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1,3,8-Trisubstituted Xanthines. Effects of Substitution Pattern upon Adenosine Receptor A_1/A_2 Affinity

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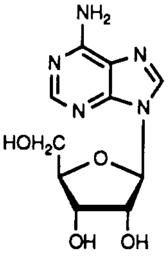
A series of 11 8-substituted xanthines having three different substitution patterns on the 1- and 3-positions [pattern **a** ($R_1 = R_3 = CH_2CH_2CH_3$), **b** ($R_1 = CH_2CH_2CH_3$, $R_3 = CH_3$), and **c** ($R_1 = CH_3$, $R_3 = CH_2CH_2CH_3$)] was prepared. These compounds were assessed for affinity and selectivity in binding to adenosine A_1 and A_2 receptors. Compounds with greatest affinity at the A_1 receptor had the 1,3-substitution pattern **a**. With one exception, compounds with pattern **a** also exhibited the most potent binding at the A_2 receptor; however, several compounds with pattern **c** were equipotent at the A_2 receptor with those having pattern **a**. Additionally, the substituents on the 1- and 3-positions of these 8-substituted xanthines were equally important for determining maximum affinity to the A_1 receptor, while the substituent at the 3-position is more important than the substituent at the 1-position for potency at the A_2 receptor. As a result of this, it is possible to maximize selectivity for the A_1 receptor by choice of the 1- and 3-position substituents. However, the R_1/R_3 substitution pattern required for maximum A_1 selectivity is also dependent upon the substituent in the 8-position in a manner which is not fully understood.

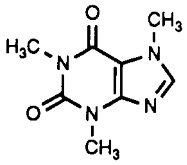
Adenosine (1) is an endogenous purine that has many pharmacological and physiological effects upon various organs and tissues, such as the vasculature, kidney, heart, smooth muscle, striated muscle, adrenals, blood cells, and brain.[1-5] Based upon radioligand binding data and adenylate cyclase studies, two subtypes of adenosine receptors (A_1 and A_2) have been identified and characterized. Initially A_1 and A_2 receptors were distinguished by their ability to inhibit and stimulate adenylate cyclase activity, respectively.[6-8] Currently, they are differentiated upon the basis of the pharmacological profile of adenosine agonists at each receptor subtype.[7] The A_2 stimulatory site has been further divided on the basis of potencies of agonists in different tissues. In the striatum Bruns et al. has characterized a high affinity site,[9] and in human fibroblasts a lower affinity site was found in earlier studies.[10] These have been termed the A_{2a} and A_{2b} receptors, respectively. The discovery that members of the xanthine class of compounds, such as caffeine (2) and theophylline (3), inhibit many of the effects of adenosine by acting as antagonists at the A_1 and A_2 receptors[1,4,5,11,12] has resulted in research to develop more potent and selective antagonists for the adenosine receptors.

Structure–activity studies in the theophylline series led to the discovery of 1,3-dipropyl-8-aryl, 8-heterocyclic, or 8-cycloalkyl derivatives, such as 1,3-dipropyl-8-cyclopentylxanthine, that have more than 10000 times greater affinity for adenosine receptors than does caffeine or theophylline.[13-16] Although it was discovered that changing the 1,3-substituents of theophylline from methyl to propyl increased the potency of the compounds at adenosine receptors, no study has been reported that determined whether *both* propyl groups are needed for activity at the receptors. As part of a program to develop xanthines as adenosine antagonists, we synthesized a series of compounds to examine the effect of the substituents on the 1- and 3-positions of xanthines on A_1 and A_2 adenosine receptor binding.



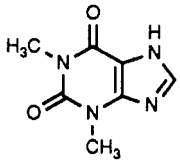
Adenosine

1



Caffeine

2



Theophylline

3

Results and Discussion. A series of 11 different 8-substituted xanthines that had three different substitution patterns on the 1- and 3-positions [pattern **a** ($R_1 = R_3 = CH_2CH_2CH_3$), **b** ($R_1 = CH_2CH_2CH_3$, $R_3 = CH_3$), and **c** ($R_1 = CH_3$, $R_3 = CH_2CH_2CH_3$)] was prepared by the route outlined in Scheme I. Nitrosation of the appropriately substituted 6-amino-1,3-dialkyluracil (4)[17] gave 6-amino-1,3-dialkyl-5-nitrosouracil (5). These compounds were reduced by catalytic hydrogenation to the 1,3-dialkyl-5,6-diaminouracils (6) which then were transformed to the 1,3,8-trisubstituted xanthines by one of three methods. Method A consisted of condensing diaminouracil 6 with an aldehyde to form the imine 7 which was oxidatively cyclized by treatment with diethyl azodicarboxylate (DEAD) in a modification of a general procedure reported by Yoneda et al.[18] to give the xanthine. Method B con-

