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Caffeine Analogs: Structure-Activity Relationships at Adenosine Receptors

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Abstract. Caffeine and analogs that contain ethyl, propyl, allyl, propargyl and other substituents in place of methyl at 1-, 3- and 7-positions were antagonists at the two major classes (A_1 and A_2) of adenosine receptors. Potency at both receptors increased as methyls were replaced with larger substituents. Certain analogs with only one of the three methyl groups of caffeine replaced by larger substituents were somewhat selective for A_2 receptors. None of the analogs were particularly selective for A_1 receptors. The presence of polar entities in the substituent at the 1- or 7-position was poorly tolerated at adenosine receptors. Activity of caffeine analogs at A_1 and A_2 adenosine receptors in a variety of systems and cell types is presented and summarized.

The pharmacological actions of caffeine, theophylline and other xanthines are consonant with a major contribution from blockade of adenosine receptors [1, 2]. However, inhibition of phosphodiesterase and mobilization of calcium undoubtedly also contribute to the pharmacological profile of xanthines. Caffeine is relatively weak, both as an adenosine receptor antagonist and as a phosphodiesterase inhibitor. Efforts have been made to develop more potent and/or selective analogs of caffeine by replacement of the methyls at the 1-, 3- and 7-positions with other substituents [3, 4]. Certain of these analogs proved to be more potent than caffeine, and some are selective for A_2 adenosine receptors. However, activity as phosphodiesterase inhibitors is in many cases also increased [5]. Behavioral stimulation by xanthines appears to be correlated with blockade of adenosine receptors [6], but only for xanthines that are not potent inhibitors of the calcium-independent phosphodiesterase [5]. Such xanthines are be-

havioral depressants. The potencies of caffeine and theophylline analogs as tracheal relaxants appear to correlate with potency as inhibitors of calcium-dependent phosphodiesterases and not with potency as adenosine receptor antagonists [7]. Caffeine analogs have potential as therapeutic agents as antiasthmatics, antithrombotics and cognitive enhancers. The present report summarizes and extends data on the affinity of caffeine and analogs for A_1 and A_2 adenosine receptors.

Table 1. Synthesis of caffeine analogs: general procedures I, II and III

Product	Starting xanthine (X)	Halide	Time h	Temperature °C	Solvent
2	1,3-DiethylX	Ethyl bromide	5	80	DMF
4[a]	1,3-DiallylX	Allyl bromide	2	40	DMF
6	1,3-DiallylX	Ethyl bromide	24	45	DMF
7	1,3-DipropylX	Allyl bromide	4	37	DMF
8[b]	1,3-DipropylX	Propargyl bromide	2	40	DMF
9	1,3-DipropylX	Benzyl bromide	12	35	DMF
11	7-MethylX	Ethyl bromide	24	45	DMF
12	1,3-DipropylX	Methyl iodide	3	35	DMF
13	1,3-DipropylX	Methyl iodide	3	35	DMF
15	3-MethylX	Ethyl bromide	4	80	DMF
17	3-MethylX	Allyl bromide	4	80	DMF
18	3-MethylX	Propargyl bromide	5	80	DMF
21	1-MethylX	Propargyl bromide	24	35	DMF
23	3-Isobutyl-1-methylX	Allyl bromide	4	35	DMF
24	3-Isobutyl-1-methylX	Propargyl bromide	24	35	DMF
28	3,7-DimethylX	Allyl bromide	22	80	EtOH-H_2O (2:1)
29	3,7-DimethylX	Propargyl bromide	72	80	EtOH-H_2O (2:1)
32	3,7-DimethylX	Benzyl bromide	10	80	EtOH-H_2O (2:1)
34	3,7-DimethylX	Bromoacetonitrile	5	60	DMF
35	3,7-DimethylX	Bromomethyl methylether	5	100	DMF
36	3,7-DimethylX	Chloroacetone	10	100	DMF
38	3,7-DimethylX	Ethyl iodoacetate	5	100	DMF
40	3-PropylX	Methyl iodide	4	35	DMF
42	1-Methyl-3-isobutylX	Methyl iodide	3	35	DMF
45	1,3-DimethylX	Allyl bromide	4	35	DMF
46	1,3-DimethylX	Propargyl bromide	24	45	DMF
47	1,3-DimethylX	Benzyl bromide	12	35	DMF
54	1,3-DimethylX	Bromoacetonitrile	24	60	DMF
55	1,3-DimethylX	Bromomethyl methyl ether	24	60	DMF
56	1,3-DimethylX	Chloroacetone	24	60	DMF
59	1,3-DimethylX	Ethyl iodoacetate	12	60	DMF

[a] mp 61 °C.
[b] mp 87 °C.

