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Selective, high affinity A_{2B} adenosine receptor antagonists: N-1 monosubstituted 8-(pyrazol-4-yl)xanthines

Rao V. Kalla,[a,*] Elfatih Elzein,[a] Thao Perry,[a] Xiaofen Li,[a] Art Gimbel,[b] Ming Yang,[b] Dewan Zeng[b] and Jeff Zablocki[a]

[a]*Department of Bioorganic Chemistry, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA*
[b]*Department of Drug Research and Pharmacological Sciences, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA*

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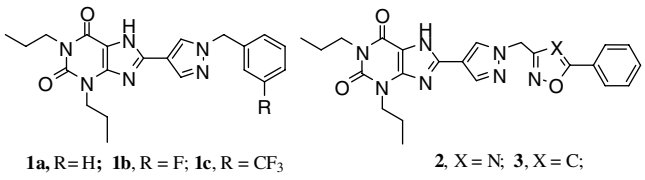
Abstract—A series of N-1 monosubstituted 8-pyrazolyl xanthines have been synthesized and evaluated for their affinity for the adenosine receptors (AdoRs). We have discovered two compounds **18** (**CVT-7124**) and **28** (**CVT-6694**) that display good affinity for the A_{2B} AdoR (K_i = 6 nM and 7 nM, respectively) and greater selectivity for the human A_1, A_{2A}, and A_3 AdoRs (>1000-, >830-, and >1500-fold; >850-, >700-, and >1280-fold, respectively). **CVT-6694** has been shown to block the release of interleukin-6 and monocyte chemotactic protein-1 from bronchial smooth muscle cells (BSMC), a process believed to be promoted by activation of A_{2B} AdoR.

In our recent publications,[1–3] we have reported on the discovery of novel 8-pyrazolyl xanthine derivatives that display high affinity and selectivity for the A_{2B} adenosine receptors (AdoRs). While exploring the SAR for this series of compounds, we initially focused on symmetrical disubstitution at the N-1 and N-3 positions of the xanthine core. Hayallah and co-workers have shown that N-1 monosubstituted xanthines that have a phenyl substitution at the 8-position display higher A_{2B} AdoR selectivity compared to the 1,3-disubstituted xanthines.[4] The goal of the present study is to explore the effect of N-1 or N-3 monosubstitution of the xanthine core on A_{2B} AdoR affinity and selectivity for the 8-pyrazolyl xanthines. For the purpose of this study, we synthesized the *m*-F benzyl and *m*-CF_3 benzyl-pyrazol-4-yl groups, as these substitutions imparted good affinity and selectivity in the 1,3-diethyl and 1,3-dipropyl derivatives compared with other substitution patterns.[1] For comparison purposes, the corresponding unsubstituted benzyl derivatives were prepared as well (Fig. 1 and Table 1). The N-1 monosubstituted (5-phenyl oxadiazolyl)-1-methyl pyrazolyl and (5-phenylisoxazolyl)-1-methyl pyrazolyl derivatives were also synthesized as these substitutions on the pyrazole ring displayed good affinity and selectivity in the 1,3-disubstituted xanthines (Fig. 1 and Table 2).[2]



1a, R = H; **1b**, R = F; **1c**, R = CF_3 **2**, X = N; **3**, X = C;

Figure 1. Structures of disubstituted 8-pyrazolyl xanthines.

The N-1 and N-3 monosubstituted 8-pyrazolyl xanthine derivatives were synthesized following the synthetic route illustrated in Scheme 1. The monosubstituted urea **I** was treated with cyanoacetic acid in the presence of freshly prepared sodium ethoxide to afford the 6-amino N-1 substituted uracil derivative **II**.[5] The 6-amino uracil **V** was treated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) at high temperature and quenched with the corresponding halide to provide the N-3 substituted 6-amino uracil derivatives **VI**.[6]

The 6-amino uracils **II** and **VI** were converted to the corresponding diamines by nitrosation with $NaNO_2$ followed by reducing with $Na_2S_2O_4$.[1] The diamines **IV** and **VIII** were selectively acylated at the 5-position by coupling with the pyrazole acid **4** using 1-[3-(dimethyl-

Keywords: A_{2B}; Adenosine; Antagonist.
* Corresponding author. Tel.: +1 650 384 8568; fax: +1 650 858 0390; e-mail: rao.kalla@cvt.com

