Effects of 8-Phenyl and 8-Cycloalkyl Substituents on the Activity of Mono-, Di-, and Trisubstituted Alkylxanthines with Substitution at the 1-, 3-, and 7-Positions

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The effects of 8-phenyl and 8-cycloalkyl substituents on the activity of theophylline, caffeine, 1,3-dipropylxanthine, 1,3-dipropyl-7-methylxanthine, 3-propylxanthine, and 1-propylxanthine at A_1 adenosine receptors of rat brain and fat cells and at A_2 adenosine receptors of rat pheochromocytoma PC12 cells and human platelets are compared. An 8-phenyl substituent has little effect on the activity of caffeine or 1,3-dipropyl-7-methylxanthine at adenosine receptors, while markedly increasing activity of theophylline, 1,3-dipropylxanthine, 1-isoamyl-3-isobutylxanthine, 1-methylxanthine, and 3-propylxanthine. 8-Phenyl-1-propylxanthine is potent (K_i = 20–70 nM) at all receptors. A *p*-carboxy or *p*-sulfo substituent, which is introduced on the 8-phenyl ring to increase water solubility, in most cases decreases the activity and selectivity for the A_1 receptor. Among the 8-*p*-sulfo analogues, only 8-(*p*-sulfophenyl)theophylline and 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine are selective for the A_1 receptors. 8-*p*-Sulfophenyl derivatives of caffeine, 1,3-dipropyl-7-methylxanthine, and 3-propylxanthine are somewhat selective for the A_2 receptors. 8-Cycloalkyl substituents (cyclopentyl, cyclohexyl) markedly increase activity of caffeine and 1,3-dipropyl-7-methylxanthine at the A_2 receptor. 8-Cyclohexylcaffeine is potent (K_i = 190 nM) and very selective for the human platelet A_2 receptors, but is not as selective for the rat PC12 cell A_2 receptor. Such A_2 selectivity is in contrast to the marked A_1 selectivity of 8-cycloalkyltheophyllines and 8-cycloalkyl-1,3-dipropylxanthines. The apparent selectivity of *certain* xanthines is dependent on the assay systems that are compared.

The methylxanthines caffeine and theophylline exhibit a variety of pharmacological actions, many of which are undoubtedly due to antagonism of A_1 and/or A_2 adenosine receptors.[1] Certain actions of xanthines, for example, bronchodilation and resultant antiasthmatic effects, may, however, be due to inhibition of phosphodiesterases[2] rather than to blockade of adenosine receptors. Thus, enprofylline (3-propylxanthine) is much more potent than theophylline as a bronchodilator,[3] but has very weak activity as an adenosine antagonist compared to theophylline.[4] Enprofylline is a more potent phosphodiesterase inhibitor than theophylline.[4] 8-Phenyl or 8-cycloalkyl substituents markedly enhance the activity of theophylline and 1,3-dipropylxanthine at A_1 adenosine receptors and to a lesser extent at A_2 adenosine receptors.[5-13] The 8-phenyl- and 8-cycloalkyl-1,3-dialkylxanthines have low activity as phosphodiesterase inhibitors.[13-15]

The effects of varying the alkyl substituents at the 1,3-positions and of substituents at the 8-position on the activity of xanthines at adenosine receptors are well known.[5-13] Propyl moieties at 1- and 3-positions confer higher potency than ethyl or methyl moieties, particularly at the A_1 receptors; thus, 1,3-dipropylxanthine (**14**) is significantly more potent than theophylline (**1**).[5,11] Larger alkyl groups at the 1- and 3-positions are not as well tolerated and 1-isoamyl-3-isobutylxanthine (**27**) has virtually no activity as an antagonist at a brain A_2 adenosine receptor[26] and is no more potent than theophylline at brain A_1 adenosine receptors.[6] The very high potency of 1,3-dipropyl-8-phenylxanthine (**15**) at adenosine receptors is attributed mainly to the presence of a phenyl group at the 8-position. 8-Cycloalkyl moieties (cyclopentyl, cyclohexyl) in theophylline and 1,3-dipropylxanthine markedly increase activity at A_1 receptors, while increasing activity only moderately at A_2 receptors, resulting in highly potent and selective A_1 receptor antagonists.[10-13] 8-Cycloalkyl-1,3-dialkylxanthines have moderate solubilities in water and have, because of marked potency and selectivity for A_1 receptors, proven to be valuable research tools.[10-13] The 8-phenyl-1,3-dialkylxanthines have not proven completely satisfactory as research tools, probably because of very low water solubility. Polar substituents, such as *p*-carboxy and *p*-sulfo on the 8-phenyl ring increase the water solubility significantly,[6,10] but in most cases result in a loss of activity and selectivity at A_1 receptors.[6] The highly water soluble 8-(*p*-sulfophenyl)theophylline and 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine in spite of a lack of marked selectivity for A_1 or A_2 receptors have proven useful research tools in a number of physiological systems.[1,16-25] The sulfo

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(1) Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.
(2) Polson, J. B.; Krazanowski, J. J.; Szentivanyi, A. *Biochem. Pharmacol.* **1982**, *31*, 3403.
(3) Persson, C. G. A.; Karlsson, J.-A.; Erjefält, I. *Life Sci.* **1982**, *30*, 2181.
(4) Ukena, D.; Schirren, C. G.; Schwabe, U. *Eur. J. Pharmacol.* **1985**, *117*, 25.
(5) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547.
(6) Daly, J. W.; Padgett, W. L.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem.* **1985**, *28*, 487.
(7) Daly, J. W.; Padgett, W. L.; Shamim, M. T. *J. Med. Chem.* **1986**, *29*, 1520.
(8) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2077.
(9) Ukena, D.; Daly, J. W.; Kirk, K. L.; Jacobson, K. A. *Life Sci.* **1986**, *38*, 297.
(10) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W. *J. Med. Chem.* **1988**, *31*, 613.
(11) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, J. J.; Huang, C. C. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59.
(12) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(13) Martinson, E. A.; Johnson, R. A.; Wells, J. M. *Mol. Pharmacol.* **1987**, *31*, 247.
(14) Smellie, F. W.; Davis, C. W.; Daly, J. W.; Wells, J. N. *Life Sci.* **1979**, *24*, 2475.
(15) Wu, P. H.; Phillis, J. W.; Nye, M. J. *Life Sci.* **1982**, *31*, 2857.
(16) Burnstock, G.; Hoyle, C. H. V. *Br. J. Pharmacol.* **1985**, *85*, 291.
(17) Collis, M. G.; Palmer, D. B.; Saville, V. L. *J. Pharm. Pharmacol.* **1985**, *37*, 278.
(18) Evoniuk, G.; Von Borstel, R. W.; Wurtman, R. J. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 428.
(19) Wiklund, N. P.; Gustafsson, L. E.; Lundin, J. *Acta Physiol. Scand.* **1985**, *125*, 681.
(20) Gustafsson, L. E.; Wiklund, N. P. *Br. J. Pharmacol.* **1986**, *88*, 197.
(21) Gustafsson, L. E.; Wiklund, N. P.; Cederquist, B. *Eur. J. Pharmacol.* **1986**, *120*, 179.