Methods

Materials

N-Ethylcarboxamidoadenosine (NECA) and *R*-N^6-phenylisopropyladenosine (*R*-PIA) were from Research Biochemicals (Natick, Mass., USA); [^{3}H]*R*-PIA (50 Ci/mmol) and [^{3}H]NECA (18 Ci/mmol) from New England Nuclear (Boston, Mass., USA); and [α-^{32}P]ATP (30 Ci/mmol) from Amersham (Arlington Heights, Ill., USA). Rolipram was provided by Schering (Berlin, FRG).

7-(2-Chloroethyl)theophylline (49) and 7-(2-hydroxyethyl)theophylline (50) were from Aldrich Chemical (Milwaukee, Wisc., USA). 1,3,7-Tripropargylxanthine (5), 1,7-dipropargyl-3-methylxanthine (18) and 3-propargyl-1,7-dimethylxanthine (41) were kindly provided by Dr. J. Neumeyer of Research Biochemicals and 1-ethyl-3,7-dimethylxanthine (26) and 1-butyl-3,7-dimethylxanthine (30) by Dr. R.F. Bruns (Lilly Research Laboratories, Indianapolis, Ind., USA). Syntheses of 3, 7–9, 12, 13, 22–25, 27–29, 40, 42, 44–47 and 51 have been described [3].

General procedures for synthesis of remaining caffeine analogs are described below. In most cases these involved alkylation of a mono- or dialkylxanthine. Purity of products was ascertained by thin-layer chromatography and mass spectral analysis. Mass spectra were determined with a Finnegan 1015 quadrapole (chemical ionization with CH_4 or NH_3) and with VG 70/70 (electron impact, 70 eV) mass spectrometers. Melting points (mp) are reported for compounds (4, 8, 16, 20), where analyses for carbon, hydrogen and nitrogen provided further evidence for purity.

Procedure and isolation method	Recrystallization solvent	Yield, %
IB	EtoH/Me_2CO	46
IE	Me_2CO	96
IA	DMF/H_2O	46
IA	EtOH/H_2O	55
IA	EtOH/H_2O	89
IA	DMF/H_2O	84
IB	DMF/Me_2CO	54
IB	DMF/Me_2CO	67
IIB	DMF/H_2O	68
IC	DMF/H_2O	43
IB	EtOH/Me_2CO	63
IC	DMF/H_2O	66
IB	DMF/H_2O	55
IA	EtOH/H_2O	72
IB	MeOH/H_2O	78
IIC	DMF/H_2O	74
IIC	DMF/MeOH	71
IIA	DMF/H_2O	80
IIIF	MeOH/ether	93
IIIE	DMF/MeOH	89
IIIE	DMF/MeOH	86
IIIE	DMF/MeOH	90
IB	DMF/H_2O	79
IB	DMF/H_2O	78
IB	Me_2CO/H_2O	76
IB	$CHCl_3$/MeOH	90
IA	DMF/H_2O	80
IIIF	MeOH/Et_2O	97
IIIE	MeOH/Et_2O	43
IIIF	MeOH/Et_2O	73
ID	DMF/Et_2O	90

Synthesis of Caffeine Analogs

General Procedure I. To a stirred suspension of 4.5 mmol of mono- or dialkylxanthine and 0.7 g of anhydrous K_2CO_3 in 8 ml of dimethylformamide (DMF) was added dropwise 5 mmol of alkyl halide. The reaction temperature and time are indicated in table 1. Volatile materials were removed in vacuo, and product was isolated by one of the following methods. (A) H_2O was added to precipitate the product, followed by chilling in ice, filtration and drying. The product was purified when necessary by recrystallization from an appropriate solvent (table 1). (B) H_2O was added and the H_2O-DMF mixture was evaporated in vacuo. The residue was extracted with several portions of EtOAc. The combined extracts were dried over $MgSO_4$, filtered and solvent evaporated in vacuo. Recrystallization with the solvent indicated in table 1 provided pure compound. (C) The residue was dissolved in H_2O followed by acidification with concentrated HCl to give a precipitate, which was removed by filtration and dried. Recrystallization with the solvent, indicated in table 1, gave a pure com-

Table 2. Hydrogenation of xanthines

Product	Starting xanthine (X)	Time	Solvent	Recrystallization solvent	Yield %
3	7-Propargyl-1,3-dipropylX	2.5 h	EtOH	$EtOH/H_2O$	58
22	7-Propargyl-1-methyl-3-isobutylX	20 min	EtOH	$EtOH/H_2O$	83
27	1-Allyl-3,7-dimethylX	20 min	DMF-EtOH (1:2)	DMF/H_2O	85
44	7-Allyl-1,3-dimethylX	20 min	DMF	$CHCl_3/Me_2CO$	85
52	7-Cyanomethyl-1,3-dimethylX	24 h	EtOH-$CHCl_3$ (5:1)	MeOH/EtOAc	86

pound. (D) After removal of solvent in vacuo, the residue was dissolved in water, followed by extraction with $CHCl_3$. After drying with Na_2SO_4, $CHCl_3$ was removed to give crude product. Recrystallization with appropriate solvent provided pure compound. (E) After removal of solvent in vacuo, the residue was triturated with acetone, filtered and solvent removed in vacuo, and the residue allowed to crystallize in freezer. Recrystallization provided pure compound.

