Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 18, pp. 2349-2352, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00440-X

ADENOSINE A_{2A} ANTAGONISTS WITH POTENT ANTI-CATALEPTIC ACTIVITY

Junichi Shimada,* Nobuaki Koike, Hiromi Nonaka, Shizuo Shiozaki, Koji Yanagawa, Tomoyuki Kanda, Hiroyuki Kobayashi, Michio Ichimura, Joji Nakamura, Hiroshi Kase, and Fumio Suzuki

Pharmaceutical Research Institute, Drug Discovery Research Laboratories, Kyowa Hakko Kogyo Co., Ltd. 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

Abstract: Structure–activity relationships of 8-styrylxanthines for *in vivo* adenosine A_{2A} antagonism were explored. Diethyl substitution both at the 1- and 3-position was found to dramatically potentiate the anti-cataleptic activity.

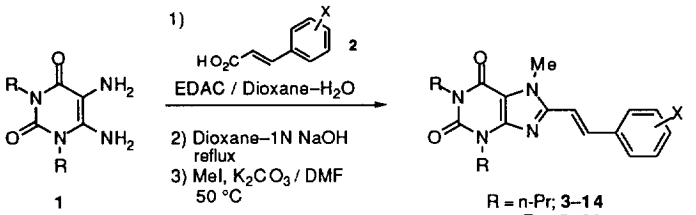
Adenosine modulates a great variety of biological functions both in the nervous system and peripheral tissues. Most of these effects appear to be mediated via specific cell surface receptors. On the basis of both pharmacological and biochemical studies, these receptors have been divided into four subtypes, termed adenosine A_1, A_{2A}, A_{2B} and A_3 receptors which belong to the superfamily of receptors coupled to G proteins.[1] The adenosine A_1 and A_2 receptor subtypes are differentially distributed in the central nervous system. In contrast to the wide distribution of the A_1 and A_{2B} receptors in brain, A_{2A} receptors appear to be confined to the striatum, nucleus accumbens, and olfactory tubercle demonstrated by the binding assay of the selective agonist CGS 21680.[2] This discrete distribution of A_{2A} receptors suggests a specific functional role of A_{2A} receptors in neuronal communication in basal ganglia. Methylxanthines such as theophylline and caffeine have been well-known to enhance locomotor activity and the stimulant effects are related, at least in part, to their ability to block adenosine receptors.[3] However, these methylxanthines are nonselective antagonists and have weak affinity for A_1 and A_{2A} receptors. Therefore the role of receptor subtypes in the behavioral effects associated with these methylxanthines remains unclear.

The major obstacle in defining the role of adenosine A_{2A} receptors *in vivo* has been the lack of reliable and selective pharmacological probes which can be trusted to provide correct information when used *in vivo*.[4] We have reported that 1,3,7-trialkylxanthine derivatives substituted with (*E*)-styryl groups at the 8-position act as selective A_{2A} antagonists *in vitro*[5]. Oral administration of KF17837 (**6**)[6], a highly selective A_{2A} antagonist, ameliorated the cataleptic response induced by dopamine D_1/D_2 antagonist (haloperidol).[7] In this study,

structure–activity relationships of 8-styrylxanthines were explored by varying substituents on the phenyl ring and the 1- and 3-positions of the xanthine moiety to optimize *in vivo* efficacy.

8-Styrylxanthine derivatives were synthesized as shown in Scheme 1. A 5,6-diaminouracil (**1**) was condensed with a substituted (*E*)-cinnamic acid (**2**). Cyclization of the resulting amide under strongly basic conditions followed by methylation with methyl iodide gave the desired 8-styrylxanthines.