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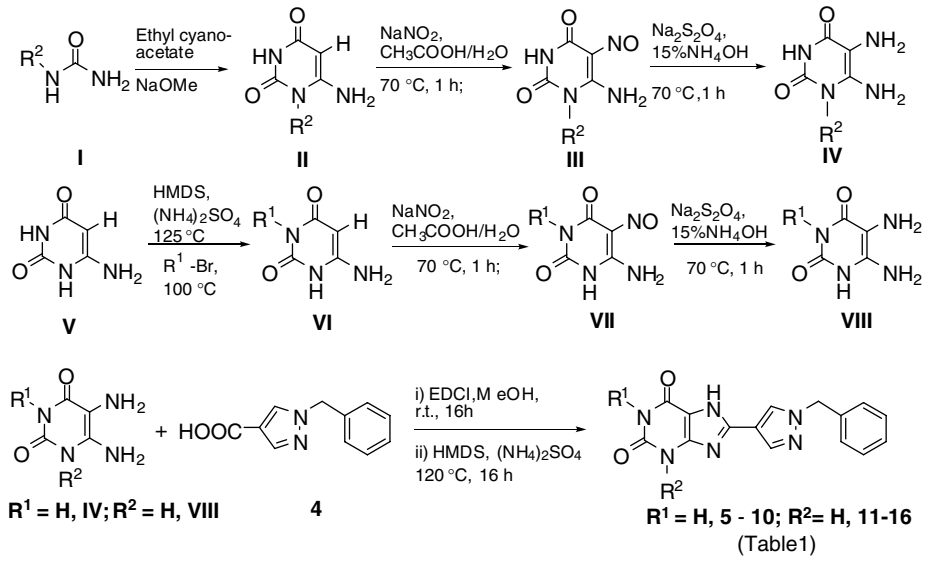
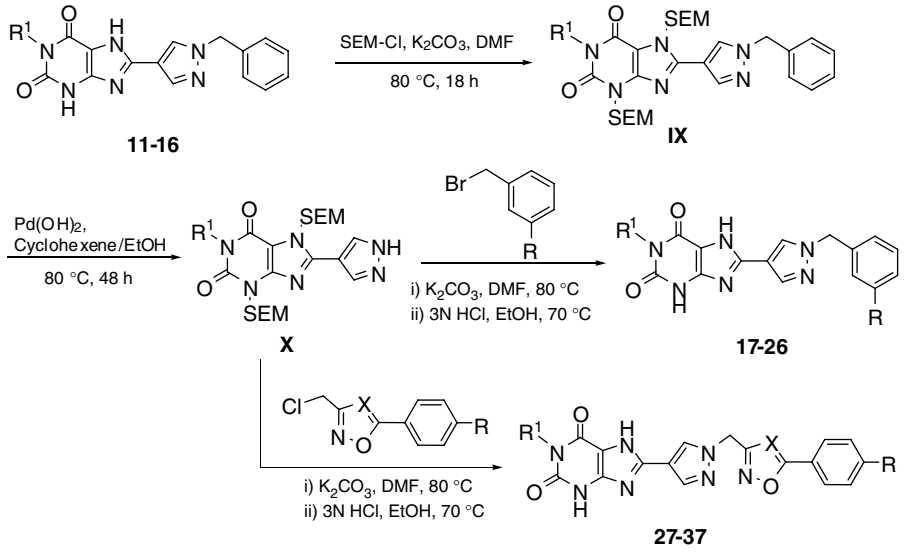


Scheme 1. Synthesis of N-1 and N-3 monosubstituted 8-pyrazolyl xanthines.

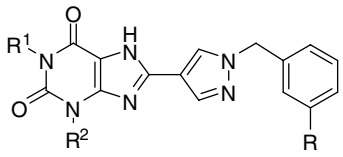


Scheme 2. Synthesis of N-1 monosubstituted 8-pyrazolyl xanthines.