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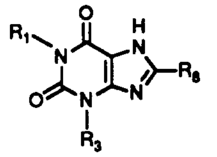
Table I. 1,3,8-Trisubstituted Xanthines



no.[a]	method of synthesis	recrystallization solvent	yield, %[b]	mp, °C	formula	analysis[c]
9a	A	EtOH	40	252–253[d]	$C_{17}H_{20}N_4O_2$	C,H,N
9b	A	MeOH	22	277–279	$C_{15}H_{16}N_4O_2$	C,H,N
9c	A	EtOH	31	284–286	$C_{15}H_{16}N_4O_2$	C,H,N
10a	A	EtOH	19	>330	$C_{17}H_{20}N_4O_3$	C,H,N
10b	A	EtOH	36	323–327	$C_{15}H_{16}N_4O_3 \cdot H_2O$	C,H,N
10c	A	EtOH	24	>310	$C_{15}H_{16}N_4O_3$	C,H,N
11a	A	EtOH	59	297–300	$C_{18}H_{19}N_5O_2$	C,H,N
11b	A	EtOH	85	314–318	$C_{16}H_{15}N_5O_2$	C,H,N
11c	A	DMF/water	57	>350	$C_{16}H_{15}N_5O_2$	C,H,N
12a	A	EtOH	74	270–272[e]	$C_{15}H_{18}N_4O_2S$	C,H,N,S
12b	A	EtOH	57	293–296	$C_{13}H_{14}N_4O_2S$	C,H,N,S
12c	A	*f*	66	285–287	$C_{13}H_{14}N_4O_2S$	C,H,N,S
13a	A	EtOH	36	261–262[g]	$C_{15}H_{18}N_4O_2S$	C,H,N
13b	A	EtOH	77	285–286	$C_{13}H_{14}N_4O_2S$	C,H,N,S
13c	A	*f*	71	294–295	$C_{13}H_{14}N_4O_2S$	C,H,N,S
14a	A	*f*	46	261–262	$C_{16}H_{19}N_5O_2$	C,H,N
14b	A	*f*	60	>270	$C_{14}H_{15}N_5O_2$	C,H,N
14c	A	*n*-PrOH	46	272–274	$C_{14}H_{15}N_5O_2$	C,H,N
15a	A	*f*	70	259.5–260.5	$C_{16}H_{19}N_5O_2$	C,H,N
15b	A	*f*	27	>270	$C_{14}H_{15}N_5O_2$	C,H,N
15c	A	*f*	68	>300	$C_{14}H_{15}N_5O_2$	C,H,N
16a	C	EtOH	11	193–194[h]	$C_{16}H_{24}N_4O_2$	C,H,N
16b	C	EtOH	37	187–189	$C_{14}H_{20}N_4O_2$	C,H,N
16c	B	EtOH	20	193–195	$C_{14}H_{20}N_4O_2$	C,H,N
17a	B	EtOAc/hexane	69	155–156[i]	$C_{17}H_{26}N_4O_2$	C,H,N
17b	C	EtOH	7	193–196	$C_{15}H_{22}N_4O_2$	C,H,N
17c	B	water	62	189–191	$C_{15}H_{22}N_4O_2$	C,H,N
18a	C	EtOH	36	193–195	$C_{19}H_{24}N_4O_2$	C,H,N
18b	C	EtOH	40	198–201	$C_{17}H_{20}N_4O_2$	C,H,N
18c	B	EtOH	30	165–167	$C_{17}H_{20}N_4O_2$	C,H,N
19a	C	EtOH	27	261–263	$C_{19}H_{22}N_4O_2$	C,H,N
19b	C	EtOH	12	283–286	$C_{17}H_{18}N_4O_2$	C,H,N
19c	A	*f*	44	294–296	$C_{17}H_{18}N_4O_2$	C,H,N

[a] R_1, R_3, and R_8 are defined in Table II. [b] Yields are for the two-step transformation from diaminouracil **6** to xanthines **9–19** and are for analytically pure products. [c] All analyses were within ±0.4% of the theoretical values. [d] lit. 260–261 °C (ref 25). [e] lit. 259 °C (ref 16). [f] Not recrystallized. [g] lit. 267 °C (ref 16). [h] lit. 199–200 °C (ref 15), 191–193 °C (ref 28). [i] lit. 159–160 °C (ref 15).

sisted of reacting diaminouracil **6** with a carboxylic acid chloride to give the amide **8**, which was then cyclized with aqueous sodium hydroxide to give the xanthine. Method C involved forming **8** by melting a mixture of the carboxylic acid and diaminouracil **6**. Again, cyclization of the resulting amide with sodium hydroxide gave the desired xanthine (Table I).

