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

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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. © 1997 Elsevier Science Ltd.

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



Affinities of the 8-styrylxanthine derivatives at adenosine A_1 and A_{2A} receptors were determined by standard radioligand binding procedures.⁸ Previous studies⁹ 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 catalepsy¹⁰ 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.

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			K_i (nM)		K_{i} ratio	on Haloperidol-Induced
no	. X	E:Z	A ₁	A _{2A}	A_1 / A_{2A}	Catalepsy (ED ₅₀ , mg/kg, po)
3	Н	50 : 50	$220 \pm 78^{*}$	15 ± 5.9^{a}	15	> 10
			100 ± 8.4^{b}	$4.9 \pm 1.0^{b,c}$	20	
4	4-OMe	17:83	$340 \pm 46^{*}$	$18 \pm 6.3^{*}$	19	> 10
			63 ± 13 ^b	$4.5 \pm 0.23^{b,c}$	14	
5	4-Cl	95 : 5	(> 10000) ^{a, d}	49 "	> 200	> 10
			470 ^b	35 ^{b,c}	13	
6	3,4-diOMe	18: 82	390 ± 68*	7.8 ± 2.7 [*]	50	2.7 (1.5-4.1)
	(KF 17837)		$62 \pm 11^{b,e}$	$1.0 \pm 0.057^{b,c,e}$	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}	$1.7 \pm 0.16^{b,c}$	71	
102,3,4-triOMe		16 : 84	160	6.8	24	1.2 (0.58-1.9)
11	l 2,4-diOMe, 3-Me	23:77	90 / 92 ^f	7.4		> 10
12	2 4-OMe, 2,3-diMe	14 : 86	560	3.9 ± 1.6	140	3.0 (2.1-4.4)
132,4,5-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	$2.2 \pm 0.34^{b,c}$	68	
162,4-diOMe		30 : 70	2500	57	44	1.7 (1.2–1.5)
17	73,4,5-triOMe	18:82	47 / 68 ^f	6.2 ± 2.5		0.25 (0.14-0.41)
			190 ± 5.8^{b}	$2.4 \pm 0.43^{b,c}$	79	
18	32,3,4-triOMe	12:88	37	3.9	9.5	0.23 (0.14-0.39)
			61 ± 10^{b}	$2.2 \pm 0.41^{b,c}$	28	
19	2,4-diOMe, 3-Me	12:88	110	1.6	69	0.63 (0.41-0.99)
			83 ± 12 ^b	$0.67 \pm 0.081^{b,c}$	120	
2(4-OMe, 2,3-diMe	5 : 95	78	2.0	39	0.24 (0.12-0.44)
			120 ± 49^{b}	$0.67 \pm 0.064^{b,c}$	180	

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

^a Data taken from ref. 5. ^bAssay was performed with all apparatus shielded from light. ^c[³H]CGS 21680 was used instead of [³H]NECA. ^dA₁ binding was carried out with [³H]CHA in guinea pig forebrain membranes. ^cData taken from ref. 9. ^f% inhibition at $10^{-5} / 10^{-4}$ M

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- 8. A₁ binding with [³H]CHA in rat forebrain membranes and A_{2A} binding with [³H]NECA (+CPA) were performed as described before.⁵
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- 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; ¹H-NMR (CDCl₃) 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 C₂₀H₂₄N₄O₄: C, 62.48; H, 6.29; H, 14.57. Found: C, 62.52; H, 6.53, N, 14.56.

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