amino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) to furnish the amides that were subsequently cyclized in the presence of HMDS and ammonium sulfate to provide the 8-pyrazolyl derivatives **5**–**16**.[7] For the synthesis of the substituted benzyl derivatives (Scheme 2) the N-3 and N-7 positions of the N-1 monosubstituted derivatives **11**–**16** were protected with 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl) using K_2CO_3 to provide derivatives **IX**. Debenzylation was accomplished by hydrogenolysis (Pearlman's catalyst) affording the 8-(*N*-1*H*-pyrazol-4-yl) derivative **X** with the N-3 and N-7 positions SEM protected. Alkylation of the derivative **X** with 3-F and 3-CF_3 benzyl groups followed by SEM deprotection with 3 N HCl furnished the 8-(1-substituted benzyl-pyrazol-4-yl)-*N*-1 substituted xanthine derivatives **17**–**26** in good yields. The 5-phenyl-(1,2,4-oxadiazoles) and 5-phenyl-isooxazoles required for the synthesis of the derivatives **27**–**37** were prepared as previously described.[2,8] Compound **X** was then alkylated with the corresponding oxadiazoles and isoxazoles, followed by SEM deprotection with 3 N HCl to afford the target molecules **27**–**37**.

The human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor binding affinities for the monosubstituted derivatives **5**–**16** and **17**–**37** were evaluated (Tables 1 and 2). To identify whether the N-3 or N-1 position of the xanthine core provides the highest A_{2B} affinity and selectivity we initially synthesized the benzyl-pyrazolyl derivatives. Compounds **5**–**10**, that are N-3 monosubstituted xanthine derivatives, demonstrated lower affinity toward the A_{2B} AdoR regardless of the substitution at the N-3 position (Table 1). The N-1 monosubstituted 8-pyrazolyl xanthine derivatives **11**–**16** have displayed good A_{2B} AdoR affinity and selectivity over the other adenosine receptor subtypes (Table 1). Compound **12** the N-1 pro-

Table 1. Binding affinities of disubstituted and monosubstituted analogues for the A_1, A_{2A}, A_{2B}, and A_3 AdoRs



Compound	R^1	R^2	R	K_i nM[#]				A_{2B} selectivity		
				hA_{2B}[a]	hA_1[b]	hA_{2A}[c]	hA_3[d]	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
1a	Propyl	Propyl	H	11	76	290	170	7	26	16
1b	Propyl	Propyl	F	14	170	230	58	13	18	4
1c	Propyl	Propyl	CF_3	14	160	400	140	12	27	10
5	H	Methyl	H	>6000	nd	nd	nd	nd	nd	nd
6	H	Ethyl	H	>6000	nd	nd	nd	nd	nd	nd
7	H	Propyl	H	>6000	nd	nd	nd	nd	nd	nd
8	H	*n*-Butyl	H	>6000	nd	nd	nd	nd	nd	nd
9	H	*i*-Butyl	H	>6000	nd	nd	nd	nd	nd	nd
10	H	Benzyl	H	>6000	nd	nd	nd	nd	nd	nd
11	Ethyl	H	H	37	5600	3800	1500	150	100	40
12	Propyl	H	H	13	1600	>5000	120	120	>380	10
13	*n*-Butyl	H	H	34	5100	3400	110	150	100	3
14	*i*-Butyl	H	H	13	>6000	>5000	1100	>460	>380	90
15	Cyclopropyl methyl	H	H	6	3290	2760	180	550	460	30
16	Benzyl	H	H	>6000	nd	nd	nd	nd	nd	nd
17	Ethyl	H	F	73	>6000	>5000	2117	>80	>70	30
18	**Ethyl (CVT-7124)**	**H**	**CF_3**	**6**	**>6000**	**>5000**	**>9000**	**>1000**	**>830**	**>1500**
19	Propyl	H	F	11	1800	1730	160	160	160	15
20	Propyl	H	CF_3	8	>6000	>5000	700	>750	>620	80
21	*n*-Butyl	H	F	18	4200	>5000	270	230	>280	15
22	*n*-Butyl	H	CF_3	30	>6000	>5000	>9000	>200	>170	>300
23	*i*-Butyl	H	F	24	>6000	>5000	1600	>250	>200	70
24	*i*-Butyl	H	CF_3	28	>6000	>5000	>9000	>210	>180	>320
25	Cyclopropyl methyl	H	F	5	210	100	nd	42	20	nd
26	Cyclopropyl methyl	H	CF_3	3	>6000	>5000	1000	>2000	>1600	300

[a] Binding affinity for the A_{2B} AdoR was determined by competition for binding sites labeled by ^{3}H-ZM241385 (14 nM) in membranes prepared from HEK-A_{2B} cells.

[b] Binding affinity for the A_1 AdoR was determined by competition for binding sites labeled by ^{3}H-CPX (0.5 nM) in membranes prepared from CHO-A_1 cells.

