N^6,9-Disubstituted Adenines: Potent, Selective Antagonists at the A_1 Adenosine Receptor

Robert D. Thompson,† Sherrie Secunda,‡ John W. Daly,‡ and Ray A. Olsson*,†,§

Department of Internal Medicine and Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida 33612, and Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, Maryland 20892.
Received April 1, 1991

N^6-Substituted 9-methyladenines are potent antagonists of the activation of A_1 adenosine receptors. The present study assessed the effect of N^6 and N-9 substituents on the binding of adenines to the A_1 and A_2 receptors, respectively, of rat brain cortex and striatum and also on the antagonism of the A_2 receptor mediated stimulation of the adenylate cyclase of PC12 cells by *N*-ethyladenosine-5′-uronamide. The potency ranking of 9-substituted adenines varied directly with the hydrophobicity of the substituent: cyclopentyl > phenyl > tetrahydrofuryl > ethyl > methyl > 2-hydroxyethyl. The 9-substituted adenines showed little selectivity for either receptor and the *R* enantiomer of N^6-(1-phenyl-2-propyl)-9-methyladenine was only 4-fold more potent than the *S* enantiomer at the A_1 receptor. An N^6-cyclopentyl substituent increased potency at the A_1 receptor and decreased potency at the A_2 receptor, resulting in selectivity for the A_1 receptor of up to 39-fold. The N^6-cyclopentyl group completely overshadowed the effect of the hydrophobicity of the 9-substituent. A 2-chloro substituent did not alter the potency of an N^6-substituted 9-methyladenine.

Exocyclic substituents at N^6 and C-2 profoundly influence the activities of adenosine derivatives at A_1 and A_2 adenosine receptors (A_1AR, A_2AR). Thus, certain alkyl and cycloalkyl substituents at N^6 can increase the affinity of adenosine for the A_1AR,[1-3] whereas some aralkyl substituents at N^6 or at C-2 can promote affinity for the A_2AR.[4-9] A 2-chloro substituent further increases the potency and selectivity of the selective A_1AR agonist N^6-cyclopentyladenosine.[10] A ribose moiety at N-9 is present in most adenosine receptor agonists. Modification of the ribose usually reduces or even abolishes activity at either receptor.[11,13] The potent adenosine-5′-ribofuranuronamides and certain other ribosides modified at C-5′ are exceptions to this rule. The combination of an N^6 substituent that confers selectivity for the A_1AR, together with a 2′,3′-dideoxyribose residue at N-9, generates a potent, highly selective A_1AR antagonist.[14] Replacement of the ribose moiety of adenosine with a methyl group yields 9-methyladenine, a relatively unselective antagonist of only modest potency.[15,16] Adenine itself is a very weak adenosine antagonist. As in the case of adenosines, alkyl and cycloalkyl substituents on N^6 increase the affinity of the 9-methyladenines for the A_1AR.[16] The 7-deaza-9-phenyladenines are also potent adenosine receptor antagonists.[17] A 2-phenyl substituent greatly increases affinity of a 7-deazaadenine for the A_1AR.[18]

This report compares the antagonist potency and selectivity of some N^6-substituted 9-methyladenines with N^6-substituted adenines containing 9-substituents other than methyl, namely, ethyl, 2-hydroxyethyl, cyclopentyl, 2-tetrahydrofuryl, and phenyl. Additionally, a series of N^6-substituted 2-chloro-9-methyladenines examines the effect of the 2-chloro substituent on antagonist potency. A prior paper on N^6-substituted 9-methyladenines[16] reports data from two assays of affinity for the A_1AR, namely, radioligand binding to the receptor in rat cerebral cortex and antagonism of the A_2AR-mediated stimulation of the adenylate cyclase in PC12 cells by *N*-ethyladenosine-5′-uronamide (NECA). The present report includes only the analogues in that prior report for which there is new information about affinity for the A_2AR in rat striatum.

* Address for correspondence: Dept. of Internal Medicine, Box 19, USF College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612.

† Department of Internal Medicine.

‡ Department of Biochemistry and Molecular Biology.

§ Laboratory of Bioorganic Chemistry.

(1) Moos, W. H.; Szotek, D. S.; Bruns, R. F. N^6-Cycloalkyladenosines. Potent, A_1-selective adenosine agonists. *J. Med. Chem.* **1985**, *28*, 1383-1384.

(2) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. Structure-activity relationships for N^6-substituted adenosines at a brain A_1-adenosine receptor with a comparison to an A_2-adenosine receptor regulating coronary blood flow. *Biochem. Pharmacol.* **1986**, *35*, 2467-2481.

(3) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.* **1986**, *29*, 331-346.

(4) Kusachi, S.; Thompson, R. D.; Bugni, W. J.; Yamada, N.; Olsson, R. A. Dog coronary artery adenosine receptor: structure of the N^6-alkyl subregion. *J. Med. Chem.* **1985**, *28*, 1636-1643.

(5) Bridges, A. J.; Bruns, R. F.; Ortwine, D. F.; Priebe, S. F.; Szotek, D. S.; Trivedi, B. K. N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine and its uronamide derivatives. Novel adenosine agonists with both high affinity and high selectivity for the A_2 receptor. *J. Med. Chem.* **1988**, *31*, 1282-1285.

(6) Hutchinson, A. J.; Webb, R. L.; Oei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. CGS 21680C, an A_2 selective adenosine receptor agonist with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 47-55.

(7) Hutchinson, A. J.; Williams, M.; DeJesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. 2-(Arylalkylamino)adenosin-5′-uronamides: a new class of highly selective adenosine A_2 receptor ligands. *J. Med. Chem.* **1990**, *33*, 1919-1924.

(8) Ueeda, M.; Thompson, R. D.; Arroyo, L.; Olsson, R. A. 2-Alkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* **1991**, *34*, 1334-1339.