General Procedure II. A mixture of 20 mmol of mono- or dialkylxanthine, 20 ml of 10% NaOH, 50 ml of H_2O and 100 ml of EtOH was refluxed for 15 min. The solution was cooled to room temperature, and 40 mmol of alkyl halide were added dropwise with stirring. Reaction temperature and time are indicated in table 1. The solvent was removed in vacuo and the product was purified by one of the isolation methods described in General Procedure I.

General Procedure III. A mixture of 2.5 mmol of dialkylxanthine, 2.5 mmol of NaOH, 15 ml H_2O and 25 ml EtOH was refluxed for 25 min. The solvent was removed in vacuo and the residue dried at 80 °C. To the resulting sodium salt of the dialkylxanthine in 15 ml DMF was added a 5-fold excess of the appropriate halide, and the reaction mixture was heated for the time and temperature indicated in table 1. Solvent was removed in vacuo and the product was isolated by one of the following isolation methods. (F) The residue was dissolved in saturated $NaHCO_3$, extracted with $CHCl_3$, dried with Na_2SO_4 and solvent removed to yield a crude product. Recrystallization with the appropriate solvent provided pure compound (table 1). (G) The residue was dissolved in small quantity of MeOH and Et_2O was added to precipitate the product, which was filtered and dried. Recrystallization with the appropriate solvent provided pure compound (table 1).

Hydrogenation of Xanthines

General Procedure. A mixture of xanthine, solvent, and 10% Pd on C was hydrogenated at 40 pounds per square inch H_2 for the time indicated in table 2. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. H_2O was added and the precipitate was filtered and dried. Recrystallization with the appropriate solvent provided pure compound (table 2).

Hydrolysis of Esters

Compounds 39 and 60 were obtained by hydrolysis as follows: a mixture of 0.3 mmol of 1-(ethylcarboxymethyl)-3,7-dimethylxanthine (38), 2 ml 10% NaOH and 3 ml DMF was refluxed for 20 min. The solvent was removed in vacuo and the residue was dissolved in H_2O. The aqueous solution was acidified and extracted with $CHCl_3$, the organic layer was dried with Na_2SO_4, and the $CHCl_3$ was removed to give crude product. Recrystallization with MeOH/ether provided pure 1-(carboxymethyl)-3,7-dimethylxanthine (39) in 82% yield. In a similar procedure, a mixture of 0.3 mmol of 7-(ethylcarboxymethyl)-1,3-dimethylxanthine (59), 2 ml of 10% NaOH and 3 ml DMF was refluxed for 30 min. The solvent was removed in vacuo and the residue was dissolved in H_2O. The aqueous solution upon acidification and cooling gave needle-like crystals which were filtered and dried to provide pure 7-(carboxymethyl)-1,3-dimethylxanthine (60) in 85% yield.

1,7-Dipropyl-3-Methylxanthine (16)

1-Methyl-3-propyl-6-aminouracil was prepared according to Papesch and Schroeder [8] by condensation of methylurea with cyanoacetic acid followed by alkylation with propyl bromide. The precipitated product was dissolved in 40% acetic acid, the solution

was warmed to 80 °C and an excess of $NaNO_2$ was added. The mixture was stirred for 1 h at room temperature, and the precipitated purple crystals were collected by filtration and air-dried. The 1-methyl-3-propyl-5-nitroso-6-aminouracil was dissolved in absolute methanol and catalytically reduced with H_2/Pt. After removal of the catalyst by filtration and evaporation, the diaminouracil was refluxed with 98% formic acid for 1 h. The excess of acid was removed, 10% NaOH solution was added and the mixture was refluxed for 15 min, followed by neutralization with concentrated HCl. Solvent was evaporated in vacuo, and residue triturated in hot acetone. After removal of the acetone, the oily product 3-methyl-1-propylxanthine crystallized in the freezer in 64% yield. A portion (0.150 g, 0.72 mmol) of the 3-methyl-1-propylxanthine was dissolved in 3 ml DMF. Equivalent amounts of K_2CO_3 and propyl bromide were added. The mixture was kept at 40 °C for 2 h and then stirred overnight. The solvent was evaporated and water was added. The oily product crystallized upon cooling, was collected by filtration and recrystallization from DMF/H_2O. Yield: 54%, mp 71 °C.

1-Methyl-3,7-Dipropylxanthine (20)

1-Propyl-3-methyl-6-aminouracil was prepared according to Papesch and Schroeder [8] by condensation of propylurea with cyanoacetic acid followed by alkylation with methyl iodide. The synthesis of 1-methyl-3-propylxanthine in 72% yield by nitrosation, reduction and cyclization with formic acid was as described above for 3-methyl-1-propylxanthine. Alkylation with propyl bromide was as described above for synthesis of 16 h. Yield: 83%, mp 88 °C.

