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The discovery of a selective, high affinity A_{2B} adenosine receptor antagonist for the potential treatment of asthma

Jeff Zablocki,[a,*] Rao Kalla,[a] Thao Perry,[a] Venkata Palle,[a] Vaibhav Varkhedkar,[a] Dengming Xiao,[d] Anthony Piscopio,[d] Tenning Maa,[b] Art Gimbel,[b] Jia Hao,[c] Nancy Chu,[c] Kwan Leung[c] and Dewan Zeng[b]

[a]*Department of Bioorganic Chemistry, CV Therapeutics, Inc., 3172 Porter Dr., Palo Alto, CA 94304, USA*
[b]*Department of Drug Research and Pharmacological Sciences, CV Therapeutics, Inc., 3172 Porter Dr., Palo Alto, CA 94304, USA*
[c]*Department of Preclinical Development, CV Therapeutics, Inc., 3172 Porter Dr., Palo Alto, CA 94304, USA*
[d]*Array Biopharma, Inc., 3200 Walnut Street, Boulder, CO 80301*

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Abstract—Adenosine has been suggested to play a role in asthma, possibly via activation of A_{2B} adenosine receptors on mast cells and other pulmonary cells. We describe our initial efforts to discover a xanthine based selective A_{2B} AdoR antagonist that resulted in the discovery of CVT-5440, a high affinity A_{2B} AdoR antagonist with good selectivity (A_{2B} AdoR K_i = 50 nM, selectivity A_1 > 200: A_{2A} > 200: A_3 > 167).

Adenosine is an endogenous agonist that can activate all four adenosine receptor subtypes—A_1, A_{2A}, A_{2B}, and A_3.[1] Activation of the A_{2B} adenosine receptors (AdoR) on mast cells may play a putative role in asthma by enhancing mast cell degranulation and releasing inflammatory cytokines (e.g. interleukin-4, 8, and 13).[2] Furthermore, activation of A_{2B} AdoR on bronchial smooth muscle cells (BSMC) has been demonstrated to lead to the release of interleukin-6 and monocytic chemotactic peptide-1 (MCP-1).[3]

Theophylline **1**, is used for the treatment of asthma in both IV rescue therapy for acute asthma attacks and chronic oral treatment.[4–6] Although the mechanism of action of theophylline is not completely understood, it is known to be a nonselective inhibitor for phosphodiesterases (PDE) and a nonselective AdoR antagonist (A_{2B} AdoR K_i = 7059 nm, selectivity A_1 0.6: A_{2A} 0.6: A_3 14, Fig. 1).

We hypothesize that the low therapeutic index of theophylline, due to both CNS and cardiac side effects, may be the result of its poor selectivity (for both PDEs and AdoRs). Based on our understanding of the role of A_{2B} AdoRs in asthma,[2,3] we hypothesize that a potent and selective A_{2B} AdoR would provide a better

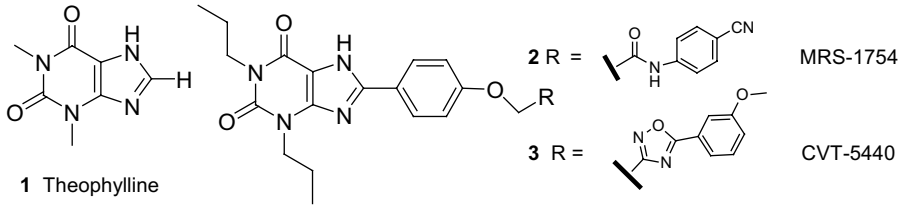

Figure 1. Structure of theophylline, MRS-1754 (**2**), and CVT-5440 (**3**).