(9) Ueeda, M.; Thompson, R. D.; Arroyo, L.; Olsson, R. A. 2-Aralkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* **1991**, *34*, 1340-1344.

(10) Lohse, M. J.; Klotz, K.-N.; Schwabe, U.; Cristalli, G.; Vitton, S.; Griffantini, M.; 2-Chloro-N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *337*, 687-689.

(11) Olsson, R. A.; Khouri, E. M.; Bedynek, J. L., Jr.; McLean, J. Coronary vasoactivity of adenosine in the conscious dog. *Circ. Res.* **1979**, *45*, 468-478.

(12) Bruns, R. F. Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. *Can. J. Physiol. Pharmacol.* **1980**, *58*, 673-691.

(13) Taylor, M. D.; Moos, W. H.; Hamilton, H. W.; Szotek, D. S.; Patt, W. C.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Heffner, T. G.; Mertz, T. E. Ribose-modified adenosine analogues as adenosine receptor agonists. *J. Med. Chem.* **1986**, *29*, 346-353.

0022-2623/91/1834-2877$02.50/0

Table I. Analytical and Physical Data of 9-Substituted Adenines

no.	N^6 substituent	formula	anal.	purific[a]	% yield	mp, °C	UV, $\lambda_{max}(\epsilon)$
4	H	$C_6H_7N_5$	C,H,N	C	50	300–303	262 (14 300)
5	$HC(C_2H_5)$	$C_{11}H_{17}N_5$	C,H,N	L45-60	77	86–89	270 (16 500)
6	c-C_4H_7	$C_{10}H_{13}N_5$	C,H,N	L40	87	125	272 (18 700)
7	c-C_5H_9	$C_{11}H_{15}N_5$	C,H,N	C	65	109	271 (19 900)
8	1-CH_3-c-C_5H_8	$C_{12}H_{17}N_5$[b]	C,H,N	C	50	32	272 (19 000)
9	c-C_6H_{11}	$C_{12}H_{17}N_5$	C,H,N	L45-60	60	109–110	271 (20 500)
10	C_6H_5	$C_{12}H_{11}N_5$	C,H,N	C	30	156–158	288 (20 400)
11	2F-C_6H_4	$C_{12}H_{10}FN_5$[c]	C,H,N	C	45	155	279 (21 000)
12	$C_6H_5CH_2$	$C_{13}H_{13}N_5$	C,H,N	C	23	116–118	270 (19 000)
13	$C_6H_5(CH_2)_2$	$C_{14}H_{15}N_5$	C,H,N	L45-60	90	116–117	271 (17 200)
14	$(C_6H_5)_2CHCH_2$	$C_{20}H_{21}N_5$	C,H,N	C	39	135	270 (17 600)
15	$(CH_3O)_3C_6H_2(CH_2)_2$	$C_{17}H_{21}N_5O_3$	C,H,N[f]	L60	52	123–124	272 (13 800)
16	2-pyridyl$(CH_2)_2$	$C_{13}H_{14}N_6$	C,H,N	L50	77	108–109	270 (17 900)
17	3-thienyl$(CH_2)_2$	$C_{12}H_{13}N_5S$·HCl	C,H,N,S,Cl	A	50	205–206	242 (7700) 270 (16 100)
18	1-C_6H_5-2R-C_3H_6	$C_{15}H_{17}N_5$·HCl	C,H,N,Cl	A	89	221–222	271 (17 100)
19	1-C_6H_5-2S-C_3H_6	$C_{15}H_{17}N_5$·HCl	C,H,N,Cl	A	61	215–216	271 (16 900)
			N^6-Substituted 9-Ethyladenines				
20	H	$C_7H_9N_5$[e]	C,H,N	B	50	189–191	261 (12 600)
21	c-C_5H_9	$C_{12}H_{17}N_5$	C,H,N	C	61	107	270 (17 900)
22	c-C_6H_{11}	$C_{13}H_{19}N_5$	C,H,N	L55	37	65–67	271 (22 200)
			N^6-Substituted 9-(2-Hydroxyethyl)adenines				
23	H	$C_7H_9N_5O$	C,H,N	C	13	240–243	261 (16 700)
24	c-C_5H_9	$C_{12}H_{17}N_5O$	C,H,N	H40	69	117	271 (18 800)
			N^6-Substituted 9-Cyclopentyladenines				
25	H	$C_{10}H_{13}N_5$	C,H,N	H70	35	165	260 (15 400)
26	c-C_5H_9	$C_{15}H_{21}N_5$	C,H,N	H70	63	oil	270 (21 800)
			N^6-Substituted 9-(Tetrahydrofuryl)adenines				
27	H	$C_9H_{11}N_5O$	C,H,N	H40	28	165	260 (15 400)
28	c-C_5H_9	$C_{14}H_{19}N_5O$	C,H,N	H50	31	oil	
			N^6-Substituted 9-Phenyladenines				
29	H	$C_{11}H_9N_5$	C,H,N	C	60	242–244	261 (16 300)
30	c-C_5H_9	$C_{16}H_{17}N_5$	C,H,N	L70-80	83	119	270 (20 400)
31	C_6H_5	$C_{17}H_{13}N_5$	C,H,N	C	80	172–174	290 (23 300)
32	1-C_6H_5-2(*R*)-C_3H_6	$C_{20}H_{19}N_5$	C,H,N	L70-80	88	38–40	270 (19 700)
33	1-C_6H_5-2(*S*)-C_3H_6	$C_{20}H_{19}N_5$	C,H,N	L70-80	93	46–48	271 (19 400)
			N^6-Substituted 2-Chloro-9-methyladenines				
36	c-C_5H_9	$C_{11}H_{14}ClN_5$	C,H,Cl,N	C	84	92–93	274 (18 900)
37	C_6H_6	$C_{12}H_{10}ClN_5$	C,H,N	C	79	193	295 (26 000)
38	1-C_6H_5-2(*R*)-C_3H_6	$C_{15}H_{16}ClN_5$	C,H,N,Cl	L60-80	77	51–52	275 (17 900)
39	1-C_6H_5-2(*S*)-C_3H_6	$C_{15}H_{16}ClN_5$	C,H,N,Cl	L60-80	76	48–51	274 (17 800)