The potency of the 1,3,8-trisubstituted xanthines at adenosine A_1 and A_2 receptors was determined by standard radioligand binding procedures. Adenosine A_1 receptor binding was determined in rat cortex by the displacement of tritiated cyclohexyladenosine ([^{3}H]CHA) in a modification of the procedure described by Bruns et al.[19] Adenosine A_2 binding was determined in rat striatum by the displacement of tritiated *N*-ethyladenosin-5′-uronamide ([^{3}H]NECA) according to the method of Bruns et al.[9] The results are shown in Table II.

In all cases, compounds with substitution pattern **a** ($R_1 = R_3 = CH_2CH_2CH_3$) exhibited the most potent binding at the adenosine A_1 receptor and, except for **19a–c**, compounds with this substitution pattern also gave the most potent binding at the adenosine A_2 receptor. Differences were seen, however, between compounds with pattern **b** and pattern **c** in their selectivity for the two types of adenosine receptors. Compounds with patterns **b** ($R_1 = CH_2CH_2CH_3$, $R_3 = CH_3$) and **c** ($R_1 = CH_3$, $R_3 = CH_2CH_2CH_3$) generally had equal affinity for the A_1 receptor, but were weaker in potency than those with pattern **a**. These data indicate that both the 1- and the 3-positions are important for binding at the adenosine A_1 receptor. This trend was also observed with 8-phenyltheophylline (**20**). Changing either the 1- or the 3-position methyl group to a propyl group (**20** vs **9b** or **9c**) gave a 10-fold increase in potency at the adenosine A_1 receptor.

At the adenosine A_2 receptor, however, comparison of compounds with patterns **b** and **c** to those with pattern **a** shows that the 3-position substituent is more important than the 1-position substituent for binding. Changing the substituent on the 3-position from a propyl (pattern **a**) to a methyl group (pattern **b**) caused a loss of affinity for the adenosine A_2 receptor that averaged 6.5-fold. The same change on the 1-position (pattern c) had no effect on A_2 affinity for five of the 11 series of compounds (**9–11**, **16**, **17**) and only a 2–3-fold loss of affinity for five of the remaining six series of compounds (**12–15**, **18**). Only compounds **19a–c** (the 8-styrylxanthines) did not follow these trends. Here both **19b** and **19c** were slightly more potent at the A_2 site than **19a**. Again, comparison with 8-phenyltheophylline supports the importance of the 3-

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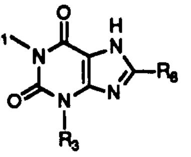
Table II. Adenosine Receptor Binding of 1,3,8-Trisubstituted Xanthines