Table I. Effects of 8-Phenyl and 8-Cycloalkyl Substituents on the Activity of Caffeine and Theophylline Analogues at A_1 and A_2 Adenosine Receptors

no.	xanthine	A_1: K_i, μM, versus[a] [^{3}H]*R*-PIA binding rat brain membrane	A_2: K_i, μM, versus[b] NECA stimulation human platelet membrane	ratio A_2/A_1
1	theophylline	13 (11–15)	14 (4–18)[c]	1.1
2	8-phenyltheophylline	0.76 (0.58–0.98)	1.9 (0.5–6.8)[c]	2.5
3	8-(*p*-carboxyphenyl)theophylline	3.0 (2.3–3.9)	1.5 (0.48–4.6)	0.5
4	8-(*p*-sulfophenyl)theophylline	1.0 (0.77–1.4)	5.5 (1.6–9)[c]	5.5
5	8-cyclopentyltheophylline	0.024 (0.017–0.034)	0.14 (0.14–0.15)	5.8
6	8-cyclohexyltheophylline	0.11 (0.07–0.16)	0.15 (0.076–0.29)	1.4
7	caffeine	44 (31–63)	30 (16–54)[c]	0.68
8	8-phenylcaffeine	15 (12–18)	14 (10–20)	0.93
9	8-(*p*-carboxyphenyl)caffeine	61 (48–77)	150 (110–230)	2.4
10	8-[*p*-(methylcarboxy)phenyl]caffeine	43 (38–49)	25 (12–53)	0.58
11	8-(*p*-sulfophenyl)caffeine	inactive	57 (28–116)	
12	8-cyclopentylcaffeine	33 (27–40)	2.4 (0.72–7.8)	0.073
13	8-cyclohexylcaffeine	28 (15–51)	0.19 (0.12–0.32)	0.007
14	1,3-dipropylxanthine[c]	0.71 (0.67–0.75)	7.4 (1.7–32)	10
15	1,3-dipropyl-8-phenylxanthine[c]	0.01 (0.006–0.018)	2.1 (1.3–3.6)	210
16	8-(*p*-carboxyphenyl)-1,3-dipropylxanthine[c]	0.2 (0.17–0.25)	0.32 (0.23–0.44)	1.6
17	1,3-dipropyl-8-(*p*-sulfophenyl)xanthine[c]	0.14 (0.11–0.2)	1.9 (1.3–3.9)	14
18	8-cyclopentyl-1,3-dipropylxanthine[c]	0.0009 (0.0008–0.0011)	0.14 (0.12–0.17)	160
19	8-cyclohexyl-1,3-dipropylxanthine[c]	0.0015 (0.0011–0.0021)	0.19 (0.17–0.21)	130
20	1,3-dipropyl-7-methylxanthine	3.4 (3.5–4.4)	2.8 (1.4–5.7)[c]	0.82
21	1,3-dipropyl-7-methyl-8-phenylxanthine	2.3 (1.1–5.0)	2.4 (1.3–4.6)	1.0
22	8-(*p*-carboxyphenyl)-1,3-dipropyl-7-methylxanthine	4.8 (3.4–6.9)	13 (9–19)	2.7
23	1,3-dipropyl-7-methyl-8-[*p*-(methylcarboxy)phenyl]xanthine	3.1 (2.8–3.3)	26 (23–31)	8.4
24	1,3-dipropyl-7-methyl-8-(*p*-sulfophenyl)xanthine	15 (10–22)	2.1 (1.9–2.4)	0.14
25	8-cyclopentyl-1,3-dipropyl-7-methylxanthine	2.3 (2.1–2.4)	0.22 (0.065–0.73)	0.098
26	8-cyclohexyl-1,3-dipropyl-7-methylxanthine	2.7 (1.6–4.5)	0.085 (0.038–0.19)	0.031
27	1-isoamyl-3-isobutylxanthine	1.6 (1.1–2.1)[d]	6.5 (3.8–11)	4.0
28	1-isoamyl-3-isobutyl-8-phenylxanthine	1.8 (1.5–2.2)[e]	2.3 (1.5–3.5)	1.3
29	1-isoamyl-3-isobutyl-8-(*p*-sulfophenyl)xanthine	1.2 (0.34–3.8)	0.52 (0.18–1.5)	0.43
30	3-methylxanthine	35 (32–39)	240 (110–520)	6.9
31	3-propylxanthine	81 (64–102)	130 (108–156)	1.6
32	8-phenyl-3-propylxanthine	9.8 (8.0–12.4)	11 (6.7–20)	1.2
33	8-(*p*-carboxyphenyl)-3-propylxanthine	57 (29–111)	14 (8.0–24)	0.24
34	3-propyl-8-(*p*-sulfophenyl)xanthine	58 (38–88)	2.7 (1.5–5)	0.05
35	8-cyclohexyl-3-propylxanthine	0.85 (0.36–2)	1.1 (0.32–3.9)	1.3
36	1-methylxanthine	17 (15–20)	1.9 (1.7–2.1)	0.11
37	1-methyl-8-phenylxanthine	0.3 (0.12–0.75)	0.86 (0.39–1.9)	2.9
38	8-phenyl-1-propylxanthine	0.067 (0.04–0.116)	0.02 (0.006–0.07)	0.33

[a] Values (n = 3) are means (95% confidence limits) for inhibition of [^{3}H]-N^6-(phenylisopropyl)adenosine binding to rat brain membranes as described.[31] [b] Values (n = 3) are means (95% confidence limits) for inhibition of NECA-elicited stimulation of adenylate cyclase in human platelet membranes as described.[32] [c] Value from ref 9, 10, or 27. [d] The K_i value is significantly lower than the K_i value reported for inhibition of [^{3}H]cyclohexyladenosine binding in ref 6. [e] Value for inhibition of binding of [^{3}H]cyclohexyladenosine from ref 6.

analogues do not penetrate into cells,[25] thus eliminating any side effects on intracellular enzymes.

In further attempts to develop selective A_1 or A_2 adenosine receptor antagonists, 8-phenylcaffeine was synthesized.[6] The 8-phenyl substituent did not markedly or selectively enhance antagonist activity at A_1 or A_2 adenosine receptors. The effects of the 8-phenyl moiety and 8-cycloalkyl moieties and the presence of *p*-carboxy and *p*-sulfo substituents on the 8-phenyl moiety in caffeines, 1,3-dipropyl-7-methylxanthines, and 3-propylxanthines have now been determined with respect to activity at the A_1 adenosine receptor in rat cerebral cortical membranes and the A_2 adenosine receptor in human platelet membranes. 8-Phenyl-1-methyl- and 8-phenyl-1-propylxanthine also are described. The biological effects are compared to those in analogous 8-phenyl and 8-cycloalkyl derivatives of theophyllines, 1,3-dipropylxanthines, and 1-isoamyl-3-isobutylxanthines. A select subgroup of xanthines has been investigated with respect to activity at the A_1 receptor in rat fat cell membranes and the A_2 receptor in rat pheochromocytoma PC12 cell membranes.

Results and Discussion

The effect of substituted 8-phenyl moieties on activities of theophylline and 1,3-dipropylxanthine has been extensively studied,[5-13] but little is known of the effects of an 8-phenyl moiety or of substituted 8-phenyl moieties on activities of other xanthines, containing a range of alkyl groups at the 1-, 3-, and 7-positions. In the present study we have compared the effects of 8-phenyl moieties alone or with *p*-carboxy and *p*-sulfo substituents and of 8-cycloalkyl moieties such as cyclopentyl and cyclohexyl on the activity of caffeine, 1,3-dipropyl-7-methylxanthine, 1-isoamyl-3-isobutylxanthine, 1-methylxanthine, 1-propylxanthine, and 3-propylxanthine.