Keywords: A_{2B}; Adenosine; Antagonist; Asthma.
* Corresponding author. Tel.: +1 650 384 8547; fax: +1 650 858 0390; e-mail: jeff.zablocki@cvt.com

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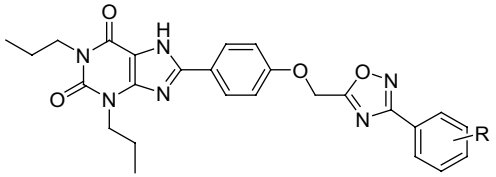
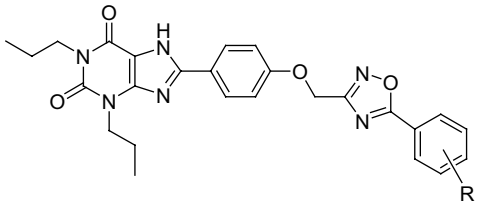
Table 1. Binding affinities of 3-phenyl (**10**–**25**) and 5-phenyl-1,2,4-oxadiazole analogues (**26**–**39**) for the A_{2B}, A_1, and A_{2A} adenosine receptors





Compd #[a]	R	A_{2B} nM ± SD or (95% CI)[b]	A_1 nM ± SD or (95% CI)[c]	A_{2A} nM ± SD or (95% CI)[d]	Compd #[a]	R	A_{2B} nM ± SD or (95% CI)[b]	A_1 nM ± SD or (95% CI)[c]	A_{2A} nM ± SD or (95% CI)[d]
10	2-Me	1120 (868–1451)	>6000	>5000	**26**	2-Me	438 ± 65	**>10,000**	>15,000
11	3-Me	>6666	>6000	>5000	**27**	3-Me	700 ± 16	—	—
12	4-Me	526 ± 264	643 (256–1610)	>5000	**28**	4-Me	91 (80–100)	113 (60–200)	1010 (640–1590)
13	2-MeO	557 (370–830)	>6000	>5000	**29**	2-MeO	127 ± 16	1160 ± 440	2940 ± 480
14	3-MeO	1680 (940–2980)	>6000	>5000	**3**[e]	3-MeO	**50 ± 22**	**>10,000**	**>5000**
15	4-MeO	2780 (1700–4530)	>6000	>5000	**30**	4-MeO	250 ± 67	2190 ± 370	—
16	2-Cl	1830 (1130–2950)	>6000	>5000	**31**	2-Cl	5830	—	—
17	3-Cl	**42 ± 10**	**6.4 (3.2–12.8)**	**407 (120–1360)**	**32**	3-Cl	2080 (1370–3170)	>6000	>5000
18	4-Cl	5170	—	—	**33**	4-Cl	3660	—	—
19	2-F	>2000	>6000	>5000	**34**	2-F	645 ± 230	—	—
20	3-F	1450 (870–2400)	2010 (890–4540)	>5000	**35**	3-F	>2000	>6000	>5000
21[e]	4-F	**215 ± 154**	**1830 ± 1505**	**>5000**	**36**	4-F	220 (140–355)	>6000	>5000
22	2-CF_3	325	473 (290–770)	3140 (2000–4940)	—	—			
23	3-CF_3	945 (540–1660)	>6000	>5000	**37**	3-CF_3	1010 (603–1697)	>6000	>5000
24	4-CF_3	485 (300–780)	1170 (350–1430)	>5000	**38**	4-CF_3	255 ± 40	5180 ± 1193	4910
25	4-CN	4520 (2730–7480)	>6000	>5000	**39**	4-CN	207 (160–270)	288 (220–380)	>5000