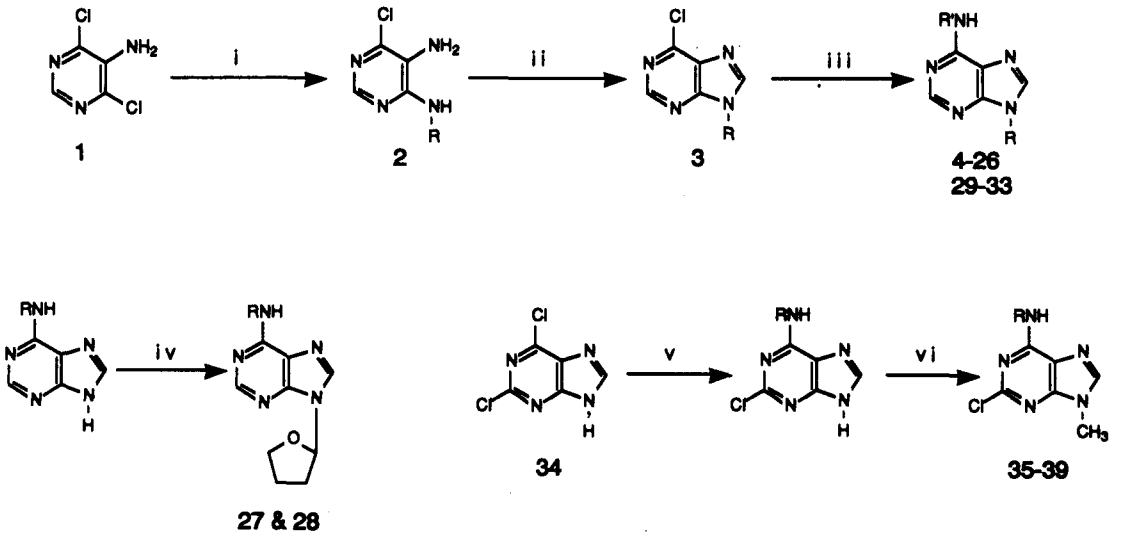
[a] Abbreviations are as follows: A, precipitation from anhydrous $(C_2H_5)_2O$ by gassing with dry HCl; B, recrystallization from C_6H_6; C, recrystallization from C_2H_5OH/H_2O; L, low-pressure chromatography; H, high-pressure chromatography; single numbers refer to % CH_3OH in water for isocratic elution; two digits refer to beginning and end concentrations for elution with gradients of CH_3OH in water. [b] Analyzed for 0.25 mol of water. [c] Analyzed for 0.75 mol of water. [d] Analyzed for 0.5 mol of water. [e] Analyzed for 1 mol of water. [f] Analysis for H: calcd 6.21, found 5.32.

Chemistry

The synthesis of the N^6,9-disubstituted adenines **4-26** and **29–33**, followed previously described general methods[19,20] (Scheme I). Briefly, the reaction of 5-amino-4,6-dichloropyrimidine (**1**) with the amino derivative of the desired N-9 substituent yielded a 4-substituted 6-chloro-4,5-diaminopyrimidine (**2**) which underwent cyclization with triethyl orthoformate to form the 9-substituted 6-chloropurine (**3**). Displacement of the chloro substituent of **3** with either ammonia or an amine yielded adenines **4–26** and **29–33**. The preparation of the 9-(2-tetrahydrofuryl)adenines **27** and **28** entailed the acid-catalyzed alkylation of either adenine or N^6-cyclopentyladenine by 2,3-dihydrofuran.[21] The synthesis of the N^6-substituted 2-chloro-9-methyladenines (**35–39**) commenced with the displacement of the 6-chloro substituent of 2,6-dichloro-

(14) Lohse, M. J.; Klotz, K.-N.; Dieckmann, E.; Friedrich, K.; Schwabe, U. 2′,3′-Dideoxy-N^6-cyclohexyladenosine: an adenosine derivative with antagonist properties at adenosine receptors. *Eur. J. Pharmacol.* 1988, *156*, 157–160.
(15) Bruns, R. F. Adenosine antagonism by purines, pteridines and benzopteridines in human fibroblasts. *Biochem. Pharmacol.* 1981, *30*, 325–333.
(16) Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W.; Daly, D. T.; Olsson, R. A. N^6-Substituted 9-methyladenines a new class of adenosine receptor antagonists. *FEBS Lett.* 1987, *215*, 203–208.
(17) Daly, J. W.; Padgett, W. L.; Eger, K. 7-Deaza-9-phenyladenines. A new class of adenosine receptor antagonists. *Biochem. Pharmacol.* 1988, *37*, 3749–3753.
(18) Muller, C. E.; Hide, I.; Daly, J. W.; Rothenhäusler, K.; Eger, K.; 7-Deaza-2-phenyladenines: structure–activity relationships of potent A_1 selective adenosine receptor antagonists. *J.Med. Chem.* 1990, *33*, 2822–2828.
(19) Robins, R. K.; Lin, H. H. Potential purine antagonists. I.V. Synthesis of some 9-methyl-6-substituted-purines. *J. Am. Chem. Soc.* 1957, *79*, 490–494.
(20) Montgomery, J. A.; Temple, C., Jr. Synthesis of potential anticancer agents. IX. 9-Ethyl-6-substituted purines. *J. Am. Chem. Soc.* 1957, *79*, 5283–5242.
(21) Bowles, W. A.; Schneider, F. H.; Lewis, L. R.; Robins, R. K. Synthesis and antitumor activity of 9-(tetrahydro-2-furyl) purine analogs of biologically important deoxynucleosides. *J. Med. Chem.* 1963, *6*, 471–480.

