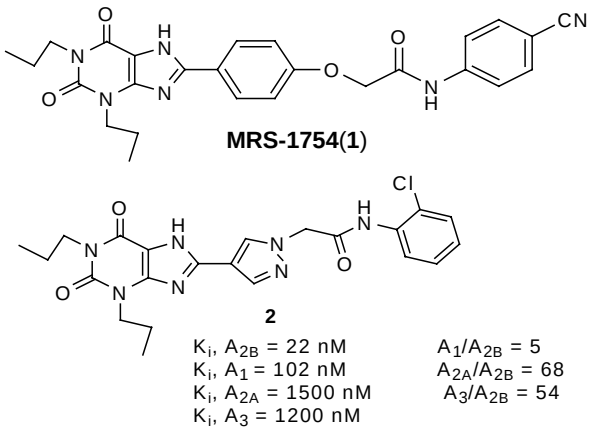


Figure 1. Structures of compounds **1** and **2**; binding affinities and selectivity of compound **2** for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs.

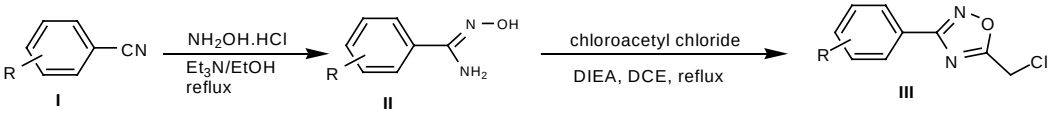
stability and oral bioavailability. Heterocyclic 5-membered rings such as 1,2,4-oxadiazoles, oxazoles, and isoxazoles have been extensively used as amide bond bioisosteres.[14] Accordingly, we replaced the amide bond in compound **2** with different oxadiazoles and isoxazoles.

5-(Chloromethyl)-3-substituted-phenyl-1,2,4-oxadiazole of general formula **III** was prepared as shown in Scheme 1.[13] Treatment of substituted benzonitrile **I** with hydroxylamine hydrochloride afforded the amide oxime **II**, which was then reacted with chloroacetyl chloride in dichloroethane to afford **III**. The oxadiazole regio isomer of **III**, compound **VII**, was prepared by reacting the chloro-oxime amide **V** with a variety of commercially available benzoyl chlorides (Scheme 2).[13]

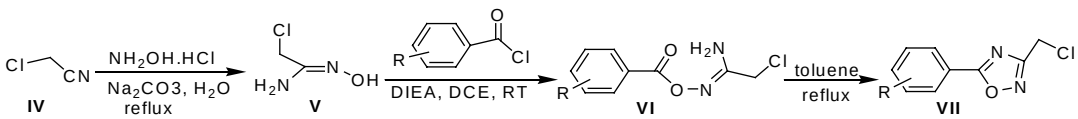
Isoxazoles of general formula **X** were obtained by condensing substituted 2-ethynylbenzene **VIII** with ethylchlorooximidoacetate in THF. The resulting ethyl 5-phenylisoxazole-3-carboxylate **IX** was then reduced using $NaBH_4$/EtOH and the primary alcohol was subsequently converted to the mesylate **X** (Scheme 3). 1-Benzyl-1*H*-pyrazole-4-carboxylic acid **38** was prepared by direct alkylation of commercially available ethyl 1*H*-pyrazole-4-carboxylate **36** with benzyl chloride in acetone using K_2CO_3 as a base followed by ester hydrolysis (Scheme 4). Preparation of the key intermediate 1,3-dipropyl-8-(1-benzyl-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione **41** is outlined in Scheme 5. 5,6-Diamino-1,3-dipropylpyrimidine **39** was synthesized following literature procedure.[15] Coupling of **39** with 1-benzyl-1*H*-pyrazole-4-carboxylic acid **38** using EDCI afforded intermediate **40**, which was cyclized in NaOH to afford compound **41**. Protection of the N-7 of compound **41** with a SEM group followed by debenzylation yielded our key intermediate **42**. Compound **42** was then alkylated with oxadiazoles of general formulas **III** and **VII**, and also with isoxazoles of general formula **X** followed by SEM deprotection to afford our target molecules **3**–**35**.

Binding affinities of compounds **3**–**35** for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs were evaluated (Tables 1–3). As shown in Table 1, replacement of the amide bond in compound **2** with 1,2,4-oxadiazole resulted in compound **3** that displayed similar binding affinity for the A_{2B}-AdoR (K_i = 19 nM) to compound **2**. However, compound **3** showed 26-fold increased selectivity for the A_{2B}-AdoR versus the A_1-AdoR and about 2-fold improved selectivity for the A_{2B}-AdoR versus the A_{2A}-AdoR relative to **2**.