				A_1		A_2		
compd	R_1	R_3	R_8	K_i, M[a]	N	K_i, nM[a]	N	A_2/A_1
9a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	phenyl	2.75 ± 0.29	4	116 ± 10	3	42
9b	$CH_2CH_2CH_3$	CH_3	phenyl	6.96 ± 0.91	4	553 ± 56	3	79
9c	CH_3	$CH_2CH_2CH_3$	phenyl	6.30 ± 0.17	3	93.3 ± 10.2	4	15
10a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	4-hydroxyphenyl	0.63 ± 0.05	5	60.8 ± 5.0	3	97
10b	$CH_2CH_2CH_3$	CH_3	4-hydroxyphenyl	1.28 ± 0.17	3	263 ± 64	3	205
10c	CH_3	$CH_2CH_2CH_3$	4-hydroxyphenyl	2.54 ± 0.22	3	81.5 ± 1.9	3	32
11a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	4-cyanophenyl	4.48 ± 0.40	3	189 ± 17	3	42
11b	$CH_2CH_2CH_3$	CH_3	4-cyanophenyl	5.95 ± 0.35	3	446 ± 87	3	75
11c	CH_3	$CH_2CH_2CH_3$	4-cyanophenyl	13.9 ± 2.8	3	254 ± 38	3	18
12a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	2-thienyl	3.20 ± 0.49	3	173 ± 8	3	54
12b	$CH_2CH_2CH_3$	CH_3	2-thienyl	9.68 ± 1.56	3	719 ± 20	3	74
12c	CH_3	$CH_2CH_2CH_3$	2-thienyl	8.93 ± 1.09	3	329 ± 55	3	37
13a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	3-thienyl	2.04 ± 0.31	6	39.4 ± 7.5	3	19
13b	$CH_2CH_2CH_3$	CH_3	3-thienyl	6.40 ± 0.96	3	315 ± 16	3	49
13c	CH_3	$CH_2CH_2CH_3$	3-thienyl	4.68 ± 0.77	3	70.3 ± 13.2	3	15
14a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	3-pyridyl	11.6 ± 0.2	3	347 ± 94	3	30
14b	$CH_2CH_2CH_3$	CH_3	3-pyridyl	70.2 ± 3.1	3	3100 ± 180	3	44
14c	CH_3	$CH_2CH_2CH_3$	3-pyridyl	72.8 ± 11.7	3	1200 ± 220	3	16
15a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	4-pyridyl	3.23 ± 0.83	3	136 ± 29	3	42
15b	$CH_2CH_2CH_3$	CH_3	4-pyridyl	27.6 ± 2.4	3	3760 ± 310	3	136
15c	CH_3	$CH_2CH_2CH_3$	4-pyridyl	21.8 ± 6.5	3	476 ± 124	3	22
16a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	cyclopentyl	0.23 ± 0.02	3	230 ± 15	6	1000
16b	$CH_2CH_2CH_3$	CH_3	cyclopentyl	1.06 ± 0.22	3	1010 ± 79	3	953
16c	CH_3	$CH_2CH_2CH_3$	cyclopentyl	0.85 ± 0.18	3	247 ± 50	3	290
17a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	cyclohexyl	0.30 ± 0.02	4	320 ± 26	3	1067
17b	$CH_2CH_2CH_3$	CH_3	cyclohexyl	5.02 ± 0.62	3	995 ± 40	3	198
17c	CH_3	$CH_2CH_2CH_3$	cyclohexyl	1.81 ± 0.34	3	403 ± 61	2	223
18a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	2-phenylethyl	57.8 ± 22.2	3	593 ± 38	3	10
18b	$CH_2CH_2CH_3$	CH_3	2-phenylethyl	275 ± 81	3	1440 ± 59	3	5
18c	CH_3	$CH_2CH_2CH_3$	2-phenylethyl	259 ± 9	3	1050 ± 76	3	4
19a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	styryl	22.2 ± 3.8	5	85.1 ± 14.5	4	4
19b	$CH_2CH_2CH_3$	CH_3	styryl	49.6 ± 3.9	3	58.0 ± 8.4	4	1.2
19c	CH_3	$CH_2CH_2CH_3$	styryl	31.1 ± 4.4	3	46.5 ± 12.1	3	1.5
20	CH_3	CH_3	phenyl	60 ± 6	3	644 ± 144	3	11

[a] A_1 binding data determined in rat cortex by the displacement of [^{3}H]cyclohexyladenosine (CHA). A_2 binding data determined in rat striatum by the displacement of [^{3}H]-*N*-ethyladenosin-5′-uronamide (NECA).

position. A change from methyl to propyl on the 3-position of 8-phenyltheophylline gave a 7-fold increase in affinity for the A_2 site (compound **20** vs **9c**), but a similar change at the 1-position caused no increase in A_2 affinity (compound **20** vs **9b**).

With regard towards selectivity for the A_1 or A_2 receptor, the compounds could be divided into two groups. The 8-aryl and 8-heteroaromatic xanthines (compounds **9**–**15**) show the greatest A_1 selectivity with substitution pattern b, whereas xanthines with 8-cycloalkyl or 8-phenethyl (compounds **16**–**18**), have the greatest A_1 selectivity with substitution pattern a. Although xanthines **9**–**15** have 8-substituents that would be expected to be coplanar with the xanthine ring system, this does not appear to be the reason for the division. Xanthines **19** have an 8-styryl substituent which would also be expected to be coplanar with the xanthine ring system, yet **19a** rather than **19b** had the better A_1 selectivity.