Scheme I[a]



[a] i. RNH_2. ii. $HC(OEt)_3$. iii. $R'NH_2$. iv. 2,3-Dihydrofuran. v. RNH_2. vi. CH_3I.

Table II. Receptor Binding and Adenylate Cyclase Stimulation[a] of N^9-Substituted Adenines

no.	N^6 substituent	K_i, μM: binding to rat cortex: A_1AR vs [^{3}H]PIA	K_i, μM: binding to rat striatum: A_2AR vs [^{3}H]NECA	selectivity ratio, A_2/A_1[b]	K_i, inhibition of rat PC12 adenylate cyclase stimulation by NECA, μM	k'[c]
		N^6-Substituted 9-Methyladenines				
4	H	69 ± 9	15 ± 3	0.22	24 (19–30)	0.11
7	c-C_5H_9	0.54 (0.45–0.67)	11 ± 1	26	25 (17–36)	1.29
9	c-C_6H_{11}	0.94 (0.4–2.2)	13 ± 1	14	21 (12–38)	
10	C_6H_5	6.9 ± 0.3	68 ± 4	9.9	107 (60–190)	
18	1-C_6H_5-2(*R*)-C_3H_6	2.5 ± 0.1	55 ± 7	22	25 (19–33)	
19	1-C_6H_5-2(*S*)-C_3H_6	11 ± 1	84 ± 11	7.6	74 (43–128)	
		N^6-Substituted 9-Ethyladenines				
20	H	46 ± 4	8.8 ± 1.7	0.19	5.6 ± 1.7	0.39
21	c-C_5H_9	0.44 ± 0.01	17 ± 2.5	39	10.3 ± 1.2	1.66
22	c-C_6H_{11}	2.0 ± 0.5	11 ± 2	5.5	8.5 ± 1.1	
		N^6-Substituted 9-(2-Hydroxyethyl)adenines				
23	H	>100 (30%)[d]	46 ± 10	<0.5	12 ± 2.6	0.04
24	c-C_5H_9	3.3 ± 0.6	47 ± 8	14	27 ± 1.9	0.68
		N^6-Substituted 9-Cyclopentyladenines				
25	H	1.8 ± 0.2	8.2 ± 1.4	4.6	3.4 ± 1.0	1.94
26	c-C_5H_9	1.2 ± 0.3	19 ± 1	16	5.1 ± 0.6	4.99
		N^6-Substituted 9-(2-Tetrahydrofuryl)adenines				
27	H	28 ± 3	23 ± 4	0.82	2.4 ± 0.5	0.21
28	c-C_5H_9	1.7 ± 0.5	39 ± 2	23	30 ± 1.4	1.81
		N^6-Substituted 9-Phenyladenines				
29	H	19 ± 1.3	38 ± 2.4	2.0	74 ± 22	0.56
30	c-C_5H_9	8.7 ± 0.3	79 ± 12	9.1	47 ± 23	3.91
31	C_6H_5	6.9 ± 1.6	111 ± 16	16	50 ± 8	
32	1-C_6H_5-2(*R*)-C_3H_6	4.7 ± 0.4	15 ± 4	3.2	11 ± 5	
33	1-C_6H_5-2(*S*)-C_3H_6	7.2 ± 0.9	18 ± 2	2.5	9.9 ± 0.8	
		N^6-Substituted 2-Chloro-9-methyladenines				
35	H	23 ± 2.3	11 ± 2	0.48	16 ± 2	0.22
36	c-C_5H_9	0.53 ± 0.0003	9.3 ± 1.1	18	6.3 ± 0.9	3.37
37	C_6H_5	7.0 ± 0.6	31 ± 5	4.4	21 ± 10	
38	1-C_6H_5-2(*R*)-C_3H_6	3.3 ± 0.1	6.8 ± 0.4	2.1	4.3 ± 0.7	
39	1-C_6H_5-2(*S*)-C_3H_6	2.8 ± 0.4	12 ± 1.7	4.3	17 ± 3	

[a] Values are means ± SEM (n = 3). Certain values are from a prior study[15] and are expressed as means with 95% confidence limits in parentheses (n = 3). [b] K_i of binding to rat brain striatum A_2AR + K_i of binding to rat brain cortex A_1AR. [c] Hydrophobicity index. See Experimental Section. [d] Value in parentheses if % inhibition at 100 μM.

purine (**34**) with either ammonia or an amine and then methylating N-9 with CH_3I.[22] Table I lists the analytical and physical data for these adenines.

(22) Sepiol, J.; Kazimierczuk, Z.; Shugar, D. Tautomerism of isoguanosine and solvent-induced keto-enol equilibrium. *Z. Naturforsch.* **1976**, *31c*, 361–370.

Affinity for A_1 and A_2 Adenosine Receptors

Table II reports the results of assays of binding to the A_1AR of rat brain cortex, of binding to the A_2AR of rat brain striatum, and of antagonism of the A_2AR-mediated

stimulation of *N*-ethyladenosine-5′-uronamide (NECA) of the adenylate cyclase in PC12 cells. The ratio of the inhibition constants derived from the radioligand binding assays is an index of the selectivity of these antagonists.