Scheme 1



EDAC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride

Affinities of the 8-styrylxanthine derivatives at adenosine A_1 and A_{2A} receptors were determined by standard radioligand binding procedures.[8] Previous studies[9] have indicated that (*E*)-8-styrylxanthines undergo rapid isomerization and give a stable equilibrium mixture when exposed to light in dilute solutions. Therefore, selected compounds were assayed in the dark for comparison. In standard 'not-dark' conditions, an *E-Z* equilibrium mixture was used for assays. The mixture was prepared by exposing 1 mM DMSO solutions of the substrates to 600 lux fluorescent light for more than 40 h, and the *E-Z* ratio was determined by HPLC.[9] Table 1 shows a series of (*E*)-1,3-dialkyl-8-styryl-7-methylxanthine with K_i values and the *E-Z* ratio at equilibrium. The *E-Z* ratio was found to be about from 2 : 8 to 3 :7 except **3**, **5** and **8**. In the dipropyl series, no apparent differences in the affinity at the A_{2A} receptors were observed except 2,4,5-trisubstituted analogs (**13**, **14**) as reported previously.[5] Diethyl substitution at the 1- and 3-position did not improve A_{2A} affinity nor selectivity in general, but **19** and **20** were the most potent A_{2A} antagonists among these series.

The ED_{50} values of inhibitory activity on haloperidol-induced catalepsy[10] are also presented in the Table. Two or three substitution of the phenyl moiety with methoxy or methyl at 2,3,4-, 3,4,5- and 3,4-position was found to be favored for *in vivo* activity. Surprisingly, diethyl substitution at the 1- and 3-position dramatically potentiated the anti-cataleptic activity without exception.

Compound **15**[11] showed about 90 times more potent anti-cataleptic effects than that of KF 17837 (**6**). This is explained by differences in oral adsorptions because bioavailability of **6** and **15** at the dose of 30 mg / kg in rats was 3.6% and 20.6%, respectively. None of the *Z*-isomer of either compound was detected in plasma.

In conclusion, diethyl substitution both at the 1- and 3-position dramatically potentiated the *in vivo* activity of (*E*)-8-styryl-1,3,7-trialkylxanthines. Compound **15** (KW-6002) was identified as an adenosine A_{2A} antagonist with the most potent anti-cataleptic activity. This new agent should open possibilities for drug development in the treatment of basal ganglia disorders, e.g. Parkinson's disease.

Table 1. A_1 and A_{2A} Adenosine Receptor Binding and Anti-Cataleptic Activity of (*E*)-8-Styryl-1,3-dialkyl-7-methylxanthines.

A) R = n-Pr

no.	X	*E* : *Z*	K_i (nM) A_1	K_i (nM) A_{2A}	K_i ratio A_1 / A_{2A}	Inhibitory Activity on Haloperidol-Induced Catalepsy (ED_{50}, mg/kg, po)
3	H	50 : 50	220 ± 78[a]	15 ± 5.9[a]	15	> 10
			100 ± 8.4[b]	4.9 ± 1.0[b,c]	20	
4	4-OMe	17 : 83	340 ± 46[a]	18 ± 6.3[a]	19	> 10
			63 ± 13[b]	4.5 ± 0.23[b,c]	14	
5	4-Cl	95 : 5	(> 10000)[a, d]	49[a]	> 200	> 10
			470[b]	35[b,c]	13	
6	3,4-diOMe	18: 82	390 ± 68[a]	7.8 ± 2.7[a]	50	2.7 (1.5–4.1)
	(KF 17837)		62 ± 11[b,c]	1.0 ± 0.057[b,c,e]	62	
7	2,4-diOMe	31 : 69	150	8.2	18	6.6 (3.7–14)
8	3,5-diOMe	64 : 36	47 / 75[f]	6.2		> 10
9	3,4,5-triOMe	15 : 85	1100 ± 380[a]	14 ± 2.6[a]	79	3.6 (1.7–6.1)
			120 ± 15[b]	1.7 ± 0.16[b,c]	71	
10	2,3,4-triOMe	16 : 84	160	6.8	24	1.2 (0.58–1.9)
11	2,4-diOMe, 3-Me	23 : 77	90 / 92[f]	7.4		> 10
12	4-OMe, 2,3-diMe	14 : 86	560	3.9 ± 1.6	140	3.0 (2.1–4.4)
13	2,4,5-triOMe	18 : 82	(3300)[d]	170	19	> 10
14	4-OMe, 2,5-diMe	22 : 78	> 100000	> 100000		> 10