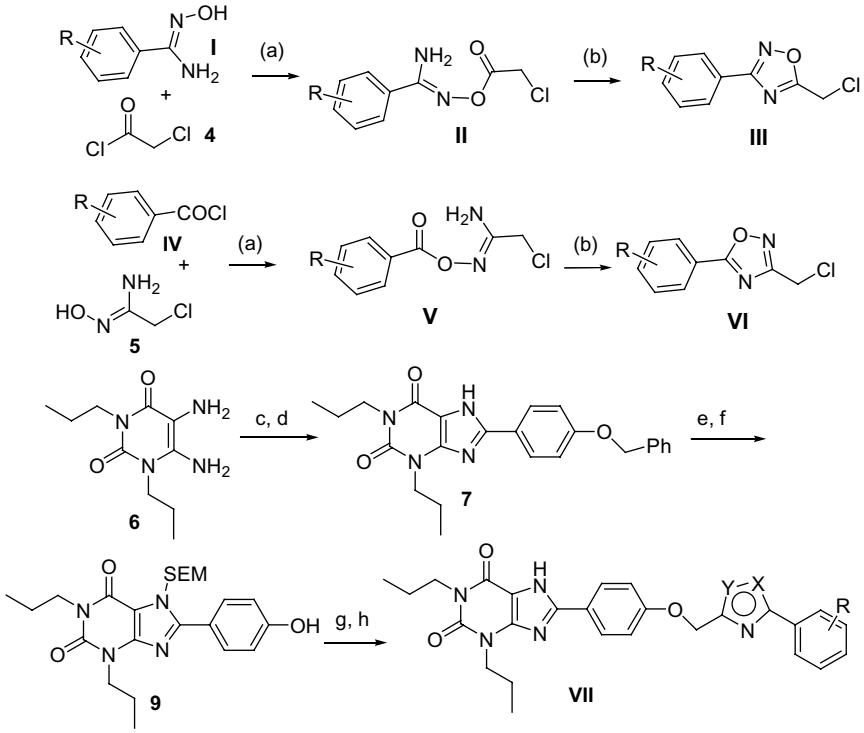
[a] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR, MS, and LC/MS. Data shown are mean with 95% confidence intervals ($n = 4$) or mean ± standard deviation ($n = 6$–8).
[b] Binding affinity for the human A_{2B} AdoR was determined by competition for binding sites labeled by ^{3}H-ZM241385 (14 nM) in membranes prepared from HEK-A_{2B} (human embryonic kidney) cells.[10]
[c] Binding affinity for the human A_1 AdoR was determined by competition for binding sites labeled by ^{3}H-CPX (0.5 nM) in membranes prepared from Chinese hamster ovary (CHO)-A_1 cells.[10]
[d] Binding affinity for the human 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.[1]
[e] Select compounds were tested for their binding affinity for human A_3 AdoR by displacement of specific binding of [^{125}I]AB-MECA in membranes prepared from CHO-A_3 cells.

therapeutic effect with fewer side effects. Jacobson and co-workers recently described a selective A_{2B} AdoR antagonist, compound **2** (A_{2B} AdoR K_i = 2 nm, selectivity A_1 210: A_{2A} 260: A_3 290);[7] however, this compound is not metabolically stable. Our goal was to discover a metabolically stable compound by replacing the anilide moiety.[7] Herein, we describe the preparation and affinity of 3-phenyl-1,2,4-oxadiazoles and 5-phenyl-1,2,4-oxadiazoles (Table 1) as amide surrogates for MRS-1754.

All of the compounds containing an amide surrogate of Table 1 were prepared by reacting the corresponding chloromethyl derivatives **III** or **VI** with phenol **9**. The 3-phenyl-1,2,4-oxadiazoles **III** and the 5-phenyl-1,2,4-oxadiazoles **VI** were prepared from the corresponding amide oximes and acid chlorides by acylation followed by condensation.[8,9] The commercially available 5,6-diaminouracil **6** was condensed with 4-benzyloxybenzoic acid followed by sodium hydroxide mediated xanthine ring closure to afford **7**.[10,11] After N-7 protection (trimethylsilylethoxymethyl, SEM) to afford **8** and hydrogenolysis of the benzyl protecting group, the resultant phenol **9** was reacted with the chloromethyl 1,2,4-oxadiazoles **III** and **VI** as shown in Scheme 1.[12] The target compounds of Table 1 were obtained by deprotection of N-7 by treatment with acid.