The adenines that lack an N^6 substituent, analogues **4**, **20**, **23**, **25**, **27**, **29**, and **35**, are weak antagonists and only two, **25** and **29**, are selective for the A_1AR. The prior study[16] showed that the N^6-cycloalkyl-9-methyladenines are potent antagonists at the A_1AR. The new data on the binding of **7** and **9** to the A_2AR shows that they are also selective for the A_1AR, the A_2/A_1 selectivity ratios being 26 and 14, respectively. N^6-(1-Phenyl-2(*R*)-propyl)adenine (**18**) is also selective for the A_1AR. The stereoselectivity as agonists at the A_1AR characteristic of certain N^6-substituted adenosines, such as the diastereomers of N^6-(1-phenyl-2-propyl)adenosine, is usually 50- to 100-fold.[23,24] Such stereoselectivity is absent in the case of the adenines **18** and **19**, **32**, and **33**, and also **38** and **39**. The enantiomers of a different class of antagonist, the 1,3-dipropyl-8-(1-phenyl-2-propyl)xanthines, have only a 5-fold difference in their affinities for the rat brain cortex A_1AR,[25] suggesting that low stereoselectivity may be an attribute of antagonists generally. However, 9-(1(*R*)-phenyl-1-ethyl)-2-phenyl-7-deazaadenine is an exception, being 35- and 22-fold more potent than the *S* enantiomer, respectively, at the A_1AR of rat brain cortex and A_2AR of rat brain striatum.[18]

At all three adenosine receptors, 9-ethyladenine (**20**) is somewhat more potent than **4**, and, like **4**, analogue **20** is selective for the A_2AR. The cyclopentyl and cyclohexyl analogues, **21** and **22**, are slightly more potent than their methyl congeners; **21** is almost twice as selective for the A_1AR than **7** but **22** is much less selective than **9**.

Like **4** 9-(2-hydroxyethyl)adenine (**23**) is a weak antagonist slightly selective for the A_2AR and, also like the 9-methyladenines, the N^6-cyclopentyl substituent of **24** greatly improves affinity and, thereby, selectivity for the A_1AR.

The activity of 9-cyclopentyladenine (**25**) is remarkable in two respects. It is by far the most potent of the 9-substituted adenines not substituted at N^6 and it shares with 9-phenyladenine (**32**) the distinction of being slightly selective for the A_1AR. Perhaps because **25** is so potent, the additional cyclopentyl group of 6,9-dicyclopentyladenine (**26**) contributes only modestly to activity. Indeed, **26** is only half as potent at the A_1AR as its 9-methyl and 9-ethyl congeners, **4** and **20**, respectively. At the A_2AR of striatum, **28** is more active than **26**.

An N^6-cyclopentyl substituent greatly enhances the potency of 9-(2-tetrahydrofuryl)adenine (**27**). The N^6-cyclopentyl derivative **28** is 16-fold more potent at the A_1AR than **27** and is 23-fold selective for the A_1AR.

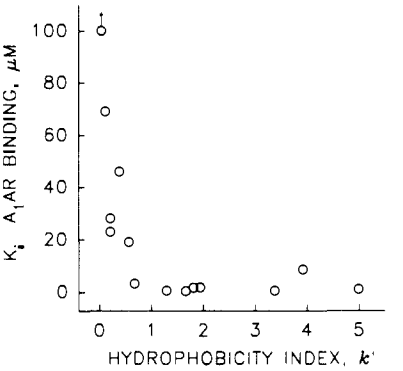


Figure 1. Relationship between K_i, the constant of the inhibition of the binding of [^{3}H]*R*-PIA to the A_1AR of the rat brain cortex, and *k′*, an index of hydrophobicity. Note that hydrophobicity strongly increases binding affinity.

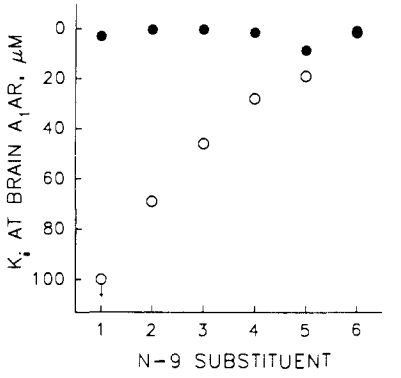


Figure 2. Effect of N^6- and N-9-substituents on the affinity of adenine for the A_1AR of rat cerebral cortex. Note that the affinity of an N-9-substituted adenine (open circles) increases according to the hydrophobicity of the substituent: 1, hydroxyethyl; 2, methyl; 3, ethyl; 4, tetrahydrofuryl; 5, phenyl; and 6, cyclopentyl. Note also that the affinity of a N^6-cyclopentyl-9-substituted-adenine (closed circles) is always higher than that of the corresponding 9-substituted adenine and also is independent of the hydrophobicity of the N-9-substituent.

9-Phenyladenine (**29**) shares with 9-cyclopentyladenine the property of slight selectivity for the A_1AR, in this instance 2- and 4-fold. N^6 substituents improve affinity for the A_1AR by as much as 4.5-fold. As in the case of the other adenines with asymmetric centers in the N^6 substituent, the stereoselectivity of **32** and **33** is low.

A 2-chloro substituent has little or no effect on the affinity of an N^6-substituted 9-methyladenine. The activity of 2-chloro-9-methyl-N^6-phenyladenine (**37**) is 3.6 times greater than that of its deschloro analogue **10**, but **36**, **38**, and **39** are essentially equipotent with **7**, **18**, and **19**, respectively. Chlorination increases affinity for the A_2AR of striatum and PC12 cells by 2- and 8-fold. As a consequence, a 2-chloro substituent tends to lower selectivity for the A_1AR and thereby abolishes any stereoselectivity conferred by an asymmetric center in the N^6 substituent.

Structure–Activity Relationships

The potency ranking of the 9-substituted adenines at the A_1AR, cyclopentyl > phenyl > 2-tetrahydrofuryl > ethyl > methyl > 2-hydroxyethyl, suggests that the hydrophobicity of the N-9 substituent determines affinity for this receptor. Size could also be important; the three adenines with cyclic substituents are more potent than the three with *n*-alkyl substituents. However, within each series as well as overall, the affinity of binding to the A_1AR of brain cortex depends strongly on hydrophobicity (Figure 1). In other words, the adenines having large substituents

(23) Smellie, F. W.; Daly, J. W.; Dunwiddie, T. V.; Hoffer, B. J. Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. *Life Sci.* **1979**, *24*, 2475–2482.