Biological Assays

(i) Inhibition of binding of [^{3}H]N^6-cyclohexyladenosine or [^{3}H]*R*-PIA to A_1 adenosine receptors in rat brain membranes was assayed as described [9, 10]. Results with either of these ligands in rat brain membranes have been nearly identical. The assay with [^{3}H]*R*-PIA now is used routinely in our laboratory. (ii) Antagonism of *R*-PIA-elicited inhibition of adenylate cyclase via an A_1 adenosine receptor in rat fat cell membranes was assayed as described [11]. (iii) Inhibition of binding of [^{3}H]NECA to A_2 adenosine receptors in rat striatal membranes was assayed as described [12] with 50 nmol/l N^6-cyclopentyladenosine present to block A_1 adenosine receptors. (iv) Antagonism of 2-chloroadenosine-elicited stimulation of adenylate cyclase via an A_2 adenosine receptor in rat striatal membranes was assayed essentially as described [13]. Further details will be published elsewhere. (v) Antagonism of NECA-elicited stimulation of adenylate cyclase via an A_2 adenosine receptor in rat pheochromocytoma PC12 membranes was assayed as described [11]. (vi) Antagonism of NECA-elicited stimulation of adenylate cyclase via an A_2 adenosine receptor in human platelets was assayed as described [11, 14]. (vii) Data for inhibition of 2-chloroadenosine-elicited accumulation of cyclic AMP in guinea pig cerebral cortical slices are from Daly et al. [3]. (viii) Data for inhibition of adenosine-elicited accumulation of cyclic AMP in human fibroblasts are from Bruns [15].

Data Analysis

EC_{50} or IC_{50} values were obtained from concentration-response curves. K_i values for binding were obtained from IC_{50} values by the Cheng-Prusoff equation [16] using a K_D for [^{3}H]*R*-PIA of 1.0 nmol/l and a K_D for [^{3}H]NECA of 8.5 nmol/l. K_B values for adenylate cyclase were calculated using the Schild equation [17], and the ratio of EC_{50} values for NECA-activation or the ratio of IC_{50} values for *R*-PIA inhibition in the presence and absence of antagonist.

Results and Discussion

Caffeine is a relatively weak and nonselective adenosine receptor antagonist showing an apparent K_i value of about 30–50 µmol/l in a variety of assay systems (table 3). These include the following.

(i) Inhibition of binding of an agonist ([^{3}H]N^6-cyclohexyladenosine or *R*-[^{3}H]N^6-phenylisopropyladenosine) to A_1 receptors in rat brain membranes.

(ii) Antagonism of *R*-PIA-elicited inhibition of adenylate cyclase via an A_1 receptor in rat fat cell membranes. Results from these two A_1 receptor assays have shown a strong correlation for many compounds [4, 11, 18].

(iii) Inhibition of binding of an agonist, [^{3}H]NECA, to A_2 receptors in rat striatal membranes. This assay affords results with many, but not all, compounds that are consonant with values obtained in assays based on adenylate cyclase with rat PC12 cell membranes (assay v). However, some compounds show a poor correlation between K_i values for this binding assay and K_i values for inhibition of A_2-receptor-mediated activation of rat adenylate cyclase (table 3). The correlation of K_i values for the binding assay in rat striatal membranes with K_i values for inhibition of A_2-receptor-mediated activation of rat striatal adenylate cyclase (assay iv) is much better, suggesting that the high-affinity A_2 receptor in striatum is a subtype different from the high-affinity A_2 receptors in other tissues. The A_2 adenosine receptor of striatal membranes has been previously proposed to be a high-affinity (A_{2a}) subtype [12, 13] to contrast it with the low-affinity (A_{2b}) receptors present in brain slices [13] and in fibroblasts [15].

(iv) Antagonism of NECA-elicited stimulation of adenylate cyclase via an A_2 receptor in rat striatal membranes. A limited number of caffeine analogs, mainly those whose K_i values in striatal binding assays (assay iii) did not correlate well with K_i values in PC12 adenylate cyclase assays (assay v), were assessed in this assay.

(v) Antagonism of NECA-elicited stimulation of adenylate cyclase via an A_2 receptor in rat PC12 membranes. Results from this assay have shown a correlation with results on stimulation of coronary blood flow with adenosine agonists [19].