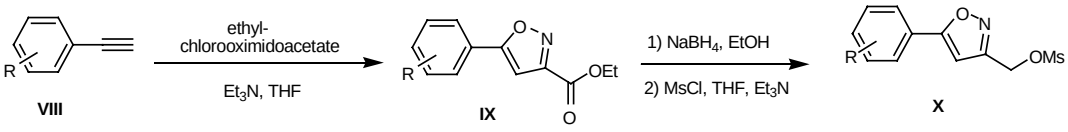
The unsubstituted phenyl analog **4** exhibited very high affinity (K_i = 1 nM) and selectivity for the A_{2B}-AdoR versus A_1, A_{2A}, and A_3-AdoRs (A_1/A_{2B}, A_{2A}/A_{2B}, and A_3/A_{2B} selectivity ratios of 370, 1100, and 480, respectively). In fact, compound **4** was our most active and selective analog among the three classes of compounds we synthesized. While the 4-Cl-phenyl analog **5** had similar binding affinity (K_i = 21 nM) to the 2-Cl analog **3**, it showed less selectivity for the A_{2B}-AdoR versus A_1, A_{2A}, and A_3-AdoRs relative to **3**. Introducing a stronger electron-withdrawing group (CF_3) in place of the 4-Cl group as in compound **6** resulted in 2-fold loss in



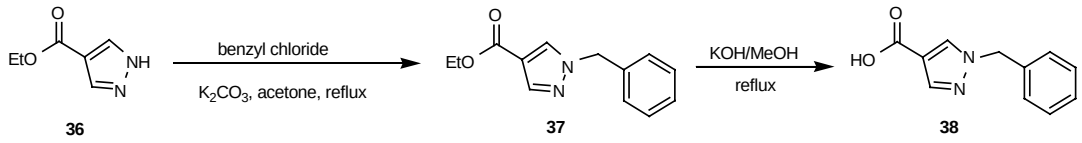
Scheme 1.



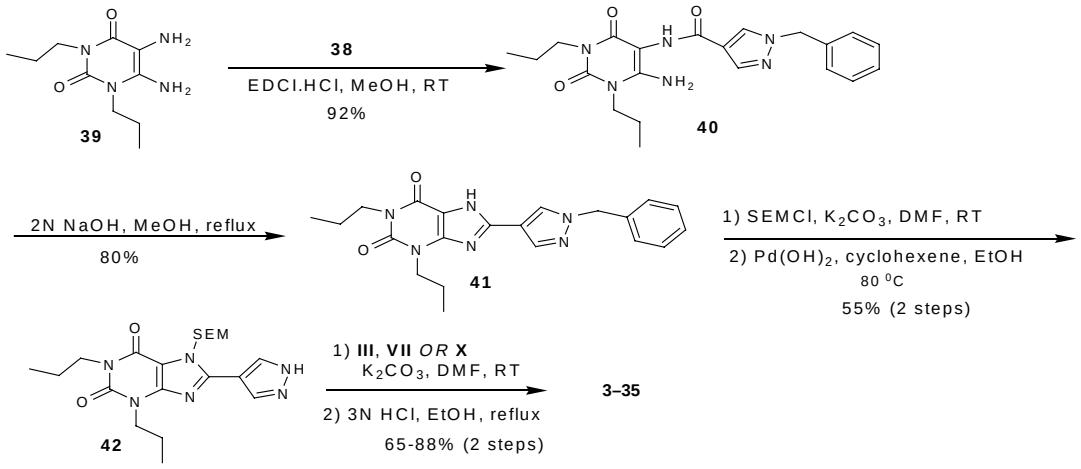
Scheme 2.



Scheme 3.

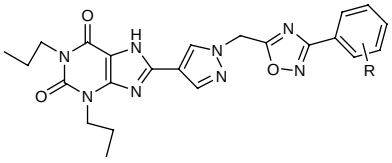


Scheme 4.



Scheme 5.