Several SAR studies of xanthines have been reported,[10,20-22] and although the main focus of these studies was not to determine the effect of the 1- and 3-substituents on adenosine A_1 and A_2 potency, some data on these effects was presented. Similarities as well as differences regarding the relative importance of the 1- and 3-substituents of xanthines for A_1 and A_2 receptor activity are apparent between these previous reports and this study. However, in those studies, compound affinity for both the A_1 and the A_2 receptors was examined in several different tissues (including both high affinity (A_{2a}) and low affinity (A_{2b}) putative subtypes) and several different assay systems (including both functional and binding assays). Further, in some cases only two of the three possible substitution patterns were examined. Therefore, it is difficult to draw close comparisons between those studies and this one.

Daly et al.[20] found that in changing either the 1- or 3-substituent of caffeine from methyl to propyl gave equal effects upon A_1 potency. However, Schwabe et al.[21] and Linden et al.[22] observed significant changes in potency at the A_1 site only when the 1-substituent was altered. These last two studies compared methyl and hydrogen substituents in a series of caffeine and theophylline analogues[21] and the effects of changing the propyl groups of 8-[4′-[(carboxymethyl)oxy]phenyl]-1,3-dipropylxanthine to

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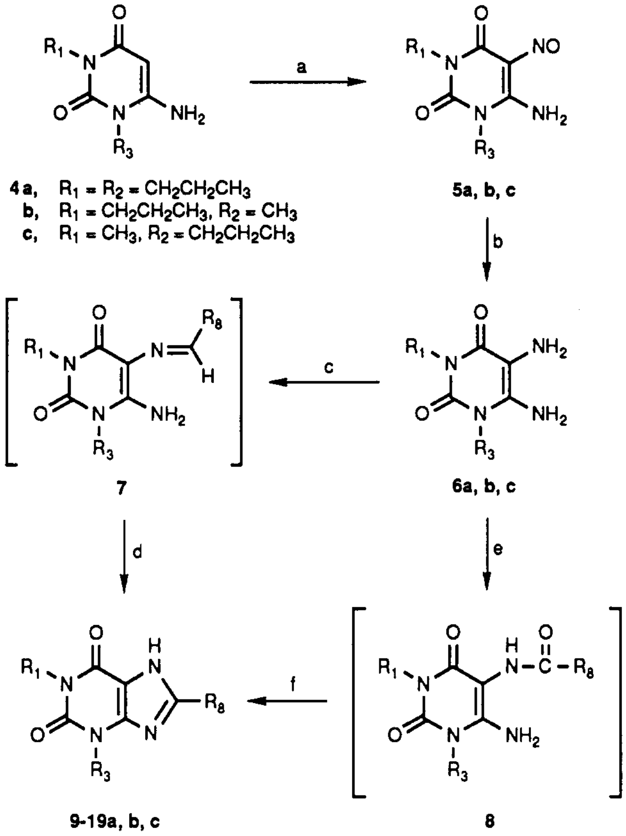
Scheme I[a]



[a] (a) $NaNO_2$, HOAc. (b) H_2, 5% Pd/C, 50 PSI. (c) Method A: R_8CHO, HOAc, EtOH. (d) DEAD, glyme, reflux. (e) Method B: R_8COCl. Method C: R_8CO_2H, melt. (f) Aqueous NaOH, reflux.

benzyl or phenethyl.[22,23] Finally, Bruns[10] and the three studies just discussed[20-22] consistently found that the 1-substituent was more important than the 3-substituent for antagonism of adenosine's effects at A_2 receptors. However, since all four studies used assays that differ from ours,[24] no close comparisons can be made.

In summary, our study found that the 1- and 3-substituents of 8-substituted xanthines were equally important for determining maximum affinity for the A_1 receptor, while the 3-position substituent is more important than the 1-position substituent for A_2 receptor affinity. As a result, it is possible to maximize selectivity for the A_1 receptor by choice of the 1- and 3-position substituents. However, the R_1/R_3 substitution pattern required for maximum A_1 selectivity is also dependent upon the substituent in the 8-position in a manner which is not fully understood at present.

Experimental Section

All melting points were obtained on a Bristoline hot-stage microscope or a Thomas Hoover capillary melting point apparatus and are reported uncorrected. IR spectra were obtained on a Beckman FT 1300 spectrophotometer. NMR spectra were obtained on either a Varian EM 360A, an IBM NR80, or a General Electric QE300 spectrometer with tetramethylsilane as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. of Atlanta, GA. 6-Amino-1,3-di-*n*-propyluracil (**4a**), 6-amino-1-methyl-3-*n*-propyluracil (**4b**), and 6-amino-3-methyl-1-*n*-propyluracil (**4c**) were prepared by the method of Papesch and Schroeder.[17] [^{3}H]CHA and [^{3}H]NECA were obtained from Dupont NEN.