(vi) Antagonism of NECA-elicited stimulation of adenylate cyclase in human platelet membranes. It has been previously noted [11, 18] that the A_2 receptors in PC12 and

Table 3. Caffeine analogs (1,3,7-trisubstituted xanthines): activity at A_1 and A_2 adenosine receptors

Product	Xanthine (X)
1	1,3,7-TrimethylX (caffeine)
2	1,3,7-TriethylX
3	1,3,7-TripropylX
4	1,3,7-TriallylX
5	1,3,7-TripropargylX
6	1,3-Diallyl-7-ethylX
7	1,3-Dipropyl-7-allylX
8	1,3-Dipropyl-7-propargylX
9	1,3-Dipropyl-7-benzylX
10	1,3-Dibutyl-7-(2-oxopropyl)X
11	1,3-Diethyl-7-methylX
12	1,3-Dipropyl-7-methylX
13	1,3-Diallyl-7-methylX
14	1,3-Dipropargyl-7-methylX
15	1,7-Diethyl-3-methylX
16	1,7-Dipropyl-3-methylX
17	1,7-Diallyl-3-methylX
18	1,7-Dipropargyl-3-methylX
19	3,7-Diethyl-1-methylX
20	3,7-Dipropyl-1-methylX
21	3,7-Dipropargyl-1-methylX
22	7-Propyl-3-isobutyl-1-methylX
23	7-Allyl-3-isobutyl-1-methylX
24	7-Propargyl-3-isobutyl-1-methylX
25	7-Benzyl-3-isobutyl-1-methylX
26	1-Ethyl-3,7-dimethylX
27	1-Propyl-3,7-dimethylX
28	1-Allyl-3,7-dimethylX
29	1-Propargyl-3,7-dimethylX
30	1-Butyl-3,7-dimethylX
31	1-Hexyl-3,7-dimethylX
32	1-Benzyl-3,7-dimethylX
33	1-(5-Oxohexyl)-3,7-dimethylX
34	1-Cyanomethyl-3,7-dimethylX
35	1-Methoxymethyl-3,7-dimethylX
36	1-(2-Oxopropyl)-3,7-dimethylX
37	1-(2-Hydroxypropyl)-3,7dimethylX
38	1-(Ethylcarboxymethyl)-3,7-dimethylX
39	1-(Carboxymethyl)-3,7-dimethylX

A_1 receptor, K_i, μmol/l		A_2 receptor, K_i, μmol/l					
i binding rat brain	ii cyclase rat fat cell	iii binding rat striatum	iv cyclase rat striatum	v cyclase rat PC12 cell	vi cyclase human platelet	vii cAMP guinea pig brain	viii cAMP human fibroblasts
44 (31–63)	59 (40–86)	45 ± 7	70 ± 7	36 ± 4	30 (16–54)	50 ± 5	13[a]
4.5 (2–11)	–	23 ± 0.7	27 ± 2	4.8 ± 1.2	4.0 ± 0.9	1.2 ± 0.2	2.5[a]
2.6 ± 0.4	9.3 (8–11)	13 ± 0.4	11 ± 0.7	4.0 ± 1.0	2.3 ± 0.3	1.4 ± 0.3	–
11.9 ± 0.6	–	22 ± 2	–	8.9 ± 0.8	–	–	–
3.0 ± 0.3	2.0 ± 0.4	4.5 ± 0.7	1.4 ± 0.3	1.6 ± 0.03	0.7 ± 0.2	–	–
10.3 ± 1.0	–	27 ± 4	–	7.8 (6–10)	–	–	–
1.9 ± 0.1	–	8.7 ± 0.3	–	2.1 ± 0.5	–	0.7 ± 0.0	–
0.6 ± 0.2	–	1.1 ± 0.3	2.6 ± 0.7	0.63 ± 0.05	–	0.4 ± 0.2	–
1.0 ± 0.2	–	5.9 ± 0.3	–	0.88 ± 0.14	–	1.5 ± 0.3	–
20[a]	–	46[a]	–	–	–	–	–
7.5 ± 0.6	–	46 ± 3	–	13 (8–22)	–	–	–
7 ± 3	12 (7–22)	10 ± 0.4	18 ± 2	5.3 (4–7)	2.8 (1.4–5.7)	1.2 ± 0.2	–
18 ± 8	–	31 ± 3	–	6.9 ± 1.3	–	4 ± 1	–
8.3 ± 1.7	4.9 ± 0.7	9.1 ± 1.0	8.6 ± 2	6.2 (4–10)	2.4 ± 0.2	–	–
29 (21–41)	–	116 ± 1	–	50 (40–63)	–	–	–
8.0 ± 0.3	–	18 ± 2	14 ± 2	9.5 ± 1.9	–	–	–
25 (15–43)	–	48 ± 3	–	31 (10–100)	–	–	–
5.8 (4–9)	5.2 ± 1.1	8.1 ± 1.0	–	9.8 (1.6–59)	–	–	–
–	–	–	–	–	–	–	28[a]
10.1 ± 1.5		26 ± 3	–	6.3 ± 0.7	–	–	–
4.4 ± 1.0	–	11 ± 1	–	5.0 (1–26)	–	–	–
13 ± 2	–	28 ± 2	–	3.7 ± 0.5	–	11 ± 5	–
13 ± 2	–	19 ± 4	–	2.9 ± 0.8	–	8.5 ± 1.4	–
3.1 ± 0.1	–	3.9 ± 0.8	–	1.5 ± 0.2	2.3 ± 0.2	2.8 ± 0.2	–
9 ± 2	–	32 ± 4	–	7.5 ± 0.6	–	11 ± 5	–
26 ± 2	–	46 ± 4	–	–	–	–	4.1[a]
38 ± 9	57 (39–82)	48 ± 4	–	28 (22–35)	16 (9–30)	5 ± 1	–
47 ± 11	–	73 ± 3	58 ± 12	9.9 ± 1.6	–	6.5 ± 0.7	–
45 ± 4	94 (68–130)	16 ± 4	12 ± 4	8.6 ± 0.4	4 (3–6)	6.1 ± 1	–
16 ± 1	–	48 ± 4	–	–	–	–	2.8[a]
–	–	–	–	–	–	–	28[a]
17 (13–23)	–	47 ± 2	–	24 (15–40)	–	–	–
> 100[a]	330[a]	> 100[a]	–	–	510[a]	–	400[a]
> 100	–	–	–	–	> 100	–	–
> 100	–	270 ± 20	–	–	71 (63–82)	–	–
> 100	–	–	–	–	> 100	–	–
410[a]	770[a]	–	–	–	1,800[a]	–	–
24 (12–48)	–	380 ± 20	–	–	87 (47–160)	–	–
> 100	–	103 ± 3	–	–	> 100	–	–