Table 1. Binding affinities of 3-phenyl-1,2,4-oxadiazole analogs **3**–**15** for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs

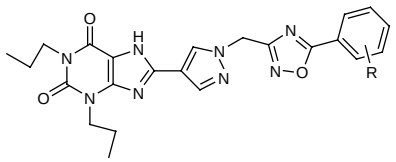


Compound	R	K_i (nM)[a]						
		(A_{2B})[b]	(A_1)[c]	(A_{2A})[d]	(A_3)[e]	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
3	2-Cl	19	2500	2500	490	130	130	25
4	H	1	370	1100	480	370	1100	480
5	4-Cl	21	740	1800	400	35	85	19
6	4-CF_3	55	3300	>5000	1300	60	>90	23
7	4-CN	18	1300	>5000	950	70	>277	53
8	3-Cl	32	652	>5000	490	20	>156	15
9	3-CF_3	42	3500	>5000	960	83	>119	22
10	2-CF_3	74	530	>5000	340	7	>67	4
11	2-OCH_3	82	1900	1600	410	23	19	5
12	4-OCH_3	106	3000	>5000	1500	28	>47	14
13	3-OCH_3	82	2200	>5000	600	26	>60	7
14	4-CH_3	54	2600	3000	8700	48	55	160
15	3-CH_3	128	1400	>5000	970	11	>39	8
2		22	102	1500	1200	5	68	54

[a] 95% confidence limits were generally ±15% of the mean value.
[b] Binding affinity for A_{2B}-AdoR was determined using HEK-A_{2B} cells with [^{3}H]ZM241385 as the radioligand.
[c] Binding affinity for A_1-AdoR was determined using CHO-A_1 cells with [^{3}H]CPX as the radioligand.
[d] Binding affinity for A_{2A}-AdoR was determined using HEK-A_{2A} cells with [^{3}H]ZM241385 as the radioligand.
[e] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]AB-MECA as the radioligand.

binding affinity (**6**, K_i = 55 nM). However, the 4-CN analog **7** showed affinity and selectivity for the A_{2B}-AdoR comparable to those of compound **5**. In general, compounds that incorporated electron-withdrawing groups in the phenyl ring displayed higher affinity and selectivity for the A_{2B}-AdoR relative to those having electron-donating substituents (e.g., **3** vs. **11**, **5** vs. **12**). The high binding affinity and selectivity of compound

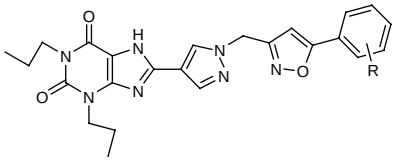
Table 2. Binding affinities of 5-phenyl-1,2,4-oxadiazole analogs **16**–**26** for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs



Compound	R	K_i (nM)[a]						
		(A_{2B})[b]	(A_1)[c]	(A_{2A})[d]	(A_3)[e]	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
16	H	21	1000	1800	630	47	85	30
17	2-Cl	38	630	2500	330	16	65	9
18	4-Cl	39	2900	>5000	>8300	74	>128	>213
19	4-CN	14	570	>5000	640	40	>357	45
20	4-CF_3	21	>6000	>5000	1300	>285	>238	61
21	3-Cl	134	3000	>5000	3000	22	>37	22
22	3-CF_3	64	>6000	>5000	1800	>93	>78	27
23	2-CF_3	100	1900	>5000	620	19	>50	6
24	4-OCH_3	123	3900	>5000	2300	32	>40	19
25	3-OCH_3	136	810	>5000	2000	6	>37	15
26	2-OCH_3	83	930	3400	>8300	11	40	>100
2		22	102	1500	1200	5	68	54

[a] 95% confidence limits were generally ±15% of the mean value.
[b] Binding affinity for A_{2B}-AdoR was determined using HEK-A_{2B} cells with [^{3}H]ZM241385 as the radioligand.
[c] Binding affinity for A_1-AdoR was determined using CHO-A_1 cells with [^{3}H]CPX as the radioligand.
[d] Binding affinity for A_{2A}-AdoR was determined using HEK-A_{2A} cells with [^{3}H]ZM241385 as the radioligand.
[e] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]AB-MECA as the radioligand.

Table 3. Binding affinities of 5-phenylisoxazole analogs **27**–**35** for the A_{2B}, A_1, A_{2A}, and A_3-AdoRs



Compound	R_1	K_i (nM)[a]						
		(A_{2B})[b]	(A_1)[c]	(A_{2A})[d]	(A_3)[e]	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
27	H	14	1500	420	1800	107	30	128
28	2-Cl	111	>6000	2700	2300	>54	24	20
29	4-Cl	82	540	1200	3300	6	14	40
30	4-CF_3	48	2300	1700	1200	48	35	25
31	3-Cl	163	4600	>5000	4400	28	>30	27
32	2-CF_3	121	3700	>5000	4200	30	>41	34
33	3-OCH_3	131	3500	>5000	>5000	27	>38	>38
34	4-OCH_3	63	2000	>5000	3000	31	>79	48
35	2-OCH_3	195	3300	>5000	4900	16	>25	24
2		22	100	1500	1200	5	68	54

[a] 95% confidence limits were generally ±15% of the mean value.
[b] Binding affinity for A_{2B}-AdoR was determined using HEK-A_{2B} cells with [^{3}H]ZM241385 as the radioligand.
[c] Binding affinity for A_1-AdoR was determined using CHO-A_1 cells with [^{3}H]CPX as the radioligand.
[d] Binding affinity for A_{2A}-AdoR was determined using HEK-A_{2A} cells with [^{3}H]ZM241385 as the radioligand.
[e] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]AB-MECA as the radioligand.