1,3-Dialkyl-5,6-diaminouracils (6). The synthesis of 5,6-diamino-3-methyl-1-*n*-propyluracil is a typical procedure for the preparation of these compounds. A related synthesis of 5,6-diamino-1,3-di-*n*-propyluracil has been described by Daly et al.[25]

5,6-Diamino-3-methyl-1-*n*-propyluracil (6c). 6-Amino-3-methyl-1-*n*-propyluracil (10 g, 58 mmol) was dissolved in about 10 mL of acetic acid and warmed to 90 °C. Then, with stirring, a solution of 4.2 g (61 mmol) of sodium nitrite in 100 mL of water was added in 10-mL portions over 10 min. The reaction mixture was cooled to approximately 10 °C and the product collected by vacuum filtration. The filter cake was washed with water (2 × 30 mL) and acetone (2 × 10 mL) to give 10 g (83%) of the nitrosouracil as a purple solid.

The nitrosouracil (5.8 g, 27 mmol) was suspended in 100 mL of ethanol and was hydrogenated on a Parr shaker-type apparatus by using 0.35 g of 5% Pd/C at 60 psi for 24 h. The reaction mixture was then filtered through Celite. The filtrate was evaporated to give a sticky yellow solid which was triturated with 50 mL ether/ethanol (9:1). The mixture was filtered to give 3.2 g of the diaminouracil as a light yellow solid. The mother liquors were concentrated to 5 mL and an additional 0.1 g of diaminouracil was recovered. Additional material was recovered by washing the filter cake with hot ethanol (6 × 25 mL). The combined washes were evaporated to give a sticky brown solid that was triturated with 25 mL of ether/ethanol (9:1). Filtration gave 1.0 g of the diaminouracil as a light yellow solid (combined yield 4.4 g, 67%). If not used immediately, the material was stored in the freezer: ^{1}H NMR (DMSO-d_6) δ 0.84 (t, J = 7.3 Hz, 3 H), 1.52 (sextet, J = 7.3 Hz, 2 H), 2.89 (br s, 2 H), 3.11 (s, 3 H), 3.74 (t, J = 7.3 Hz, 2 H), 6.17 (s, 2 H).

5,6-Diamino-1,3-di-*n*-propyluracil (6a). The compound was prepared by the above method from 6-amino-1,3-di-*n*-propyluracil (140 g, 0.68 mol) to give 42 g (27%) of the desired diaminouracil: ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7.4 Hz, 3 H), 0.84 (t, J = 7.3 Hz, 3 H), 1.41 (m, 4 H), 3.10 (br s, 2 H), 3.71 (t, J = 7.3 Hz, 2 H), 3.78 (t, J = 7.4 Hz, 2 H), 6.21 (s, 2 H).

5,6-Diamino-1-methyl-3-*n*-propyluracil (6b). The compound was prepared by the above method from 6-amino-1-methyl-3-*n*-propyluracil (13 g, 7.3 mmol) to give 3.7 g (27%) of the desired diaminouracil as a light yellow solid: ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7.4 Hz, 3 H), 1.47 (sextet, J = 7.4 Hz, 2 H), 2.94 (br s, 2 H), 3.28 (s, 3 H), 3.70 (t, J = 7.4 Hz, 2 H), 6.15 (s, 2 H).

Method A. Illustrated by the Synthesis of 3-Methyl-8-phenyl-1-*n*-propylxanthine (9b). A mixture of benzaldehyde (2.5 mL, 25 mmol), **6b** (4.4 g, 22 mmol), and 1 mL of acetic acid was refluxed overnight in 60 mL of ethanol. Upon cooling of the mixture to room temperature, the precipitate was filtered and washed with ethanol (2 × 5 mL) and ether (2 × 5 mL) to give the imine as a pale yellow solid (5.4 g, 84%) which was used directly in the next step: ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7 Hz, 3 H), 1.50 (sextet, J = 7 Hz, 2 H), 3.34 (s, 3 H), 3.74 (t, J = 7 Hz, 2 H), 7.35 (m, 3 H), 7.75 (m, 2 H), 9.57 (s, 1 H). The imine was heated in 60 mL of glyme. As the mixture began to reflux, the imine dissolved. Diethyl azodicarboxylate (DEAD)[26] (4.4 mL, 28 mmol) was added through the condenser. Within 5 min a white

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(23) Daly et al. determined compound potency at A_1 receptors by the inhibition of binding of [^{3}H]CHA to rat cerebral cortical membranes. Schwabe et al. determined compound potency at A_1 receptors by the inhibition of binding of [^{3}H]PIA to either rat or bovine brain. Linden et al. determined compound potency at A_1 receptors by the inhibition of binding of [^{125}I](aminobenzyl)adenosine to either rat or bovine brain.