[c] Binding affinity for the A_{2A} AdoR was determined by competition for binding sites labeled by ^{3}H-ZM241385 (2 nM) in membranes prepared from HEK-A_{2A} cells.

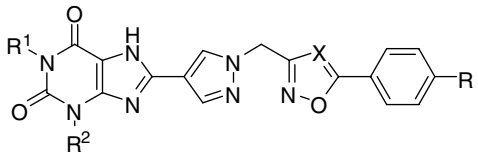
[d] Binding affinity for A_3 AdoR was determined using CHO-A_3 cells with ^{125}I-AB-MECA as the radioligand.

[#] 95% Confidence intervals are generally within 15% of the mean value.

pyl derivative has A_{2B} affinity of 13 nM that is similar to the disubstituted derivative **1a** (K_i = 11 nM, Table 1) but displayed higher selectivity compared to **1a**. The N-1 ethyl (**11**) and N-1 butyl (**13**) derivatives exhibited lower affinity than the N-1-propyl derivative (**12**). Branched alkyl chain N-1-isobutyl derivative **14** showed similar affinity (K_i = 13 nM) compared to the propyl derivative and also displayed higher selectivity. Similarly, the cyclopropyl methyl derivative **15** has good A_{2B} affinity (K_i = 6 nM) and greater selectivity compared to **12**. Introduction of a bulky benzyl group at the N-1-position as in **16** has a significantly lower affinity for the A_{2B} receptor. These findings clearly demonstrate that the N-1 monosubstituted 8-(1-benzyl-pyrazol-4-yl) xanthine derivatives show good affinity and greater selectivity related to the N-3-monosubstituted derivatives. Encouraged by these results, we further explored the effect of *m*-F and *m*-CF_3 benzyl substitution on the pyrazoles with respect to the N-1 monosubstituted xanthines (Table 1). In comparison N-1 ethyl analogue **17** with a 3-F substitution has lower affinity than the benzyl analogue **11**, but the *m*-CF_3 analogue **18** (**CVT-7124**) displayed good affinity (K_i = 6 nM) for the A_{2B} AdoR and greater selectivity against the other AdoRs (Table 1). The F (**19**) and CF_3 (**20**) analogues in the N-1 propyl series have good affinity for the A_{2B} AdoR (11 and 8 nM), respectively, and good selectivity over the A_1 and A_{2A} AdoRs but not against the A_3 AdoR. The F (**21**) and CF_3 (**22**) derivatives in the N-1 butyl series have similar affinity and selectivity compared to the corresponding benzyl derivative **13** (Table 1). The isobutyl analogues **23** and **24** have displayed lower A_{2B} AdoR affinity and lower selectivity than the benzyl analogue **14**. Similarly the cyclopropyl methyl derivative **26** with CF_3 substitution showed good A_{2B} AdoR affinity and selectivity. In this series of compounds substitution of *m*-CF_3 benzyl group on the pyrazole ring led to a high affinity and selective A_{2B} AdoR antagonist **18** (**CVT-7124**).

The results of the N-1 monosubstituted 8-(1-benzyl-pyrazol-4-yl)xanthines persuaded us to further expand our SAR by replacing the benzyl group with 5-phenyl oxadi-

Table 2. Binding affinities of N-1 monosubstituted 5-phenyl oxadiazoles and 5-phenyl-isoxazole analogues **33–43**



Compound	R^1	R^2	X	R	$K_i^{\#}$ (nM)				A_{2B} selectivity		
					hA_{2B}[a]	hA_1[b]	hA_{2A}[c]	hA_3[d]	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
2	Propyl	Propyl	N	H	21	1000	1800	630	50	85	30
27	Propyl	H	N	H	15	3500	>5000	>9000	200	>300	>600
28	**Propyl (CVT-6694)**	**H**	**N**	**Cl**	**7**	**>6000**	**>5000**	**>9000**	**>850**	**>700**	**>1280**
29	Propyl	H	N	CF_3	15	1400	>5000	>9000	90	>300	>600
30	*n*-Butyl	H	N	Cl	23	>6000	>5000	>9000	>260	>210	>400
31	*i*-Butyl	H	N	CF_3	14	>6000	>5000	>9000	>400	>350	>650
32	Cyclopropyl methyl	H	N	Cl	48	>6000	>5000	>9000	>120	>100	>190
33	Cyclopropyl methyl	H	N	CF_3	13	>6000	>5000	>9000	>460	>380	>700
3	Propyl	Propyl	C	H	14	1500	420	1800	110	30	130
34	Propyl	H	C	H	22	>6000	>5000	>9000	>270	>230	>400
35	Propyl	H	C	Cl	170	>6000	>5000	nd	>30	>30	nd
36	*i*-Butyl	H	C	H	12	>6000	>5000	>9000	>500	>410	>750
37	Cyclopropyl methyl	H	C	CF_3	15	>6000	>5000	>9000	>400	>300	>600