B) R = Et

15	3,4-diOMe	19 : 81	580 ± 59	13 ± 0.88	45	0.03 (0.02–0.06)
	(KW-6002)		150 ± 22[b]	2.2 ± 0.34[b,c]	68	
16	2,4-diOMe	30 : 70	2500	57	44	1.7 (1.2–1.5)
17	3,4,5-triOMe	18 : 82	47 / 68[f]	6.2 ± 2.5		0.25 (0.14–0.41)
			190 ± 5.8[b]	2.4 ± 0.43[b,c]	79	
18	2,3,4-triOMe	12 : 88	37	3.9	9.5	0.23 (0.14–0.39)
			61 ± 10[b]	2.2 ± 0.41[b,c]	28	
19	2,4-diOMe, 3-Me	12 : 88	110	1.6	69	0.63 (0.41–0.99)
			83 ± 12[b]	0.67 ± 0.081[b,c]	120	
20	4-OMe, 2,3-diMe	5 : 95	78	2.0	39	0.24 (0.12–0.44)
			120 ± 49[b]	0.67 ± 0.064[b,c]	180	

[a] Data taken from ref. 5. [b]Assay was performed with all apparatus shielded from light. [c][^{3}H]CGS 21680 was used instead of [^{3}H]NECA. [d]A_1 binding was carried out with [^{3}H]CHA in guinea pig forebrain membranes. [e]Data taken from ref. 9. [f] % inhibition at 10^{-5} / 10^{-4} M

Acknowledgment We thank M. Akimoto and T. Ohta for technical assistance.

References and notes

1. Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. *Pharmacol. Rev.* **1994**, *46*, 143–156.
2. (a) Jarvis, M. F.; Williams, M. *Eur. J. Pharmacol.* **1989**, *168*, 243–246. (b) Parkinson, F. E.; Fredholm, B. B. *Naunyn Schmiedebergs Arch. Pharmacol.* **1990**, *342*, 85–89. (c) Martinez-Mir, M. I.; Probst, A.; Palacios, J. M. *Neurosciene* **1991**, *42*, 697–706.
3. Fredholm, B. B. *Trends Pharmacol. Sci.* **1980**, *1*, 129–132.
4. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. *Cur. Med. Chem.* **1995**, *2*, 707–722.
5. Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A.; Ichikawa, S. *J. Med. Chem.* **1992**, *35*, 2342–2345.
6. (a) Jackson, E. K.; Herzer, W. A.; Suzuki, F. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1304–1310. (b) Nonaka, H.; Ichimura, M.; Takeda, M.; Nonaka, Y.; Shimada. J.; Suzuki, F.; Yamaguchi, K.; Kase, H. *Eur. J. Pharmacol.* **1994**, *267*, 335–341.
7. Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F.; Nakamura, J. *Eur. J. Pharmacol.* **1994**, *256*, 263–268.
8. A_1 binding with [^{3}H]CHA in rat forebrain membranes and A_{2A} binding with [^{3}H]NECA (+CPA) were performed as described before.[5]
9. Nonaka, Y.; Shimada, J.; Nonaka, H.; Koike, N.; Aoki, N.; Kobayashi, H.; Kase, H.; Yamaguchi, K.; Suzuki, F. *J. Med. Chem.* **1993**, *36*, 3731–3733.
10. The anti-cataleptic effect in mice was evaluated as described before.[7] Mouse given the scoreless than 3 was defined as effective. The ED_{50} values with 95% confidence limits were calculated by means of the Probit method (n = 10).
11. Physical data for compound **15**: a pale yellow powder; mp 191 °C; IR(KBr) 1697, 1655, 1518 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.74(1 H, d, *J* = 15.5 Hz), 7.18(1H, dd, *J* = 8.3, 1. 9 Hz), 7.08 (1 H, d, *J* = 1. 9 Hz), 6. 89 (1 H, d, *J* = 8. 3 Hz), 6.77 (1 H, d, *J* = 15. 5 Hz) , 4.21(2 H, q, *J* = 6. 9 Hz), 4.09 (2 H, q, *J* = 6.9 Hz), 4.06(3 H, s), 3.96(3 H, s), 3.93(3 H, s), 1.39(3 H, t, *J* = 6. 9 Hz), 1. 27 (3 H, t, *J* = 6. 9 Hz). Anal. Calcd for $C_{20}H_{24}N_4O_4$: C, 62.48; H, 6.29; H, 14.57. Found: C, 62.52; H, 6.53, N, 14.56.

(*Received in Japan* 15 *May* 1997; *accepted* 11 *August* 1997)