(24) Bruns determined compound potency at A_1 receptors by the inhibition of adenosine elicited accumulation of cyclic AMP in human fibroblasts. Daly et al. determined compound potency at A_2 receptors by inhibition of 2-chloroadenosine elicited accumulation of cyclic AMP in guinea pig cortex. Schwabe et al. determined compound potency at A_2 receptors by inhibition of NECA stimulation of adenylate cyclase in human platelets or by the inhibition of binding of [^{3}H]NECA to human platelets. Linden et al. determined compound potency at A_2 receptors by the inhibition of NECA stimulation of adenylate cyclase in human platelets.

(25) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem.* **1985**, *28*, 487.

(26) The substitution of diisopropyl azodicarboxylate (which costs about one-third as much per mole as DEAD) was found to be equally effective for this step.

solid was formed. After an additional 25 min, the solution was cooled and filtered, and the filter cake was washed with ethanol (2 × 5 mL) and ether (2 × 5 mL) to give a white solid (4.3 g) which was pure by ^{1}H NMR analysis. An analytical sample was obtained by refluxing with 750 mL of methanol, filtering hot (2.3 g did not dissolve), and cooling the filtrate to give a precipitate which was collected by vacuum filtration and washed with methanol (3 × 5 mL) and ether (3 × 5 mL) to give 1.4 g (22%) of **6b** was a white solid with the consistency of cotton: mp 277–279 °C; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7 Hz, 3 H), 1.60 (sextet, J = 7 Hz, 2 H), 3.51 (s, 3 H), 3.88 (t, J = 7 Hz, 2 H), 7.50 (m, 3 H), 8.12 (m, 2 H); IR (KBr) 3175, 1702, 1650 cm^{-1}. Anal. ($C_{15}H_{16}N_4O_2$) C, H, N.

Method B. Illustrated by the Synthesis of 8-Cyclohexyl-1-methyl-3-*n*-propylxanthine (17c). To a stirred solution of **6c** (0.80 g, 4.0 mmol) and pyridine (0.37 mL, 4.5 mmol) in anhydrous dichloromethane (10 mL) under argon at 5 °C was added cyclohexanecarbonyl chloride (0.56 mL, 4.1 mmol). After 15 min at 5 °C and 30 min at room temperature, the mixture was diluted with 0.5 N hydrochloric acid, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL) and the combined organics were washed with saturated aqueous sodium bicarbonate and dried (sodium sulfate). Concentration gave a yellow solid which was suspended in water (10 mL). Sodium hydroxide (1.2 g, 30 mmol) was added and the mixture was refluxed for 30 min. The solution was cooled to room temperature, acidified to pH 2 with 5.0 N hydrochloric acid, and stirred at room temperature for 30 min. After the solution stood overnight, the solids were collected, washed with water (4 × 20 mL), and dried under vacuum at room temperature. Recrystallization from water gave **17c** (0.73 g, 62%) as a fibrous white solid: mp 189–191 °C; ^{1}H NMR (CDCl$_3$) δ 0.99 (t, J = 7.4 Hz, 3 H), 1.39 (m, 3 H), 1.80 (m, 7 H), 2.07 (m, 2 H), 2.91 (m, 1 H), 3.49 (s, 3 H), 4.12 (m, 2 H), 12.46 (br s, 1 H); IR (KBr) 3165, 2931, 2856, 1710, 1656, 1545, 1494, 1391, 1280, 995, 761, 748 cm^{-1}. Anal. ($C_{15}H_{22}N_4O_2$) C, H, N.