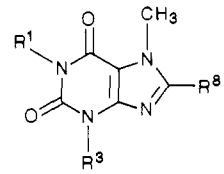
An 8-phenyl group in caffeine does not result in a large increase in activity at either A_1 or A_2 receptors.[6] Thus, 8-phenylcaffeine (**8**) is only slightly more potent than caffeine (**7**) and is nonselective (Table I). An 8-(*p*-carboxyphenyl)caffeine (**9**) is even less active than caffeine (**7**) and is only 2-fold selective for the rat brain A_1 receptor. 8-(*p*-Sulfophenyl)caffeine (**11**, Figure 1) has virtually no activity at the rat brain or fat cell A_1 receptor and modest activity at the A_2 receptors, making it weak but selective for the A_2 receptors (Tables I and II). In contrast to

(22) Hoffman, B. B.; Dall'aglio, E.; Hollenbeck, C.; Chang, H.; Reaven, G. M. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 715.
(23) Wiklund, N. P.; Samuelson, U. E.; Brundin, J. *Eur. J. Pharmacol.* **1986**, *123*, 11.
(24) Westerberg, V. S.; Geiger, J. D. *Life Sci.* **1987**, *41*, 2201.
(25) Heller, L. J.; Olsson, R. A. *Am. J. Physiol.* **1985**, *248*, 907.

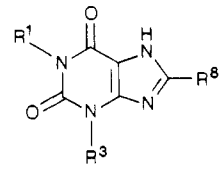
Table II. Comparison of Effects of Certain Xanthines at A_1 Adenosine Receptors and A_2 Adenosine Receptors

no.	A_1: K_i, μM, versus [^{3}H]R-PIA binding rat brain membrane[a]	A_1: K_i, μM, versus PIA inhibtn of adenylate cyclase rat fat cell membrane[b]	A_2: K_i, μM, versus NECA stimulation of adenylate cyclase	
			rat PC12 membrane[c]	human platelet membrane[d]
1	13 (11–15)	8.7 (5.1–15)	17 (16–19)	14 (4–18)
2	0.76 (0.58–0.98)	0.35 (0.2–0.6)	1.6 (0.4–6.1)	1.9 (0.5–6.8)
4	1.0 (0.77–1.4)	1.5 (1.3–1.7)	5.0 (1.4–10)	5.5 (1.6–9)
6	0.11 (0.07–0.16)	0.041 (0.027–0.062)	0.45 (0.29–0.69)	0.15 (0.076–0.29)
7	44 (31–63)	59 (40–86)	37 (26–53)	30 (16–54)
11	inactive	inactive	150 (67–360)	57 (28–116)
12	33 (27–40)	14.9 (6.29–35.5)	3.8 (1.9–7.7)	2.4 (0.72–7.8)
13	28 (15–51)	2.0 (1.3–3.1)	4.1 (2.5–6.6)	0.19 (0.12–0.32)
14	0.71 (0.67–0.75)	1.6 (0.66–3.7)	5.4 (4–7.3)	7.4 (1.7–32)
15	0.01 (0.006–0.018)	0.0059 (0.0026–0.0134)	2.3 (0.6–8.7)	2.1 (1.3–3.6)
17	0.14 (0.11–0.20)	0.43 (0.28–0.66)	11 (3.1–30)	1.9 (1.3–3.9)
18	0.0009 (0.0008–0.0011)	0.0006 (0.0004–0.001)	0.25 (0.1–0.59)	0.14 (0.12–0.17)
19	0.0015 (0.0011–0.0021)	0.0013 (0.0007–0.0022)	0.21 (0.062–0.71)	0.19 (0.17–0.21)
20	3.4 (3.5–4.4)	12 (6.7–22)	5.3 (3.9–7.2)	2.8 (1.4–5.7)
24	15 (10–22)	4.9 (2–12)	5.6 (3.6–8.7)	2.1 (1.9–2.4)
26	2.7 (1.6–4.5)	4.2 (3.3–5.2)	1.0 (0.44–2.4)	0.085 (0.038–0.19)
34	58 (38–88)	33 (22–51)	17 (5.9–51)	2.7 (1.5–5)
37	0.3 (0.12–0.75)	0.13 (0.042–0.43)	0.25 (0.053–0.12)	0.86 (0.39–1.9)
38	0.067 (0.04–0.116)	0.036 (0.01–0.13)	0.073 (0.018–0.29)	0.02 (0.006–0.07)

[a] Values (n = 3) are means (95% confidence limits) for inhibition of [^{3}H]-N^6-(phenylisopropyl)adenosine binding to rat brain membranes as described[31] (data from Table I). [b] Values (n = 3) are means (95% confidence limits) for inhibition of N^6-(phenylisopropyl)adenosine-elicited inhibition of adenylate cyclase in rat fat cell membranes as described.[32] [c] Values (n = 3) are means (95% confidence limits) for inhibition of NECA-elicited stimulation of adenylate cyclase in rat PC12 cell membranes or human platelet membranes as described[32] (data on platelets from Table I).



11: $R^1 = R^3 = CH_3$, $R^8 = C_6H_4SO_3H$
13: $R^1 = R^3 = CH_3$, R^8 = cyclohexyl
24: $R^1 = R^3 = n\text{-}C_3H_7$, $R^8 = C_6H_4SO_3H$
26: $R^1 = R^3 = n\text{-}C_3H_7$, R^8 = cyclohexyl



34: $R^1 = H$, $R^3 = n\text{-}C_3H_7$, $R^8 = C_6H_4SO_3H$

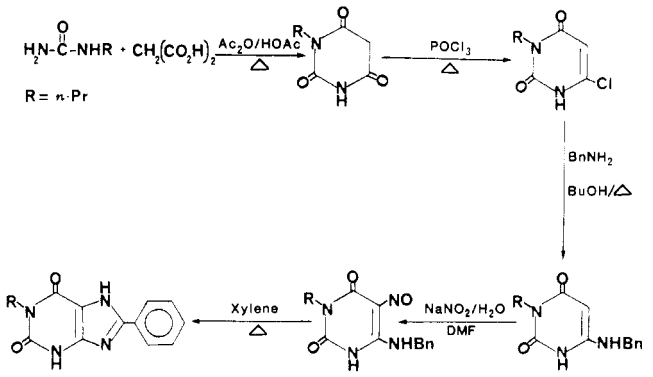
Figure 1. Prototypic xanthines with selectivity for human platelet A_2 adenosine receptors: comparison to A_1 adenosine receptors of rat brain and fat cells and to A_2 adenosine receptors of rat PC12 cells.