(24) Ukena, D.; Olsson, R. A.; Daly, J. W. Definition of subclasses of adenosine receptors associated with adenylate cyclase:interaction of adenosine analogues with inhibitory A_1 receptors and stimulatory A_2 receptors. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365–376.

(25) Peet, N. W.; Lentz, N. L.; Meng, E. C.; Dudley, N. W.; Ogden, A. M. L.; Demeter, D. A.; Weintraub, H. J. R.; Bey, P. A. A novel synthesis of xanthines: support for a new binding mode for xanthines with respect to adenosine at adenosine receptors. *J. Med. Chem.* **1990**, *33*, 3130–3127.

seem to be more potent because these substituents are hydrophobic, not because they are large. Neither the K_i of inhibition of A_2AR-mediated stimulation of the adenylate cyclase of PC12 cells nor the K_i of inhibition of the binding of [^{3}H]NECA to the A_2AR of striatum correlated with the hydrophobicity index (data not shown).

Figure 2 shows that whereas the affinities of the 9-substituted adenines vary over 2 orders of magnitude according to the nature of the 9-substituent. The N^6-cyclopentyladenines have higher affinities for the A_1AR, those affinities vary over a narrower range and the potency ranking among the N^6-cyclopentyl analogues is unrelated to that of the 9-substituted adenines. Such a result suggests that the contribution of the N^6-cyclopentyl group to affinity overshadows that of the 9-substituent.

At the A_2AR, the N^6,9-disubstituted adenines are usually less active than adenines without an N^6 substituent, probably because those substituents were chosen on the basis of the affinity of the corresponding N^6-substituted adenosines for the A_1AR.

In summary, certain N^6,9-disubstituted adenines are potent antagonists at the A_1AR of rat cerebral cortex and are active in the low micromolar and submicromolar range. Selectivity for the A_1AR over the A_2ARs of rat striatum or PC12 cells is an attribute of the 9-methyladenines. Although a 2-chloro substituent increases the agonist potency of N^6-substituted adenosines at the A_1AR, such a substituent has no effect on the antagonist potency of N^6-substituted 9-methyladenines at this receptor.

Experimental Section

Chemistry. Melting points were estimated on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra of samples dissolved in DMSO-d_6 were recorded on a Varian EM360L spectrometer and were consistent with the putative structures of the purines. For UV spectroscopy, purines were dissolved in absolute ethanol and diluted with 19 volumes of 125 mM NaCl, 25 mM $NaHPO_4$, pH 7.0. MHW Laboratories, Tucson, AZ, performed the elemental analyses, which agreed within ±0.4% of calculated composition. Assays of purity by reverse-phase HPLC revealed that product accounted for >99% of the UV-absorbing material in samples submitted for assay.

N^6-Cyclopentyl-9-methyladenine [N-Cyclopentyl-9-methyl-9H-purin-6-amine, 7]. A mixture of **3** (2.0 g, 11.9 mmol) cyclopentylamine (1.02 g, 12 mmol), N,N-disopropylethylamine (1.55 g, 12 mmol) and 100 mL of absolute ethanol was refluxed overnight and evaporated and the residue recrystallized from ethanol–water to yield 1.56 g (65%) of a white solid: ^{1}H NMR δ 1.62 (br s, 8 H, cyclopentyl), 3.74 (s, 3 H, CH_3N), 4.68 (m, 1 H, C*H*NH), 7.35 (br d, 1 H, NH), 8.00 (s, 1 H, H-2), 8.22 (s, 1 H, H-8).

2-Chloro-N^6-cyclopentyl-9-methyladenine [2-Chloro-N-cyclopentyl-9-methyl-9H-purin-6-amine, 36]. A solution of 2,6-dichloropurine (3.0 g, 15.9 mmol), cyclopentylamine (1.5 g, 17.6 mmol) and N,N-diisopropylethylamine (3.1 mL, 17.8 mmol) in 100 mL of 1-propanol was refluxed for 20 h and evaporated in vacuo to give a light yellow solid. The residue was suspended in water and filtered, and the precipitate was washed with water. Recrystallization from ethanol–water yielded 2.8 g (74%) of colorless product. A mixture of 2.7 g (11.36 mmol) of this product and K_2CO_3 (1.7 g, 12.3 mmol) in 20 mL of DMF was heated to dissolve the purine, cooled, and treated overnight with CH_2I (1.0 mL, 16.06 mmol). The solvent was evaporated and the yellow residue was triturated with water and recrystallized from ethanol–water to yield 2.4 g (84%) of product: ^{1}H NMR δ 1.40–2.20 (br s, 8 H, cyclopentyl). 3.75 (s, 3 H, CH_3), 4.68 (m, 1 H, C*H*NH) 7.02 (m, 1 H, NH), 7.70 (s, 1 H, H-8).

N^6-(1-Phenyl-2(R)-propyl)-9-phenyladenine [N-(1-Phenyl-2(R)-propyl)-9-phenyl-9H-purin-6-amine, 32]. A solution of 6-chloro-N^4-phenyl-4,5-pyrimidinediamine (4.1 g, 18.6 mmol) and 1 drop of ethanesulfonic acid in 80 mL of triethyl orthoformate was stirred for 48 h at room temperature and diluted with 150 mL of hexane. After 2–3 h the precipitate was filtered and washed with hexane to give 3.8 g (89%) of 6-chloro-9-methylpurine, mp 197 °C. A solution of the chloropurine (1.2 g, 5.2 mmol), (R)-amphetamine (0.84 g, 6.2 mmol), and N,N-diisopropylethylamine (1.1 mL, 6.2 mmol) in 50 mL of dry 1-propanol was refluxed for 24 h and evaporated in vacuo. The residue was dissolved in 70% methanol in water and purified by low-pressure LC as described in Table I. Evaporation of fractions containing product yielded 1.5 g (88%) of a white foam: ^{1}H NMR δ 1.30 (d, 3 H, C*H*$_3$), 3.00 (d, 2 H, C*H*$_2$), 4.85 (m, 1 H, CHNH), 7.10–8.12 (m, 6 H, phenyl and NH) 8.36 (s, 1 H, H-2), 8.60 (s, 1 H, H-8).