Method C. Illustrated by the Synthesis of *trans*-3-Methyl-8-(2-phenylethenyl)-1-*n*-propylxanthine (19b). A mixture of **6b** (5.00 g, 25.3 mmol) and *trans*-cinnamic acid (7.47 g, 50.6 mmol) was heated at 130 °C overnight. The solid was removed from the flask, ground with a mortar and pestle, and then stirred with 75 mL of ether. Filtration gave 7.50 g of the amide which was refluxed for 1 h in 50 mL of water containing 1.25 g of sodium hydroxide. The mixture was filtered while hot and the pH was adjusted to 2 with use of hydrochloric acid. The resulting white precipitate was collected and recrystallized from ethanol to give 0.95 g (12%) of **19b**: mp 283–286 °C; ^{1}H NMR (DMSO-d_6) δ 0.86 (t, J = 7.5 Hz, 3 H), 1.56 (sextet, J = 7.5 Hz, 2 H), 3.46 (s, 3 H), 3.83 (t, J = 7.5 Hz, 2 H), 7.02 (d, J = 16.5 Hz, 1 H), 7.38 (m, 3 H), 7.62 (d, J = 7.5 Hz, 2 H), 7.63 (d, J = 16.5 Hz, 1 H); IR (KBr) 3132, 3083, 3024, 2960, 1692, 1651, 1594, cm^{-1}. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

Radioligand Binding Assays. Potency of xanthine analogues at adenosine A$_1$ and A$_2$ receptors was assessed by using standard radioligand binding procedures. The A$_1$ binding assay was performed by using a modification of the procedure described by Bruns et al.[19] Fresh rat cerebral cortex (male Sprague-Dawley) was homogenized (Brinkman Polytron, setting 5.5, 10 s) in 50 vol (w/v) of ice cold Tris-HCl buffer (50 mM, pH 7.4 at 25 °C), centrifuged at 48000g (10 min; 4 °C) and the pellet resuspended in 50 vol (w/v) of buffer containing adenosine deaminase (ADA; 2 IU/mL). Following a 30-min incubation at 37 °C to remove endogenous adenosine, the suspension was cooled on ice, recentrifuged as before, and the final pellet was resuspended in fresh buffer (7.5 mg tissue/mL) for use in the binding assay.

Inhibition of specific binding of [^{3}H]CHA to A$_1$ receptors of cerebral cortical membranes was performed by using triplicate incubations in a total assay volume of 2 mL containing 1.0 nM [^{3}H]cyclohexyladenosine ([^{3}H]CHA; sp act. = 25 Ci/mmol), 7.5 mg of tissue, 50 mM Tris-HCl buffer (pH 7.4 at room temperature), and 12 concentrations of the xanthine analogues. Nonspecific binding was defined by 10 μM 2-chloroadenosine (2-CADO). Following a 2-h incubation at 25 °C, the binding reaction was stopped by vacuum filtration (Whatman GF/B filters) and the samples were counted (Beckman Ready Safe scintillation fluid) in a Beckman LS 3801 counter.

Adenosine A$_2$ binding of [^{3}H]-*N*-ethyladenosin-5′-uronamide ([^{3}H]NECA) to rat striatal membranes was performed using the method described by Bruns et al.[9] which incorporates 50 nM cyclopentyladenosine (CPA) to block binding to the A$_1$ receptor population. Striatal membranes were prepared from rat brain (male Sprague-Dawley) by homogenization in 10 vol (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) and centrifugation at 48000g (10 min; 4 °C). The tissue pellet was washed once, suspended in 50 mM Tris-HCl buffer containing 10 mM MgCl$_2$, 0.1 unit/mL ADA, and 50 nM CPA.

The A$_2$ assay was performed by using triplicate incubations in a total volume of 0.5 mL containing 4 nM [^{3}H]NECA (sp act. = 18 Ci/mmol), 5 mg of tissue, 50 mM Tris-HCl buffer (pH 7.7) containing 10 mM MgCl$_2$, 0.1 unit/mL ADA, 50 nM CPA, and 12 concentrations of the xanthine analogues. Nonspecific binding was defined by 100 μM CPA. Following a 1-h incubation at room temperature, the binding reaction was stopped by vacuum filtration, and samples were quantified as above.

For both assays, the IC$_{50}$ values were calculated by using computer-assisted log-logit analyses. Apparent affinity constants (K_i) were calculated according to the method of Cheng and Prusoff.[27] The apparent K_d values for [^{3}H]CHA and [^{3}H]NECA were 1 and 4 nM, respectively, as calculated from 3–5 separate saturation experiments.

Supplementary Material Available: IR and ^{1}H NMR data for xanthines 9–19 (except 9b, 17c, and 19b which are reported above) (2 pages). Ordering information is given on any current masthead page.

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