4 for the A_{2B}-AdoR suggest that in this 3-phenyl-1,2,4-oxadiazole class of compounds, unsubstituted phenyl ring is optimal for both A_{2B}-AdoR binding affinity and selectivity. The binding affinity and selectivity of 5-phenyl-1,2,4-oxadiazole class of compounds are shown in Table 2. Compound **16**, which is a 1,2,4-oxadiazole regio isomer of **4**, displayed 20-fold decreased A_{2B}-AdoR binding affinity relative to **4**. In addition, compound **16** was 7-, 12-, and 16-fold less selective for the A_{2B}-AdoR versus A_1, A_{2A}, and A_3-AdoRs, respectively, relative to **4**. Compound **17**, which is an oxadiazole regio isomer of **3**, showed a 2-fold decrease in A_{2B}-AdoR binding affinity (K_i = 38 nM) and 8-fold less selectivity for the A_{2B}-AdoR versus the A_1-AdoR

relative to **3**. Even though, the 4-Cl phenyl analog **18** showed 2-fold decreased A_{2B}-AdoR binding affinity (K_i = 39 nM) than its oxadiazole regio isomer **5**, it displayed more selectivity for the A_{2B}-AdoR versus other three receptor subtypes (A_1/A_{2B}, A_{2A}/A_{2B}, and A_3/A_{2B} selectivity ratios of 74, >128, and >218, respectively). The 4-CF_3 analog, compound **20**, showed at least 2-fold increase in A_{2B}-AdoR binding affinity (K_i = 21 nM) relative to its regio isomer **6**. In addition, compound **20** showed much improved selectivity profile for the A_{2B}-AdoR relative to **6** (A_1/A_{2B}, A_{2A}/A_{2B}, and A_3/A_{2B} selectivity ratios of >285, >238, and 61, respectively). Unlike in the 3-phenyl-1,2,4-oxadiazole class of compounds (Table 1, where unsubstituted phenyl ring was optimal for both, binding affinity and selectivity for the A_{2B}-AdoR) and in this series of 5-phenyl-1,2,4-oxadiazoles (Table 2), a strong electron-withdrawing group in the 4-position of the phenyl ring provided the most active and selective analog (compound **20**). One could envision the 5-phenylisoxazole series (Table 3) as a direct analog of the 3-phenyl-1,2,4-oxadiazole series where the 4-nitrogen atom in the 3-phenyl-1,2,4-oxadiazole ring is isosterically replaced with a carbon atom. The isoxazole analog **30** showed 2-fold decreased A_{2B}-AdoR binding affinity (K_i = 48 nM) relative to the oxadiazole analog **20**. In addition, compound **30** displayed much lower selectivity for the A_{2B}-AdoR versus A_1, A_{2A}, and A_3-AdoRs relative to **20**. A similar trend was observed when comparing the isoxazole analog **29** to the oxadiazole analog **18**. In general, the 5-phenylisoxazole series was less active and selective for the A_{2B}-AdoR in comparison to the other two oxadiazole classes of compounds. It is noteworthy that some of these new analogs showed good oral bioavailability in rats. Compound **5**, for instance, displayed 22% oral bioavailability in rats when dosed at 1 mg/Kg (in DMSO/ethanol/PEG300/50 mM *n*-methylglucamine) (2.5:10:20:67.5) with AUC of 2330 ng h/mL and $t_{1/2}$ of 1.5 h.

In summary, we have discovered a novel class of A_{2B}-AdoR antagonists. Bioisosteric replacement of the amide bond in compound **2** with different heterocyclic 5-membered rings (1,2,4-oxadiazoles and isoxazoles) resulted in compounds with high affinity and selectivity for the A_{2B}-AdoR. Compound **4** is among the most active and selective A_{2B}-AdoR antagonist known to date. Considering the fact that only a few high affinity and selective A_{2B}-AdoR antagonists are available, this new series of compounds constitutes a significant addition to the field and might be useful as pharmacological tools. In addition, these new analogs may serve as leads to discover additional potent and selective A_{2B}-AdoR antagonists that may have potential use as therapeutic agents for treatment of asthma.

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