N^6-Cyclopentyl-9-(2-tetrahydrofuryl)adenine [N-Cyclopentyl-9-(2-tetrahydrofuryl)-9H-purin-6-amine, 28]. A solution of N^6-cyclopentyladenine (0.95 g, 4.66 mmol), 2,3-dihydrofuran (0.38 g, 5.42 mmol), and 6 drops of ethanesulfuric acid in 20 mL of dry ethyl acetate was heated overnight at 50 °C. Workup consisted of neutralization with 1 mL ammonia diluted with 20 mL of water, back-extraction of the water layer with 2 × 20 mL of ethyl acetate, drying ($MgSO_4$), and evaporation of the ethyl acetate. Purification as described in Table I yielded 400 mg (31%) of product: ^{1}H NMR ($CDCl_3$) δ 1.60–2.68 (m, 12 H, cyclopentyl and furyl 3-H and 4-H), 4.16 (m, 2 H, furyl 5-H), 4.70 (m, 1 H, C*H*NH), 5.80 (br d, 1 H, CH N*H*), 6.25 (t, 1 H, furyl 2-H), 7.88 (s, 1 H, H-2), 8.40 (s, 1 H, H-8).

Assays. The inhibition of the binding of [^{3}H]N^6-(1-phenyl-2R-propyl)adenosine to rat cortical membranes[26] and inhibition of the binding of [^{3}H]-N-ethyladenosine-5′-uronamide to rat striatal membranes in the presence of 50 nM N^6-cyclopentyladenosine[3] measured affinity for the A_1AR and A_2AR, respectively. In both assays, binding in the presence of 5 mM theophylline defined unspecific binding. The Cheng–Prusoff equation[27] calculated the inhibition constant, K_i, from measurements of IC_{50}. We used previously described methods[3,28] to assay antagonism of the A_2AR-mediated stimulation of adenylate cyclase of PC12 cells by N-ethyladenosine-5′-uronamide. The retention time of a nucleoside on a reverse-phase HPLC column served for the calculation[29] of a hydrophobicity index k', by the formula $k' = (t - t_o)/t_o$, where t is the retention time of the solute and t_o is the transit time of the solvent. In the present experiments the mobile phase consisted of a mixture containing 35% 10 mM $NaHPO_4$, pH 7.0, and 65% CH_3OH.

Acknowledgment. This work was supported by NIH HL-30391, by Whitby Research, Inc., Richmond, VA, and by the Ed C. Wright Chair in Cardiovascular Research at the University of South Florida. The authors thank Ms. Patricia Botero and Dr. Masayuki Ueeda for preparing the manuscript.

Registry No. **2** (R = Ph), 41259-65-8; **3** (R = Me), 2346-74-9; **3** (R = Et), 5462-86-2; **3** (R = CH_2CH_2OH), 1670-62-8; **3** (R = cyclopentyl), 5444-81-5; **3** (R = Ph), 5470-24-6; **4**, 700-00-5; **5**, 109292-94-6; **6**, 109292-90-2; **7**, 109292-91-3; **8**, 135394-01-3; **9**, 109292-93-5; **10**, 84602-82-4; **11**, 109292-95-7; **12**, 5440-16-4; **13**, 109292-96-8; **14**, 135394-02-4; **15**, 135394-03-5; **16**, 135394-04-6; **17**, 135394-05-7; **18**·HCl, 135394-06-8; **18** (free base), 109293-00-7; **19**·HCl, 135394-07-9; **19** (free base), 109293-01-8; **20**, 2715-68-6; **21**, 135394-08-0; **22**, 135394-09-1; **23**, 707-99-3; **24**, 135394-10-4; **25**, 715-91-3; **26**, 135394-11-5; **27**, 17318-31-9; **28**, 135394-12-6; **29**, 20145-09-9; **30**, 135394-13-7; **31**, 135394-14-8; **32**, 135394-15-9; **33**,

(26) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. [^{3}H]-Xanthine amine congener of 1,3-dipropyl-8-phenylxanthine: An antagonist radioligand for adenosine receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4089–4093.

(27) Cheng, Y. C.; Prusoff, W. H. Relationships between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(28) Ukena, D.; Daly, J. W.; Kirk, K. L.; Jacobson, K. A. Functionalized congeners of 1,3-dipropyl-8-phenylxanthine:potent antagonists for adenosine receptors that modulate membrane adenylate cyclase in pheochromocytoma cells, platelets and fat cells. *Life Sci.* **1986**, *38*, 797–807.

(29) Brent, D. A.; Sabatka, J. J.; Minick, D. J.; Henry, D. W. A simplified high-pressure liquid chromatography method for determining lipophilicity for structure–activity relationships. *J. Med. Chem.* **1983**, *26*, 1014–1020.

135426-10-7; **34**, 5451-40-1; **35**, 7013-21-0; **36**, 135394-16-0; **37**, 135394-17-1; **38**, 135394-18-2; **39**, 135394-19-3; $PhNH_2$, 62-53-3; $2\text{-}FC_6H_4NH_2$, 348-54-9; $PhCH_2NH_2$, 100-46-9; $PhCH_2CH_2NH_2$, 64-04-0; $H_2NCH_2CHPh_2$, 3963-62-0; $H_2NCH_2CH_2C_6H_2(OMe)_3$, 135394-20-6; (*R*)-$PhCH_2CH(NH_2)CH_3$, 156-34-3; (*S*)-$PhCH_2CH(NH_2)CH_3$, 51-64-9; MeI, 74-88-4; $HC(OEt)_3$, 122-51-0; H_2NCHEt_2, 616-24-0; cyclobutylamine, 2516-34-9; cyclopentylamine, 1003-03-8; 1-methylcyclopentylamine, 40571-45-7; cyclohexylamine, 108-91-8; 2-(2-pyridyl)ethylamine, 2706-56-1; 2-(3-thienyl)ethylamine, 59311-67-0; adenine, 73-24-5; N^6-cyclopentyladenine, 103626-36-4; 2,3-dihydrofuran, 1191-99-7; 2-chloro-N^6-cyclopentyladenine, 135394-21-7; adenosine, 58-61-7; adenylate cyclase, 9012-42-4.