The SAR of the 3-phenyl and 5-phenyl-1,2,4-oxadiazole analogues with respect to A_{2B} AdoR affinity is shown in Table 1. A general comparison of similarly substituted phenyl analogues between the 3-phenyl and 5-phenyl series (e.g. **10** vs **26**, **11** vs **27**, etc) series favors the 5-phenyl-1,2,4-oxadiazole series with the exception of the *meta*-chloro analogues (**17** vs **32**). Although these series were designed as constrained mimetics of *p*-cyanoanilide **2** (Fig. 1), the corresponding *p*-cyano analogues **25** and **39** did not have high affinity or selectivity for the A_{2B} subtype (Table 1). The oxadiazole is a constrained amide surrogate that led to a different SAR result than the anilide series previously described by Jacobson and co-workers.[7] The SAR of the 3-phenyl-1,2,4-oxadiazole series (Table 1, left panel) for the A_{2B} AdoR regarding substitution on the phenyl ring demonstrates a slight trend favoring electron withdrawing groups (EWGs), **16**–**25**, over electron donating groups (EDGs), **10**–**15**. Within the compounds containing EWGs there is not a clear preference for *ortho* versus *meta* versus *para* substitution. In fact, the *ortho*-phenyl substitution was preferred for trifluoromethyl (**22**), *meta* substitution for chloro (**17**), and *para* substitution for fluoro (**21**) with respect to A_{2B} AdoR affinity. Within the 3-phenyl-1,2,4-oxadiazole series the *meta*-chloro analogue **17** had the highest affinity for the A_{2B} AdoR (K_i = 42 ± 10 nM); however, it was found to have higher affinity also for the A_1 AdoR (K_i = 6.4 nM). The compound from the 3-phenyl-1,2,4-oxadiazole series with the best selectivity and affinity for the A_{2B} AdoR is the *para* fluoro analogue **21** (A_{2B} AdoR K_i = 215 nM, selectivity A_1 8: A_{2A} 23: A_3 39).

In direct contrast to the 3-phenyl-1,2,4-oxadiazole series, the 5-phenyl-1,2,4-oxadiazole series (Table 1, right panel) favors EDGs on the phenyl ring (**3**, **26**–**30**) over EWGs (**31**–**39**) in relation to affinity for the A_{2B} AdoR. There is a clear trend with respect to phenyl substitution



Scheme 1. Reagents and conditions: (a) DIEA, CH_2Cl_2, rt, 24 h; (b) toluene, reflux, 24 h; (c) 4-benzyloxybenzoic acid, EDCI, MeOH; (d) NaOH (2 N), MeOH; (e) SEM-Cl, K_2CO_3, DMF, 23 °C; (f) H_2, Pd–C, MeOH; (g) **III** or **VI**, K_2CO_3, 56 °C; (h) 1 N HCl, EtOH, 83 °C.

and affinity for the A_{2B} AdoR within the 5-phenyl-1,2,4-oxadiazole series: MeO > Me > F = CF_3 > Cl. Again, there is not a clear preference with regards to *ortho* versus *meta* versus *para* phenyl substitution in the 5-phenyl-1,2,4-oxadiazole series. The *para*-Me analog **28** had favorable affinity for the A_{2B} AdoR (K_i = 91 nM); however, it had similar affinity for the A_1 AdoR (K_i = 113 nM). The *ortho*-MeO analogue **29** had favorable affinity for the A_{2B} AdoR (K_i = 127 nM) and modest selectivity (A_1 9: A_{2A} 23: A_3 5). The *meta*-MeO analogue **3** had the best overall affinity for the A_{2B} AdoR (K_i = 50 ± 22 nM) and binding selectivity (A_1 > 200: A_{2A} > 200: A_3 > 167). Therefore, although the SAR did not parallel the anilide series, MRS-1754 (**2**), comparable affinity and selectivity was obtained in the 5-phenyl-1,2,4-oxadiazole series with the analogue **3**.[13]