8-phenylcaffeine (**8**), both 8-cyclopentylcaffeine (**12**) and 8-cyclohexylcaffeine (**13**) are potent and selective for the human platelet A_2 receptor. This was unexpected, since 8-cyclopentyltheophylline (**5**) and 8-cyclohexyltheophylline (**6**) are potent and selective for the A_1 receptor (Table I and ref 10–13) and led to an examination of the potency of 8-cyclohexylcaffeine with other A_1 and A_2 receptors. 8-Cyclohexylcaffeine was found to be fully 20-fold less potent at the rat PC12 cell A_2 receptor than at the human platelet receptor (Table II). Remarkably it was also found to be 14-fold more potent at the rat fat cell A_1 receptor than at the rat brain A_1 receptor (Table II). This is one of the few instances where apparent potency derived from adenylate cyclase assays for blockade of A_1 receptor responses in rat fat cell membranes are *markedly* different than apparent potency derived from inhibition of ligand binding to A_1 receptor in rat brain membranes (see Table II). It would appear that the 7-methyl group of the 8-cycloalkylcaffeines selectively reduces affinity for rat brain A_1 receptors. The caffeine analogue 1,3-dipropyl-7-methylxanthine (**20**) is much less active than 1,3-dipropylxanthine (**14**) at rat brain A_1 receptors and is nonselective.[27] An 8-phenyl group in 1,3-dipropyl-7-methylxanthine has little effect on activity at the rat brain A_1 and human platelet A_2 receptors. The resulting 1,3-dipropyl-7-methyl-8-phenylxanthine (**21**) is less active than 1,3-dipropyl-8-phenylxanthine (**15**) and is nonselective. An 8-(*p*-carboxyphenyl)-1,3-dipropyl-7-methylxanthine (**22**), as expected, is even lower in activity for adenosine receptors than **21** and is only slightly selective for the rat brain A_1 receptor (Table I). The introduction of a *p*-sulfo substituent to the 8-phenyl moiety causes a significant decrease in activity at the rat brain A_1 receptor, while having no effect on activity at the human platelet A_2 receptor. The resulting 1,3-dipropyl-7-methyl-8-(*p*-sulfophenyl)xanthine (**24**) is moderately potent (2.1 μM) and selective for the human platelet A_2 receptor. It is not selective when activity at the rat PC12 cell A_2 receptor is compared to activity at the rat fat cell A_1 receptor (Table II). In the 8-cycloalkyl-1,3-dipropyl-7-methylxanthines both the 8-cyclopentyl (**25**) and 8-cyclohexyl (**26**) analogues are potent and selective for the human platelet A_2 receptor (Table I). In contrast, 8-cyclopentyl-1,3-dipropylxanthine (**18**) and 8-cyclohexyl-1,3-dipropylxanthine (**19**) are two of the most potent and selective antagonists for A_1 receptors.[10–13] As in the 8-cycloalkylcaffeine series, the addition of a 7-methyl group selectively reduces affinity of 8-cycloalkyl-1,3-dipropylxanthines for rat brain A_1 receptors. As in the case of 8-cyclohexylcaffeine (**13**), the 8-cyclohexyl-1,3-dipropyl-7-methylxanthine (**26**) was much less potent at the rat PC12 cell A_2 receptor than at the human platelet A_2 receptor (Table II). Unlike **13**, however, **26** had nearly equivalent potency at the rat brain and rat fat cell A_1 receptors.

In the 1-isoamyl-3-isobutylxanthine series the 8-phenyl analogue (**28**) was previously shown to have weak but se-

(26) Smellie, F. W.; Daly, J. W.; Wells, J. N. *Life Sci.* **1979**, *25*, 1917.
(27) Ukena, D.; Shamim, M. T.; Padgett, W.; Daly, J. W. *Life Sci.* **1986**, *39*, 743.

Scheme I



lective A_1 receptor activity.[6] In the present study with a different A_2 receptor assay **28** is nonselective (Table I). The 8-*p*-sulfophenyl analogue (**29**) was in the prior study[6] weak and nearly nonselective for A_1 and A_2 receptors, while in the present study with a different A_2 receptor assay it has a 7-fold selectivity for the A_2 receptor (Table I).

1-Alkylxanthines have not been studied in detail as adenosine receptor antagonists. In part this reflects the lack of a simple synthetic route for the preparation of 1-alkylxanthines. The present synthetic route is presented in Scheme I. The structural confirmation of the final product was based on comparative thin-layer and NMR analysis of 8-phenyl-1-propylxanthine (**38**) and 8-phenyl-3-propylxanthine (**32**) prepared by an unambiguous route (see the Experimental Section). 1-Methylxanthine (**36**) is relatively potent and selective for the human platelet A_2 receptor (Table I). The presence of an 8-phenyl moiety in 1-methylxanthine results in a 10-fold increase in activity at the rat brain A_1 receptor, while increasing activity only slightly at the human platelet A_2 receptor. The resulting 1-methyl-8-phenylxanthine (**37**) is somewhat more potent than 8-phenyltheophylline (**2**) at both A_1 and A_2 receptors (Table II). Replacement of 1-methyl group in 1-methyl-8-phenylxanthine (**37**) with a *n*-propyl moiety increases activity at both A_1 and A_2 receptors (Table II). The resulting 8-phenyl-1-propylxanthine (**38**) is very potent (20–70 nM) and is nonselective at either receptor (Table I). In contrast, for 1,3-dialkylxanthines, replacing the methyl groups of theophylline (**1**) with *n*-propyl groups, selectively increases activity at the A_1 receptor (Table I, compare **1** and **14**).

3-Methylxanthine (**30**) is a relatively weak adenosine receptor antagonist[28] and is less potent than theophylline as a tracheal relaxant.[3] The presence of a propyl group at the 3-position instead of a methyl yields enprofylline (**31**), a potent bronchodilator, reported to have little or no antagonistic activity at A_1 and A_2 receptors.[3] In the present study 3-methylxanthine is slightly more active than caffeine at adenosine receptors and has about a 7-fold selectivity for the rat brain A_1 receptor (Table I). Enprofylline is even less potent than caffeine at both adenosine receptors and, like caffeine, is nonselective. Introduction of the 8-phenyl group in 3-propylxanthine causes a significant increase in activity at both adenosine receptors (Table I). The resulting 8-phenyl-3-propylxanthine (**32**) is about 8-fold more potent than enprofylline and is nonselective. An 8-*p*-carboxy substituent (**33**) selectively reduces activity at the rat brain A_1 receptor. An 8-*p*-sulfo substituent also reduces activity at the rat brain A_1 receptor, while increasing activity at the human platelet A_2 receptor (Table I). The resulting 3-propyl-8-(*p*-sulfophenyl)xanthine (**34**) has 21-fold selectivity for the human platelet A_2 receptor compared to the rat brain A_1 receptor. But it is only 2-fold selective for the A_2 receptor when the rat PC12 cell A_2 receptor is compared to the rat fat cell A_1 receptor (Table II). Replacement of 8-phenyl group in 3-propylxanthines with 8-cyclohexyl moiety causes a 10-fold increase in activity at both receptors (Table I). 8-Cyclohexyl-3-propylxanthine (**35**) is the most potent of the 3-propylxanthine series, but is nonselective. It has been suggested that one structural requirement for a potent tracheal relaxant is the substitution at the 3-position, and that such 3-alkylxanthines may not cause adenosine receptor antagonism.[3] The effect of an 8-phenyl and 8-cycloalkyl substituents on activity of 3-propylxanthine in trachea as yet has not been determined.

In conclusion, several 1-alkylxanthines, 3-alkylxanthines, and 1,3,7-trialkylxanthines with 8-phenyl and 8-cycloalkyl substituents have been synthesized and their activity as antagonists at A_1 and A_2 adenosine receptors has been compared to analogous 8-phenyl derivatives of theophylline, 1,3-dipropylxanthine, and 1-isoamyl-3-isobutylxanthine. Among the 8-phenyl analogues, 8-phenyl-1-propylxanthine (**38**) proved to be very potent for both A_1 and A_2 adenosine receptors (Table II). 1,3-Dipropyl-8-phenylxanthine (**14**) on the other hand is highly potent and selective for the A_1 receptors (Table II). Alkyl groups larger than propyl reduce activity at both receptors. This is evident in the low activity of 1-isoamyl-3-isobutyl-8-phenylxanthine (**28**). The differential effects of 8-phenyl moieties on the potency of the parent mono-, di-, and trisubstituted alkylxanthines suggest that the binding of such xanthines differs significantly according to the substitution pattern, thereby changing the interaction of the receptor with the 8-phenyl moiety. 1,3-Dipropyl-8-(*p*-sulfophenyl)xanthine (**17**) remains the most potent adenosine receptor antagonist of the 8-(*p*-sulfophenyl)xanthines and exhibits a marked selectivity for A_1 receptors. This xanthine (**17**) was previously noted[9] to have a much lower potency at the A_2 receptor of rat PC12 cell membranes than at the A_2 receptor of human platelet membranes. The effect was ascribed to the para substituent lowering activity at the rat PC12 cell A_2 receptor. It has been used as a selective A_1 receptor antagonist in studies on adenosine receptors in myenteric nerve endings.[30] 8-(*p*-Sulfophenyl)theophylline (**4**) is less potent and is less selective for A_1 receptors (Table II). In contrast, 8-(*p*-sulfophenyl)caffeine (**11**) is inactive at A_1 receptors, but has relatively low activity at A_2 receptors. 1,3-Dipropyl-7-methyl-8-(*p*-sulfophenyl)xanthine (**24**) is moderately potent but relatively nonselective (Table II). The effect of the presence of a 7-methyl substituent is remarkable in 8-cycloalkylxanthines, where 8-cycloalkyl analogues of caffeine and 1,3-dipropyl-7-methylxanthine are potent and selective for the human platelet A_2 receptor, while 8-cycloalkyltheophyllines and 1,3-dipropyl-8-cycloalkylxanthines are potent and selective for the A_1 receptors (Tables I and II).