(Table continued next page.)

platelet membranes do not afford identical K_i values with many compounds, suggesting that they represent different subtypes of the high-affinity (A_{2a}) A_2 adenosine receptors.

(vii) Antagonism of 2-chloroadenosine-elicited accumulations of cyclic AMP in guinea pig cerebral cortical slices [3]. This response appears to involve a low-affinity (A_{2b} subtype of adenosine receptor [12]. This assay is with guinea pig tissue rather than rat, and several studies have shown that both A_1 and A_2 receptors vary from species to species [20–22], providing another complication in comparison of results from different assay systems.

(viii) Antagonism of adenosine-elicited accumulations of cyclic AMP in human fibroblast cells [15]. This is a low-affinity (A_{2b}) A_2 adenosine receptor. It is only in this assay that caffeine is relatively potent (K_i 13 μmol/l, table 1). But theophylline is also more potent (K_i, 5 μmol/l) in this assay system than in most other A_2 assays (K_i values: iii, 17; iv, 20; v, 17; vi, 14, and vii, 14 μmol/l [3, 4, 12, 23].

An increase in the size of the substituents at the 1-, 3- and 7-positions from methyl in caffeine (1) to ethyl in 2 increased potency at both A_1 and A_2 receptors with the sole exception of the A_2 receptor in striatum where the effect of the increase in the size of the substituent on activity was minimal (table 3). A further increase in size to propyl in 3 had little further effect, except for the A_2 receptor in striatum where activity was slightly increased. The 1,3,7-tripropargyl analog 5 tended to be more potent than the corresponding 1,3,7-tripropyl analog 3 at A_2 receptors and at the fat cell A_1 receptor, but the two analogs were equipotent at the brain A_1 receptor.

Table 3 (continued)

Product	Xanthine (X)
40	3-Propyl-1,7-dimethylX
41	3-Propargyl-1,7-dimethylX
42	3-Isobutyl-1,7-dimethylX
43	7-Ethyl-1,3-dimethylX
44	7-Propyl-1,3-dimethylX
45	7-Allyl-1,3-dimethylX
46	7-Propargyl-1,3-dimethylX
47	7-Benzyl-1,3-dimethylX
48	7-Phenyl-1,3-dimethylX
49	7-(2-Chloroethyl)-1,3-dimethylX
50	7-(2-Hydroxyethyl)-1,3-dimethylX
51	7-(2-Acetoxyethyl)-1,3-dimethylX
52	7-(2-Aminoethyl)-1,3-dimethylX
53	7-(2-Diethylaminoethyl)-1,3-dimethylX
54	7-Cyanomethyl-1,3-dimethylX
55	7-Methoxymethyl-1,3-dimethylX
56	7-(2-Oxopropyl)-1,3-dimethylX
57	7-(2-Hydroxypropyl)-1,3-dimethylX
58	7-(2,3-Dihydroxypropyl)-1,3-dimethylX
59	7-(Ethylcarboxymethyl)-1,3-dimethylX
60	7-(Carboxymethyl)-1,3-dimethylX

[a] Values presented as single numbers are from other laboratories as reported [12, 15, 24, 25].