We found the *p*-cyanoanilide **2** to be metabolically unstable in liver S-9 incubations as compared to **29** and **3** (**29**≫**3** > **2**). Although the metabolic stability was enhanced by replacing the anilide by the 5-phenyl-1,2,4-oxadiazole, we did not observe any appreciable amounts of **29** in plasma following oral dosing in rats (10 mg/kg). In conducting these studies, we discovered that the 5-phenyl-1,2,4-oxadiazole series as a whole had very poor aqueous solubility, and this may be contributing to the unfavorable oral availability. The 5-phenyl-1,2,4-oxadiazole series including analogues **29** and **3** served as a starting point for subsequent series of selective, high affinity A_{2B} AdoR antagonists with improved oral bioavailability that will be described elsewhere. The discovery of selective, high affinity A_{2B} AdoR antagonists may help further define the role of the A_{2B} AdoR in asthma.[14]

Acknowledgements

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11. Alternative synthesis: 4-{2-[5-(2-methoxyphenyl)1,2,4-oxadiazol-3-yl]methoxy}benzoic acid (3.0 g), 5,6-diamino-1,3-dipropyl-1,3-dihydropyrimidine-2,4-dione (3.2 g), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (3.0 g) in DMF (50 mL) was stirred for 18 h at 23 °C. After concentration in vacuo (rotovap and high vac), the residue was treated with NaOH (2 N, 150 mL) for 2 h. After cooling to 0 °C, the reaction was acidified to pH 2–3, extracted with ethyl acetate (2 × 300 mL), washed with water, and concentrated to a small volume (20 mL). The resultant solid was filtered, washed with ethyl acetate (20 mL), and ethyl acetate–methanol (20 mL) to afford **29**: NMR (DMSO-d_6) δ 0.89 (t, J = 8.0 Hz, 3H), 0.92 (t, J = 8.0 Hz, 3H), 1.84–1.52 (m, 4H), 3.88 (t, J = 8.0 Hz, 2H), 3.96 (s, 3H), 4.03 (t, J = 8.0 Hz, 2H), 5.46 (s, 2H), 7.18 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 2H).
12. To prepare **7** 4-benzyloxybenzioc acid was substituted in place of 4-{2-[5-(2-methoxyphenyl)1,2,4-oxadiazol-3-yl]-methoxy}benzoic following the procedure outlined in Ref.11. The N-7 SEM protection was accomplished by treatment of **7** (3.8 g, 9.1 mmol), potassium carbonate (6.27 g, 45.4 mmol) in DMF (100 mL) with SEM-Cl (3.31 mL, 18 mmol) at 70 °C for 72 h. After concentration in vacuo, the residue was purified by applying flash chromatography (ethyl acetate–hexane 3/7) to afford the N-7 SEM protected **7**. The N-7-SEM protected **7** (1.74 g, 3.2 mmol) was converted to **9** by hydrogenolysis of the benzyloxy protecting group using palladium hydroxide (10%, 1.0 g) in methanol at 23 °C at 5 atm hydrogen for 16 h. The resultant suspension was filtered through Celite, washed with methylene chloride–methanol (1/1, 2 × 40 mL) to afford **9** after concentration in vacuo. Compound **9** (50 mg, 0.1 mmol) and potassium carbonate (0.5 g) in acetone was reacted with 5-[(3-methoxy)phenyl]-3-chloromethyl-oxadiazole (0.1 mmol) at 56 °C for 16 h. After concentration in vacuo, the residue was diluted with ethyl acetate, filtered, and purified by applying preparative TLC (ethyl acetate–hexane 3/7) to afford SEM protected **3**. The SEM protected **3** (50 mg) was dissolved in ethanol (2 mL) and treated with HCl (1 N, 0.5 ml) for 2 h at 86 °C. After cooling to 23 °C, the solid obtained was filtered, washed with ethanol (3 × 2 mL) to afford pure **3**: ^{1}H NMR (DMSO-d_6) δ 1.00–0.82 (m, 6H), 1.82–1.54 (m, 4H), 3.89 (s, 3H), 4.12–3.80 (m, 4H), 5.48 (s, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.63 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 8.0 Hz, 2H).
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