The present results (Table II) provide further evidence (see ref 1) that there are subclasses of A_2 receptors and perhaps subclasses of A_1 receptors. Certainly, the marked differences (>4-fold) in potency at the two A_2 receptors

(28) Daly, J. W.; Butts-Lamb, P.; Padgett, W. *Cell. Mol. Neurobiol.* **1983**, *3*, 69.

(29) L. E. Gustafsson (Karolinska Institutet, Stockholm) has also prepared 8-(*p*-sulfophenyl)enprofylline and kindly provided us with a sample for evaluation. The biological activity was similar to that of material synthesized at NIH.

(30) Christofi, F. L.; Cook, M. A. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 302.

for some xanthines (13, 17, 26, 34) indicate differences in xanthine recognition sites, especially in view of the fact that both receptors were assessed by an adenylate cyclase assay. Similarly, while activity of xanthines at a rat brain A_1 receptor, based on a ligand binding assay, usually corresponds well with activity, based on an adenylate cyclase assay in rat fat cell membranes, there is at least one xanthine (13) that shows a marked difference (14-fold) in these two systems. Further studies are needed to fully define differences among adenosine receptors presently assigned to either the A_1 or the A_2 receptor classes. At present, differences may relate to species, tissue, or assays.

Experimental Section

Mass spectra were determined with Finnegan 1015 quadrapole (chemical ionization with CH_4 or NH_3) and VG 70/70 (electron impact, 70 eV) mass spectrometers and were consistent with the structures. Melting points were taken on a Kofler block hot stage and are uncorrected. Thin-layer chromatographic analysis on silica gel with $CHCl_3$/MeOH (9:1) indicated the presence of a single compound in the final xanthine products. The synthesis of 8-(*p*-carboxyphenyl)theophylline (3), 8-(*p*-sulfophenyl)theophylline (4), 8-phenylcaffeine (8), 1,3-dipropylxanthine (14), 1,3-dipropyl-8-phenylxanthine (15), 8-(*p*-carboxyphenyl)-1,3-dipropylxanthine (16), 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine (17), 1,3-dipropyl-7-methylxanthine (20), 1-isoamyl-3-isobutylxanthine (27), 1-isoamyl-3-isobutyl-8-phenylxanthine (28), 1-isoamyl-3-isobutyl-8-(*p*-sulfophenyl)xanthine (29), 8-cyclopentyl-1,3-dipropylxanthine (18), and 8-cyclohexyl-1,3-dipropylxanthine (19) has been described elsewhere.[6,10] 8-Phenyltheophylline (2), 1-methylxanthine (36), 3-methylxanthine (30), and 3-propylxanthine (enprophylline) (31) were from Research Biochemical Inc. (Wayland, MA). 1,3-Dipropylxanthine (14) was from G. D. Searle[6] or was prepared by standard procedure.[33]

1,3-Dipropyl-7-methyl-8-[*p*-(methylcarboxy)phenyl]xanthine (23). To a solution of 0.356 g (1 mmol) of 8-(*p*-carboxyphenyl)-1,3-dipropylxanthine in 5 mL of DMF was added 0.18 g of K_2CO_3 and 0.16 mL (2.5 mmol) of methyl iodide. The reaction mixture was heated at 40 °C for 12 h and the solvent removed in vacuo. H_2O was added to precipitate the product, which was filtered and dried to give 0.35 g (91%) of 1,3-dipropyl-7-methyl-8-[*p*-(methylcarboxy)phenyl]xanthine. Purification was by recrystallization with DMF/H_2O; mp 159 °C. Anal. ($C_{20}H_{24}N_4O_4$) C, H, N.

8-(*p*-Carboxyphenyl)-1,3-dipropyl-7-methylxanthine (22). A mixture of 0.285 g of 1,3-dipropyl-7-methyl-8-[*p*-(methylcarboxy)phenyl]xanthine in 2 mL of DMF and 2 mL of 10% NaOH was refluxed for 15 min. The basic aqueous solution was allowed to cool and acidified with HCl to give a white precipitate, which was filtered and dried. Recrystallization by acidification (HCl) of a solution in aqueous NaOH (base/acid) afforded 0.24 g (88%) of 8-(*p*-carboxyphenyl)-1,3-dipropyl-7-methylxanthine; mp 219 °C. Anal. ($C_{19}H_{22}N_4O_4 \cdot 1^1/_4H_2O$) C, H, N.

1,3-Dipropyl-7-methyl-8-phenylxanthine (21). A mixture of 0.718 g (2.3 mmol) of 1,3-dipropyl-8-phenylxanthine in 6 mL of DMF, 0.35 g of K_2CO_3, and 0.25 mL (5 mmol) of methyl iodide was heated at 40 °C for 15 h. The solvent was removed in vacuo and H_2O added to precipitate the product, which was filtered and dried to give 0.63 g (84%) of 1,3-dipropyl-7-methyl-8-phenylxanthine. Purification was by recrystallization with DMF/H_2O; mp 116 °C. Anal. ($C_{18}H_{22}N_4O_2$) C, H, N.

1,3-Dipropyl-7-methyl-8-(*p*-sulfophenyl)xanthine (24). A mixture of 0.196 g (0.5 mmol) of 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine in 15 mL of H_2O and 0.04 g (0.5 mmol) of NaOH was refluxed for 25 min. Following the removal of solvent and drying, the Na salt was taken up in 15 mL of DMF. Methyl iodide (0.06 mL) was added and the reaction mixture was refluxed for 4 h. After the removal of solvent in vacuo, the residue was dissolved in H_2O and acidified. White crystals formed upon standing and were filtered and dried to afford 0.28 g (28%) of 1,3-dipropyl-7-methyl-8-(*p*-sulfophenyl)xanthine; mp >300 °C. Anal. ($C_{18}H_{22}N_4O_5S \cdot {}^1/_4H_2O$) C, H, N.

8-Cyclohexyl-1,3-dipropyl-7-methylxanthine (26). A mixture of 0.699 g (2.2 mmol) of 8-cyclohexyl-1,3-dipropylxanthine in 5 mL of DMF, 0.35 g of K_2CO_3, and 0.19 mL of methyl iodide was heated at 40 °C for 24 h. Solvent was removed under vacuo and H_2O added to precipitate the product, which was filtered and dried to afford 0.61 g (83%) of 8-cyclohexyl-1,3-dipropyl-7-methylxanthine. Recrystallization with DMF/H_2O provided an analytical sample; mp 109 °C. Anal. ($C_{18}H_{28}N_4O_2$) C, H, N.