Interphenylene 7-Oxabicyclo[2.2.1]heptane Thromboxane A_2 Antagonists. Semicarbazone ω-Chains†

Raj N. Misra,* Baerbel R. Brown, Wen-Ching Han, Don N. Harris, Anders Hedberg, Maria L. Webb, and Steven E. Hall

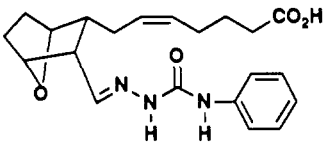
Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000.
Received March 21, 1991

A series of chiral interphenylene 7-oxabicyclo[2.2.1]heptane semicarbazones **19–26** were prepared and evaluated for their in vitro thromboxane (TxA_2) antagonistic activity and in vivo duration of action. The potency of **19–26** was found to highly dependent on the substitution pattern of the interphenylene ring and decreased in the order ortho > meta ≫ para. SQ 35,091 (**25**), [1*S*-(1α,2α,3α,4α)]-2-[[3-[[[(phenylamino)carbonyl]hydrazono]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]methyl]benzenepropanoic acid, was identified as a potent and long-acting TxA_2 antagonist. In human platelet rich plasma SQ 35,091 inhibitied arachidonic acid (800 μM) and U-46,619 (10 μM) induced aggregation with I_{50} values of 3 and 12 nM, respectively. In contrast, no inhibition of ADP (20 μM) induced aggregation was observed at >1000 μM. Receptor binding studies with [^{3}H]-SQ 29,548 showed SQ 35,091 was a competitive antagonist with a K_d value of 1.0 ± 0.1 nM in human platelet membranes. In vivo SQ 35,091 (0.2 mg/kg po) showed extended protection (T_{50} = 16 h) from U-46,619 (2 mg/kg iv) induced death in mice. These compounds have for the first time demonstrated that a metabolically stable interphenylene α-sidechain can be introduced into a prostanoid-like series of TxA_2 antagonists with the maintainance of potent antagonistic activity.

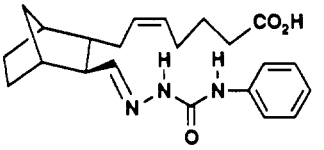
Introduction

Thromboxane A_2 (TxA_2)[1] is an extremely potent, short-lived endogenous mediator which induces both platelet activation and aggregation, and smooth muscle contraction. The biological activities of TxA_2 have implicated it as a contributor in the pathogenesis of thrombotic and vasospastic disorders.[2] However, in order to establish a definitive connection between TxA_2 and specific diseases it has been necessary to examine models in which the activities of TxA_2 can be experimentally elicited and/or suppressed. This has prompted the development of stable, selective TxA_2 agonists and antagonists as pharmacological tools. These compounds have demonstrated that TxA_2 mimics are able to elicit and TxA_2 antagonists are able to block a number of cardiovascular abnormalities and suggest that antagonists possessing suitable pharmacokinetic and pharmacodynamic properties have the important clinical potential to be developed as useful therapeutic agents.[2b,3]

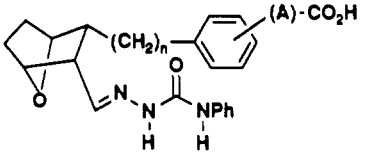
Nearly 10 years ago bicyclic semicarbazones SQ 27,825[4] and EP-045[5] were found to act as selective TxA_2 antagonists of moderate potency as measured by their ability to inhibit arachidonic acid induced platelet aggregation (AAIPA). As in the case of many prostaglandin analogues, both SQ 27,825 and EP-045 contain a metabolically labile 5(*Z*)-heptenoic acid side chain (α-chain) which is subject to in vivo β-oxidation. This process generally results in a rapid loss of antagonist activity and consequently limited in vivo duration of action.[6a,b] As part of a program to develop an orally active TxA_2 antagonist with an extended in vivo duration of action we have attempted to identify a metabolically stable surrogate for the 5(*Z*)-heptenoic acid side chain which is compatible with potent antagonist activity. Thus, we have prepared and evaluated a series of chiral 7-oxabicyclo[2.2.1]heptane analogues of SQ 27,825 (**1**) in which a metabolically stable interphenylene group



SQ 27,825



EP-045



1

has replaced the olefin α-side chain.[6c] The synthesis of this series of antagonists is described and the effect of the

† Presented at the 200th American Chemical Society National Meeting, Washington, DC, August 26–31, 1990; paper MEDI 118.

(1) Hamberg, M.; Svensson, J.; Samuelsson, B. Thromboxanes: A New Group of Biologically Active Compounds Derived From Prostaglandin Endoperoxides. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2994–2998.

(2) (a) Halushka, P. V.; Mais, D. E. Basic and Clinical Pharmacology of Thromboxane A_2. *Drugs of Today* **1989**, *25*(6), 383–393. (b) Smith, E. F., III. Thromboxane A_2 in Cardiovascular and Renal Disorders: Is There a Defined Role for Thromboxane Receptor Antagonists or Thromboxane Synthase Inhibitors? *Eicosanoids* **1989**, *2*, 199–212. (c) Ogletree, M. L. Overview of Physiological and Pathophysiological Effects of Thromboxane A_2. *Federation Proc.* **1987**, *46*(1), 133–138.

0022-2623/91/1834-2882$02.50/0