Variation of the 7-substituent from propyl to allyl, in 1,3-dipropyl series (compounds 3, 7) had little effect on activity (table 3). However, the 7-propargyl analog (8) was more potent than the propyl analog [3] in all systems. The 7-benzyl analog [9] was more potent than 3 in all systems except guinea pig brain slices. The 1,3-dipropyl-7-methylxanthine (11) was equivalent in potency or slightly less potent than 3 in all assay systems. Denbufylline, a 1,3-dibutyl analog (10) with a polar carbonyl group in the 7-substituent had relatively low affinity

A_1 receptor, K_i, μmol/l		A_2 receptor, K_i, μmol/l					
i binding rat brain	ii cyclase rat fat cell	iii binding rat striatum	iv cyclase rat striatum	v cyclase rat PC12 cell	vi cyclase human platelet	vii cAMP guinea pig brain	viii cAMP human fibroblasts
24±6	24 (12–48)	40±2	29±5	15 (13–16)	8.1 (5–13)	9±3	–
16±1.6	–	46±4	50±7	21 (9.4–45)	14±3	–	–
19±8	–	28±3	–	3.2±0.4	–	7±1	–
–	–	–	–	–	–	–	21[a]
21±6	109 (63–190)	74±6	55±12	8.2 (3.6–19)	9.3 (7.6–11)	11±3	–
33±2	–	39±0.5	–	7.7±1.6	–	10±2	–
12±3	12±2	23±0.1	23±3	3.4±0.6	5.4±0.9	4.1±0.2	–
6±1	–	46±4	–	5.6±1.0	–	14±4	–
14±1	–	20±4	–	–	–	–	39[a]
5±1	–	35±5	17±3	6.3 (3.5–11)	–	4.5±1.2	0.98[a]
105±15	195[a]	–	–	37±5.5	180[a]	135±8	160[a]
40±7	–	56±1	–	30±6.5	–	68±3	–
>100	–	110±4	–	–	>100	–	–
–	690[a]	–	–	–	520[a]	–	–
52 (40–66)	–	82±5	–	–	46±16	–	–
>100	–	78±6	–	–	>100	–	–
>100	–	88±4	–	–	>100	–	–
99[a]	280[a]	–	–	–	850[a]	–	130[a]
280[a]	810[a]	–	–	–	4,600[a]	–	>1,000[a]
24 (12–48)	–	>250	–	–	>100	–	>1,000[a]
>100	–	110±2	–	–	>100	–	250[a]

Values presented as means ± SEM or with 95% confidence limits are for 3 or more determinations from our laboratory. Certain data are from prior publications from our laboratory [3, 4].

for A_1 and A_2 receptors [24] consonant with the lack of tolerance for hydrophilic groups in the binding site for 7-substituents. Doxofylline (7-(1,3-dioxalone)-1,3-dimethyl-xanthine) also has low affinity for adenosine receptors [25].

In the series where the 1,3-substituents were varied, while the 7-methyl group was constant (compounds 11–14), the 1,3-diethyl compound 11 was the least potent at the A_2 receptor of rat PC12 cells, while being the most potent at the A_1 receptor of rat brain (table 3).

In the series where the 1,7-substituents were varied, while the 3-methyl group was held constant (compounds 15–18), 1,7-dipropyl-3-methylxanthine (16) and 1,7-dipropargyl-3-methylxanthine (18) were more potent than the 1,7-diethyl (15) and 1,7-diallyl (17) analogs. Analogs 15 and 17 were only slightly more potent than caffeine (3).

Two compounds were available for the present study in which only the 3,7-substituents were increased in size relative to caffeine (20, 21) and both were markedly more active than caffeine (table 3). 3,7-Diethyl-1-

methylxanthine (19) has been reported to be less active than caffeine at the A_2 receptor of human fibroblasts [15].

Several 7-substituted derivatives (compounds 22–25, 42) of the potent phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine [23] were assayed (table 3). The 7-propargyl derivative (24) was the most active of the series for both A_1 and A_2 receptors. 3-Isobutyl-1-methylxanthine is itself a potent, but relatively nonselective adenosine receptor antagonist (K_i values: i, 7 ± 2; ii, 3.2; iii, 14; v, 2.8; vi, 1.1; vii, 7, and viii, 3.5 μmol/l) [3, 12, 15, 23, 24].

A wide range of caffeine analogs have been prepared in which the 1-substituent was varied, while the 3- and 7-methyl groups were held constant (compounds 26–39). Several of these (26–29) were more potent than caffeine at A_2 receptors and represent somewhat selective antagonists for A_2 receptors (table 3). Indeed, 3,7-dimethyl-1-propargylxanthine (29) appeared to have A_2-selective actions in vivo [26]. A number of this group of analogs had polar groups (CO, CN, OCH_3, OH, COOH, COOEt) in the 1-substituent. Nearly all of the xanthines with a polar group in the 1-substituent, including 33 (pentoxifylline) and 37 (protheobromine), had low or no activity as adenosine receptor antagonists with the possible exception of the 1-ethylcarboxymethyl analog (38), which was more potent than caffeine at the brain A_1 receptor (table 3). Thus, the adenosine receptors do not accept polar entities in the apparent hydrophobic region of the receptor that interacts with the 1-substituent.