8-Cyclopentyl-1,3-dipropyl-7-methylxanthine (25). A mixture of 0.248 g (1 mmol) of 8-cyclopentyl-1,3-dipropylxanthine in 10 mL of DMF, 0.35 g of K_2CO_3, and 0.13 mL of methyl iodide was heated at 40 °C for 24 h. After the removal of solvent in vacuo, H_2O was added to precipitate the compound, which was filtered and dried to give 0.21 g (80%) of 8-cyclopentyl-1,3-dipropyl-7-methylxanthine. Recrystallization by acetone/H_2O afforded an analytical sample; mp 114 °C. Anal. ($C_{17}H_{26}N_4O_2$) C, H, N.

8-[(*p*-Methylcarboxy)phenyl]caffeine (10). A mixture of 0.15 g (0.5 mmol) of 8-(*p*-carboxyphenyl)theophylline in 5 mL of DMF, 0.175 g of K_2CO_3, and 0.16 mL of methyl iodide was heated at 60 °C for 4 h. Following the removal of solvent, addition of H_2O and acidification of reaction mixture, 0.15 g (94%) of 8-[*p*-(methylcarboxy)phenyl]caffeine was obtained. Recrystallization by DMF/H_2O afforded an analytical sample; mp 231 °C. Anal. ($C_{16}H_{16}N_4O_4$) C, H, N.

8-(*p*-Carboxyphenyl)caffeine (9). A mixture of 0.138 g (0.4 mmol) of 8-[*p*-(methylcarboxy)phenyl]caffeine (10) in 5 mL of DMF and 2 mL of 10% NaOH was refluxed for 15 min. After the usual workup and recrystallization by base/acid treatment, 0.13 g (99%) of 8-(*p*-carboxyphenyl)caffeine was obtained; mp >300 °C. Anal. ($C_{15}H_{14}N_4O_4 \cdot H_2O$) C, H, N.

8-(*p*-Sulfophenyl)caffeine (11). To a solution of 0.34 g (1 mmol) of 8-(*p*-sulfophenyl)theophylline in 30 mL of DMF was added 0.04 (2 mmol) of NaOH and the reaction mixture was refluxed for 25 min. The solvent was removed under vacuo and the Na salt was dried overnight. This was taken up in 30 mL of DMF and 0.13 mL (2 mmol) of methyl iodide was added to the solution. The reaction mixture was refluxed for 2 h and solvent removed under vacuo. The residue was dissolved in a small quantity of H_2O and acidified with HCl. The aqueous solution was extracted with $CHCl_3$ to remove the impurities and then refrigerated for a few days. The white crystals were filtered, washed with a small quantity of MeOH, and dried to give 0.055 g (16%) of 8-(*p*-sulfophenyl)caffeine; mp >300 °C. Anal. ($C_{14}H_{14}N_4O_5S \cdot 1^1/_2H_2O$) C, H, N.

8-Cyclopentylcaffeine (12). A mixture of 0.248 g (1 mmol) of 8-cyclopentyltheophylline in 10 mL of DMF, 0.175 g of K_2CO_3, and 0.12 mL of methyl iodide was heated at 40 °C for 24 h. Following the removal of solvent, addition of H_2O and acidification of reaction mixture, 0.21 g (80%) of 8-cyclopentylcaffeine was obtained. Recrystallization by acetone/H_2O provided an analytical sample; mp 127 °C. Anal. ($C_{13}H_{18}N_4O_2 \cdot {}^1/_4H_2O$) C, H, N.

8-Cyclohexylcaffeine (13). A mixture of 0.11 g (0.4 mmol) of 8-cyclohexyltheophylline in 4 mL of DMF, 0.058 g of K_2CO_3, and 0.03 mL of methyl iodide was heated at 80 °C for 1 h. After the removal of solvent, addition of H_2O and acidification of reaction mixture, 0.11 g (95%) of 8-cyclohexylcaffeine was obtained. Recrystallization with DMF/H_2O afforded an analytical sample; mp 212 °C. Anal. ($C_{14}N_{20}N_4O_2$) C, H, N.

8-Phenyl-3-propylxanthine (32). A mixture of 3.83 g (0.17 g-atom) of sodium in 120 mL of absolute EtOH, 10.21 g (100 mmol) of propyl urea, and 10.64 mL (100 mmol) of ethyl cyanoacetate was refluxed for 18 h. The precipitate was removed by filtration, washed with EtOH and dissolved in H_2O. The aqueous solution was acidified with HCl to give a yellowish white precipitate, which was filtered and dried to afford 11.83 g (70%) of 1-propyl-6-aminouracil.

To an ice-cold solution of 8.45 g (50 mmol) of the 1-propyl-6-aminouracil in 40% HOAC was added dropwise 4.14 g (60 mmol) of sodium nitrite in 10 mL of H_2O. The reaction mixture turned purple and after a few minutes a precipitate formed, which was filtered and dried to yield 6.87 g (69%) of 1-propyl-6-amino-5-nitrosouracil.

(31) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4089.
(32) Ukena, D.; Daly, J. W.; Kirk, K. L.; Jacobson, K. A. *Life Sci.* **1986**, *38*, 797.
(33) Kramer, C. L.; Garet, J. E.; Mitchel, S. S.; Wells, J. N. *Biochemistry* **1977**, *16*, 3316.

A suspension of 4.48 g (23 mmol) of the 1-propyl-6-amino-5-nitrosouracil in 200 mL of absolute EtOH and 0.08 g of PtO_2 was hydrogenated at 40 psi of H_2 for 30 min. The catalyst was removed by filtration and solvent removed in vacuo. Et_2O was added to precipitate the product, which was filtered and dried to yield 3.65 g (88%) of 1-propyl-5,6-diaminouracil.

To a solution of 0.57 g (3.1 mmol) of the 1-propyl-5,6-diaminouracil in 40 mL of MeOH/HOAC (1:1) was added 0.36 mL (3.5 mmol) of benzaldehyde and the reaction mixture was stirred for a few minutes. A yellowish precipitate appeared, which was filtered and dried to yield 0.66 g (78%) of 1-propyl-5-(benzylideneamino)-6-aminouracil.

A mixture of 0.66 g (2.4 mmol) of the 1-propyl-5-(benzylideneamino)-6-aminouracil and 0.389 (2.4 mmol) of anhydrous $FeCl_3$ was refluxed for 6 h. A precipitate formed on cooling, which was filtered, washed with MeOH, and dried. Recrystallization by base/acid treatment afforded 0.25 g (39%) of 3-propyl-8-phenylxanthine: mp >300 °C; ^1H NMR (Me_2SO-d_6) δ 0.92 (t, 3 H), 1.72 (m, 2 H), 3.34 (s, 1 H), 3.95 (t, 2 H), 7.5 (m, 3 H), 8.1 (m, 2 H), 11.1 (s, 1 H). Anal. ($C_{14}H_{14}N_4O_2 \cdot H_2O$) C, H, N.

8-(*p*-Carboxyphenyl)-3-propylxanthine (33). A mixture of 0.552 g (3 mmol) of 1-propyl-5,6-diaminouracil in 40 mL of EtOH/HOAC (1:1) and 0.525 g (3.5 mmol) of *p*-carboxybenzaldehyde in 20 mL of EtOH was stirred for a few minutes. The precipitate that formed was filtered and dried to afford 0.90 g (81%) of 1-propyl-5-[(*p*-carboxybenzylidene)amino]-6-aminouracil. The crude uracil was refluxed with 0.486 g (3 mmol) of $FeCl_3$ in 100 mL of EtOH and the solvent volume reduced in vacuo to yield a precipitate, which was filtered and dried. The crude product was purified by recrystallization using DMF/MeOH to yield 0.9 g (96%) of 8-(*p*-carboxyphenyl)-3-propylxanthine. Recrystallization by base/acid treatment provided an analytical sample; mp >300 °C. Anal. ($C_{15}H_{14}N_4O_4 \cdot 1^3/_4H_2O$) C, H, N.