[a] Binding affinity for the A_{2B} AdoR was determined by competition for binding sites labeled by ^{3}H-ZM241385 (14 nM) in membranes prepared from HEK-A_{2B} cells.
[b] Binding affinity for the A_1 AdoR was determined by competition for binding sites labeled by ^{3}H-CPX (0.5 nM) in membranes prepared from CHO-A_1 cells.
[c] Binding affinity for the A_{2A} AdoR was determined by competition for binding sites labeled by ^{3}H-ZM241385 (2 nM) in membranes prepared from HEK-A_{2A} cells.
[d] Binding affinity for A_3 AdoR was determined using CHO-A_3 cells with ^{125}I-AB-MECA as the radioligand.
[#] 95% confidence intervals are generally within 15% of the mean value.

azoles and 5-phenyl-isoxazoles as these groups in the 1,3-disubstituted series exhibited higher A_{2B} affinity and selectivity (Table 2).[2] The 5-phenyl oxadiazole derivative with mono N-1 propyl substitution, as in **27**, demonstrated good affinity and higher selectivity for the A_{2B} AdoR compared to the disubstituted derivative **2**. The N-1 propyl analogue with 4-chloro-5-phenyl oxadiazole **28** (**CVT-6694**) has displayed high A_{2B} AdoR affinity (K_i = 7 nM) and excellent selectivity for the human A_1, A_{2A}, and A_3 AdoRs compared to the disubstituted analogue **2**. Replacing the chloro group with CF_3 as in **29** resulted in a decrease in A_{2B} AdoR affinity and selectivity compared to the chloro analogue **28**. Replacing the propyl group of **28** with a butyl group as in **30** resulted in decrease in A_{2B} AdoR affinity and selectivity. The N-1 isobutyl analogue **31** with 4-CF_3 substitution on the phenyl group has displayed similar A_{2B} AdoR affinity and selectivity compared to the propyl analogue **29**. In the N-1 cyclopropyl methyl analogues 4-CF_3 derivative **33** presented better affinity compared to the 4-chloro analogue **32**, and both compounds displayed very good selectivity. Similarly the N-1 propyl substituted isoxazole analogue **34** showed better selectivity while retaining the A_{2B} AdoR affinity compared to the disubstituted analogue **3** (Table 2). Introduction of a 4-chloro substituent on the phenyl ring of **34** resulted in 5-fold loss in A_{2B} AdoR affinity. Substitution of isobutyl group in place of propyl group as in **36** increased the A_{2B} AdoR affinity and selectivity compared to **34**. Compound **37** that has a cyclopropyl methyl group at the N-1 position also displayed very good selectivity and A_{2B} AdoR affinity. In general, the monosubstituted 5-phenyl oxadiazole and isoxazole analogues displayed greater selectivity for the A_{2B} AdoR compared to the disubstituted compounds regardless of the substitution at the N-1 position of the xanthine.

In conclusion, we have shown that N-1 monosubstituted 8-pyrazolyl xanthine derivatives display higher selectivity while retaining the A_{2B} AdoR affinity compared to their corresponding 1,3-disubstituted analogues. We have also shown that the N-1 substitution not the N-3 substitution on the xanthines provide greater selectivity for the A_{2B} AdoRs of the 8-pyrazolyl xanthines. In this process, we have discovered two high affinity and selective A_{2B} AdoR antagonists [**18** (**CVT-7124**) and **28** (**CVT-6694**)]. Compound **28** (**CVT-6694**) has been shown to block the release of interleukin-6 and monocyte chemotactic protein-1 from bronchial smooth muscle cells (BSMC), a process believed to be promoted by the activation of A_{2B} AdoR.[9] The activation of A_{2B} AdoR in human lung fibroblasts (HLFs) increases the release of IL-6 and induces the differentiation of fibroblasts into myofibroblasts that has been shown to be attenuated by the selective A_{2B} AdoR antagonist **28** (**CVT-6694**).[10]

References and notes

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