Only three caffeine analogs were available for the present study in which the 3-position was varied, while the 1- and 7-methyl groups were held constant (40–42). None were markedly more potent than caffeine (1) at A_1 receptors, nor at the A_2 receptors of striatum (table 3). The 3-propyl (40) and 3-propargyl (41) analogs were somewhat more potent than caffeine at the A_2 receptor of PC12 cells, while the 3-isobutyl (42) analog was 12-fold more potent than caffeine (3). Both the 3-propyl (40) and 3-isobutyl (42) analogs were more potent than caffeine at the adenosine receptor of guinea pig brain. The 3-propyl (40) analog was 3-fold more potent than caffeine at the A_2 receptor of human platelets. No analogs were available with a polar group in the 3-substituent, and it is unknown whether that part of the xanthine binding site on adenosine receptors will accept a hydrophilic group.

A wide range of caffeine analogs were available in which the 7-substituent was varied and the 1- and 3-methyl groups were held constant (compounds 43–60). This is due to the ease with which theophylline can be alkylated at the 7-nitrogen. The 7-propyl (44) and 7-allyl (45) analogs were markedly more potent than caffeine at A_2 receptors, except for the A_2 receptor of striatum, while being only slightly more potent than caffeine at the A_1 receptors of brain (table 3). Both appeared to be somewhat A_2 selective. The 7-propargyl analog 46 was more potent than caffeine at all adenosine receptors, and like 43 and 44, remained somewhat A_2 selective. None of these analogs are A_2 selective when the A_2 receptor of striatum was considered. 7-Ethyl-1,3-dimethylxanthine (43) has been reported to be less potent than caffeine at the A_2 receptor of fibroblasts [15]. It may be that 7-ethyl substitution lowers affinity at fibroblast A_2 receptors compared to methyl (see also 19 vs 1). As was the case for the 1-substituent, most of the analogs of caffeine with polar groups in the 7-substituent (compounds 49–60) had very low or no activity at adenosine receptors.

Thus, the xanthine binding site of the adenosine receptors appears to accommodate hydrophilic groups rather poorly in the 7-substituent. Certain of these analogs, such as 10 (denbufylline), 50 (etofylline), 53 (etamiphylline), 57 (proxyfylline) and 58 (dipropylline) have potential as therapeutic agents [24, 25, 27, 28]. The 7-(2-acetoxyethyl) analog 52, the 7-cyanomethyl analog 54 and the 7-ethyl carboxymethyl analog 55 were as potent as caffeine at certain adenosine receptors. The 7-(2-chloroethyl) analog 49 is noteworthy in being markedly more potent than caffeine at all adenosine receptors except the A_2 receptor of striatum (table 3).

The present summary provides some insights into structure activity of caffeine analogs at adenosine receptors and should serve as a guide to future studies either aimed at more potent or selective analogs or at analogs with little or no activity at adenosine receptors. The results reinforce earlier evidence [11–13, 18, 22] for various subtypes of A_2 adenosine receptors. In this regard, most of the xanthines are more potent in blocking NECA-stimulated adenylate cyclase in PC12 cell membranes than in striatal membranes. The potency and, in some cases, selectivity of the propargyl analogs (5, 8, 18, 19, 24, 29, 41, 46) suggest that this or similar electron-deficient moieties are worthy of further investigation as xanthine substituents.

Certain of these caffeine analogs have been studied in vivo for behavioral effects and for reversal of adenosine-analog-elicited behavioral depression and hypothermia [5, 26]. Compounds 5, 29 and 41 with propargyl substituents are marked behavioral stimulants, while compounds 3, 12, 38 and 44 with n-propyl substituents are behavioral depressants, apparently because they, and not the propargyl analogs, are potent inhibitors of a brain calcium-independent phosphodiesterase [5] (for further data on inhibition of brain phosphodiesterases by xanthines see Smellie et al. [23]). Compound 25, 7-benzyl-3-isobutyl-1-methylxanthine is a weak behavioral stimulant and is only moderately active as an inhibitor of the calcium-independent phosphodiesterase [5]. An earlier study had stressed a correlation of behavioral stimulant activity of a series of xanthines with affinity for a brain A_1 receptor [6]. It is clear that such correlations will be complicated not only by pharmacokinetics, but by effects on brain phosphodiesterases. One of the inactive xanthines in that study [6], namely 8-*p*-sulfophenyltheophylline, is a potent adenosine receptor antagonist, but does not penetrate into brain [26] or cells [29]. Another inactive xanthine is isocaffeine (1,3,9-trimethylxanthine), which, unlike caffeine, has nearly no affinity for adenosine receptors (K_i: i, 1,500; viii, 3,000 μmol/l). It, unlike caffeine, does not penetrate well into brain [6]. Certain of the caffeine analogs have also been investigated as tracheal relaxants [7]. In this case potency appeared to correlate not with potency as adenosine receptor antagonists, but with potency as inhibitors of a calcium-dependent phosphodiesterase. The 1,3,7-tripropyl analog 3 and the 1,3,7-tripropargyl analog 5 were the most potent tracheal relaxants of the thirteen caffeine analogs tested, with 3 being more potent than enprofylline and 5 being equipotent with enprofylline.

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