3-Propyl-8-(*p*-sulfophenyl)xanthine (34). To a solution of 0.529 (2.2 mmol) of *p*-sulfobenzoic acid potassium salt in 15 mL of H_2O was added 0.422 g (2.2 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and 0.368 g (2 mmol) of 1-propyl-5,6-diaminouracil in 15 mL of H_2O. The reaction mixture was stirred for a few minutes until all the solid went in solution. The clear aqueous solution was allowed to stand overnight and the solvent was removed in vacuo. The residue was dissolved in a small quantity of a H_2O/MeOH mixture (1:2) and Et_2O was added to precipitate the compound, which was filtered and dried. The crude product was dissolved in 20 mL of 20% NaOH and refluxed for 15 min. The reaction mixture was allowed to cool and acidified with HCl to give a white precipitate, which was filtered and dried. Recrystallization by base/acid treatment provided 0.52 (74%) of 3-propyl-8-(sulfophenyl)xanthine; mp >300 °C. Anal. ($C_{14}H_{14}N_4O_5S \cdot 4H_2O$) C, H, N.

8-Cyclohexyl-3-propylxanthine (35). A mixture of 0.276 g (1.5 mmol) of 1-propyl-5,6-diaminouracil in 20 mL of EtOH/HOAc (1:1) and 0.25 mL (2 mmol) of cyclohexanecarboxaldehyde in 10 mL of EtOH was stirred for a few minutes. The precipitate that formed was filtered and dried to afford 0.192 g (46%) of 1-propyl-5-(cyclohexylideneamino)-6-aminouracil. The crude uracil was refluxed with 0.12 g (7 mmol) of $FeCl_3$ in 12 mL of EtOH and the reaction mixture was refluxed for 3 h. The solvent was removed in vacuo and EtOH was added to precipitate the compound, which was filtered, washed with small quantity of EtOH, and dried to give 0.065 g (34%) of 8-cyclohexyl-3-propylxanthine; mp 290 °C. Anal. ($C_{14}H_{20}N_4O_2$) C, H, N.

1-Methyl-8-phenylxanthine (37). A mixture of 22 g (212 mmol) of malonic acid and 13.33 g (180 mmol) of methylurea in 35 mL of AcOH was warmed to 60–70 °C and 72 mL of acetic anhydride was added dropwise. The reaction mixture was heated at 90 °C for 6 h and allowed to stand overnight. After removal of solvent in vacuo, the residue was triturated with EtOH and the solid filtered and dried to give 18.5 g (72%) of 1-methylbarbituric acid.

To a suspension of 12 g (84.5 mmol) of the 1-methylbarbituric acid in 25 mL of H_2O was added 60 mL of $POCl_3$ dropwise, while the flask was cooled in an ice bath. After the addition was complete, the reaction mixture was refluxed for 1 h. After cooling, the mixture was extracted with three 25-mL portions of $CHCl_3$. After drying with Na_2SO_4 the combined $CHCl_3$ extracts were concentrated in vacuo. The residue was crystallized by trituration with Et_2O, filtered, and dried to yield 4.5 g (33%) of 3-methyl-6-chlorouracil.

A mixture of 2 g (12.5 mmol) of the 3-methyl-6-chlorouracil and 3.1 mL of benzylamine in 20 mL of butanol was refluxed for 1 h. A precipitate formed upon cooling and was filtered, washed with EtOH, and dried to give 1.5 g (52%) of 3-methyl-6-(benzylamino)uracil.

To a solution of 1.5 g (6.5 mmol) of the 3-methyl-6-(benzylamino)uracil in 50 mL of H_2O/AcOH (1:1) was added 0.483 g (7 mmol) of sodium nitrite in 2 mL of H_2O. The purple solution was allowed to stand overnight to yield a precipitate, which was filtered and dried to give 1.5 g (89%) 3-methyl-5-nitroso-6-(benzylamino)uracil.

A mixture of 1.5 g (58 mmol) of the 3-methyl-5-nitroso-6-(benzylamino)uracil in 50 mL of xylene was refluxed for 2.5 h. The color disappeared and the precipitate, which appeared on cooling was filtered, washed with EtOH, and dried. Recrystallization with DMF/H_2O gave 1.33 g (95%) of 1-methyl-8-phenylxanthine; mp >300 °C. Anal. ($C_{12}H_{10}N_4O_2$) C, H, N.

8-Phenyl-1-propylxanthine (38). A mixture of 3.064 g (30 mmol) of propylurea, 3.64 g (35 mmol) of malonic acid, and 12 mL of acetic anhydride and 6 mL of acetic acid was heated at 50 °C for 24 h. The solvent was removed in vacuo and the residue was dissolved in EtOH. The EtOH solution was heated at 30 °C for 24 h. The reaction mixture was filtered and solvent removed in vacuo. The solid residue was crystallized with MeOH/Et_2O mixture to give 2.3 g (45%) of 1-propylbarbituric acid.

A mixture of 1.63 g (9.6 mmol) of 1-propylbarbituric acid, 4.4 mL of $POCl_3$, and 0.32 mL of H_2O was heated at 40 °C for 24 h. Upon cooling, the reaction mixture was poured on ice and filtered. The filtrate was extracted with $CHCl_3$ and dried with Na_2SO_4 and the solvent was removed in vacuo. The residue was crystallized with a MeOH/Et_2O mixture to give 0.55 g (31%) of 3-propyl-6-chlorouracil.

A mixture of 0.55 g (2.9 mmol) of 3-propyl-6-chlorouracil in 0.70 mL of butanol and 1.8 mL of benzylamine was refluxed gently for 2 h. The reaction mixture solidified on cooling and was diluted with absolute EtOH. The white precipitate was filtered and dried to give 0.39 g (52%) of 3-propyl-6-(benzylamino)uracil.

To a solution of 0.105 g (0.4 mmol) 3-propyl-6-(benzylamino)uracil in 4 mL of DMF was added dropwise a concentrate aqueous solution of sodium nitrite until a bright pinkish color developed. The reaction mixture was acidified with HCl and stirred for a few minutes. A pinkish precipitate, which formed on standing, was filtered and dried to give 0.04 g (35%) of 3-propyl-5-nitroso-6-(benzylamino)uracil.

A solution of 0.36 g of 3-propyl-5-nitroso-6-(benzylamino)uracil in 10 mL of xylene was refluxed for 30 min. The white precipitate, which formed on cooling, was filtered, washed with EtOH, and dried to afford 0.014 g (42%) of 8-phenyl-1-propylxanthine. Recrystallization with DMF/EtOH provided an analytical sample. Thin-layer chromatographic analysis on silica gel with $CHCl_3$/MeOH (9:1) gave an R_f value of 0.33 for 8-phenyl-1-propylxanthine (**38**) versus an R_f value of 0.17 for 8-phenyl-3-propylxanthine (**32**): mp >300 °C; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3 H), 1.55 (m, 2 H), 3.34 (s, 1 H), 3.85 (t, 2 H), 7.5 (m 3 H), 8.1 (m, 2 H), 11.9 (s, 1 H). Anal. ($C_{14}H_{14}N_4O_2 \cdot H_2O$) C, H, N.

Biochemical Assay. Inhibition of binding of 1 nM [^3H]-N^6-(phenylisopropyl)adenosine (PIA) to A_1 adenosine receptors in rat cerebral cortical membranes was assayed as described.[31] Inhibition of binding by a range of concentrations of xanthines was determined in triplicate in at least two separate experiments. K_i values were calculated from IC_{50} values by using the Cheng–Prusoff equation[34] and a K_d for [^3H]-N^6-(phenylisopropyl)-adenosine of 1 nM. Inhibition of the stimulation by 5′-(*N*-ethylcarbamoyl)adenosine (NECA) of adenylate cyclase via A_2 receptors in human platelet or rat PC12 membranes was assayed as described.[32] EC_{50} values for stimulation by NECA were determined from concentration–response curves in the absence or presence of xanthine in three experiments. K_i values were then calculated from the EC_{50} values for NECA in the presence and

(34) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

absence of fixed concentrations of xanthine by using Schild equation.[35]

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Registry No. 1, 58-55-9; 2, 961-45-5; 3, 85872-58-8; 4, 80206-91-3; 4·Na, 120362-60-9; 5, 35873-49-5; 6, 5438-77-7; 7, 58-08-2; 8, 6439-88-9; 9, 120362-45-0; 10, 120362-46-1; 11, 120362-47-2; 12, 120362-48-3; 13, 110166-60-4; 14, 31542-62-8; 15, 85872-53-3; 16, 94781-78-9; 17, 89073-57-4; 18, 102146-07-6; 19, 106686-66-2; 20, 31542-63-9; 21, 120362-49-4; 22, 120362-50-7; 23, 120362-51-8; 24, 120362-52-9; 25, 120362-53-0; 26, 120362-54-1; 27, 63908-26-9; 28, 94781-84-7; 29, 94781-85-8; 30, 1076-22-8; 31, 41078-02-8; 32, 120362-55-2; 33, 120362-56-3; 34, 120362-57-4; 35, 120362-58-5; 36, 6136-37-4; 37, 2850-37-5; 38, 120362-59-6; methyl iodide, 74-88-4; propylurea, 627-06-5; ethyl cyanoacetate, 105-56-6; 1-propyl-6-aminouracil, 53681-47-3; 1-propyl-6-amino-5-nitrosouracil, 120362-61-0; 1-propyl-5,6-diaminouracil, 76194-07-5; benzaldehyde, 100-52-7; 1-propyl-5-(benzylideneamino)-6-aminouracil, 120362-62-1; *p*-carboxybenzaldehyde, 619-66-9; 1-propyl-5-[(*p*-carboxybenzylidene)amino]-6-aminouracil, 120362-63-2; *p*-sulfobenzoic acid, potassium salt, 22959-32-6; 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, 25952-53-8; cyclohexanecarboxaldehyde, 2043-61-0; 1-propyl-5-(cyclohexylideneamino)-6-aminouracil, 120362-64-3; malonic acid, 141-82-2; methylurea, 598-50-5; 1-methylbarbituric acid, 2565-47-1; 3-methyl-6-chlorouracil, 4318-56-3; benzylamine, 100-46-9; 3-methyl-6-(benzylamino)uracil, 5759-79-5; 3-methyl-5-nitroso-6-(benzylamino)uracil, 5770-20-7; propylurea, 627-06-5; 1-propylbarbituric acid, 5496-93-5; 3-propyl-6-chlorouracil, 50721-48-7; 3-propyl-6-(benzylamino)uracil, 120362-65-4; 3-propyl-5-nitroso-6-(benzylamino)uracil, 120362-66-5.

(35) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.

6-Alkyl-*N*,*N*-disubstituted-2-pyridinamines as Anticonvulsant Agents

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The anticonvulsant effect of a series of 6-alkyl-*N*,*N*-disubstituted-2-pyridinamines is described. An investigation was carried out to optimize the anticonvulsant activity and reduce behavioral side effects in this series. Three compounds (**7**, **8**, **10**; Table I) were selected from initial screening for a more complete pharmacological evaluation. While each of these compounds was a potent anticonvulsant agent with ED_{50} values from 5 to 10 mg/kg, the activity was accompanied by significant behavioral side effects including decreased spontaneous locomotion, ataxia, and ptosis.

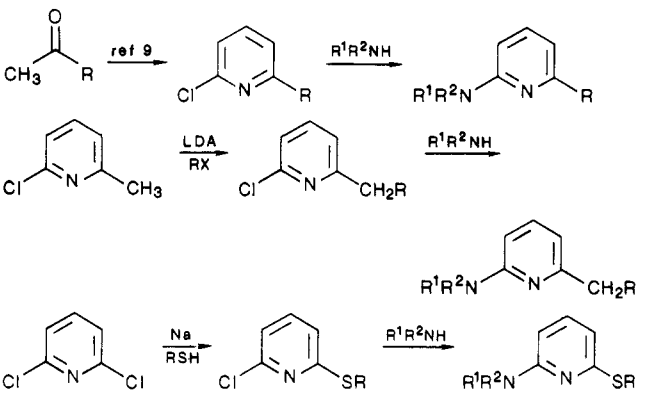
Recently, we have described the anticonvulsant activity of a series of 6-alkoxy-*N*,*N*-disubstituted-2-pyridinamines.[1] The most encouraging results were obtained with 1-[6-(2-methylpropoxy)-2-pyridinyl]piperazine, A (Figure 1). While the potency of A was nearly equal to diphenylhydantoin (phenytoin), a clinically useful anticonvulsant agent, there was insufficient separation between the efficacious dose and the dose causing central nervous system depression and hypothermia. In addition, A possessed a relatively short duration of anticonvulsant activity.

Further investigation of this structural class, in collaboration with the NIH–NINCDS Antiepileptic Drug Discovery Program,[2] revealed the potent anticonvulsant activity of the related 6-alkyl-*N*,*N*-disubstituted-2-pyridinamines, B (Figure 1).

2-Piperazinylpyridine has been reported[3,4] to be useful for the treatment of Parkinson's disease and the 3-, 5-, and 6-substituted 2-piperazinylpyridines have been reported to possess a diverse range of pharmacological properties.[5-7] To our knowledge, 6-alkyl-2-piperazinylpyridines have not been described as possessing anticonvulsant activity.

Initially we observed that 1-(6-hexyl-2-pyridinyl)-piperazine, **7** (Table I), was active against seizures induced by maximal electroshock (MES),[8] a model for generalized tonic–clonic seizures. The anticonvulsant potency of this compound was comparable to that of A but exhibited a greater separation between doses having an anticonvulsant effect and those demonstrating behavioral side effects (ataxia). Because of this encouraging result, we examined a series of 6-alkyl-*N*,*N*-disubstituted-2-pyridinamines in

Scheme I



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(1) Pavia, M. R.; Taylor, C. P.; Hershenson, F. M.; Lobbestael, S. J. *J. Med. Chem.* **1987**, *30*, 1210.
(2) Kupferberg, H. J.; Gladding, G. D.; Swinyard, E. A. In *Antiepileptic Drugs, Handbook of Experimental Pharmacology*; Frey, H.-H., Janz, D. E.; Springer Verlag: Berlin, 1985; Vol. 74, p 341.
(3) Rodriguez, R. U.S. Patent 3773951, 1973; *Chem. Abstr.* **1973**, *80*, 63860c.
(4) Rodriguez, R. U.S. Patent 3798324, 1974; *Chem. Abstr.* **1974**, *81*, 68559s.
(5) Delarge, J. E.; Thunus, L. N.; Lapiere, C. L.; Georges, A. H. U.S. Patent 3980652, 1976; *Chem. Abstr.* **1976**, *77*, 88325h.
(6) Saari, W. S. U.S. Patent 4442103, 1984; *Chem. Abstr.* **1984**, *101*, 60140j.

0022-2623/89/1832-1237$01.50/0