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8-SUBSTITUTED XANTHINES AS ANTAGONISTS AT A_1- AND A_2-ADENOSINE RECEPTORS

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Abstract—Two classes of 8-substituted analogs of theophylline (1,3-dialkylxanthines), having 8-cycloalkyl, 8-cycloalkenyl or 8-(para-substituted aryl) groups, were shown to be potent and, in some cases, receptor subtype selective antagonists at A_1- and A_2-adenosine receptors. New analogs based on a functionalized cogener approach and on classical medicinal chemical approaches were prepared. Affinity at A_1-adenosine receptors was evaluated by inhibition of binding of [^{3}H]N^6-phenylisopropyladenosine to rat brain membranes. Activity at A_2-adenosine receptors was measured by the reversal of 5′-*N*-ethylcarboxamidoadenosine (NECA)-stimulated production of cyclic AMP in membranes from rat pheochromocytoma PC12 cells. Cycloalkenyl analogs containing rigid olefinic bonds differed greatly in potency from the saturated analogs. The selectivity of phenylsulfonamide analogs depended on distal structural features. Novel xanthine analogs include diamino-, thiol-, aldehyde, and halogen-substituted derivatives, peptide conjugates of 8-[4-[2-aminoethylaminocarbonylmethyloxy]phenyl]1,3-dipropylxanthine (XAC), and a hydroxyethylamide analog of XAC.

Theophylline and other purine derivatives act as stimulants in the cardiovascular system, the central nervous system, the kidney, and at other sites, through the competitive inhibition of endogenous adenosine at A_1- and A_2-adenosine receptors [1]. In the past several years, 8-aryl analogs of theophylline having greatly enhanced potency at adenosine receptors have been described [2–10]. Such 8-arylxanthines were found to have 30- to 60-fold greater potency in binding to A_1-receptors than the corresponding non-substituted (C8-H) analog. Recently introduced antagonist ligands of particularly high potency containing 8-aryl substituents (Fig. 1) are 8-[2 - [4 - [2 - aminoethylaminocarbonylmethyloxy]-phenyl]-1,3-dipropylxanthine (XAC)¶ [4], with a K_i value of 1.2 nM at central A_1-receptors in the rat, and the sulfonamide PD 113,297 [5], with a K_i value of 5.6 nM [6]. [^{3}H]XAC is now available as a radioligand for A_1- [7] and A_2- [8] adenosine receptors. The sulfonoamide derivative PD 115,199 (Fig. 1) also has been tritiated for use as an A_2-antagonist radioligand [9]. The breadth of substitution on the aryl ring tolerated by adenosine receptors led to a functionalized congener approach to the design of xanthine drugs [10]. By this approach a chemically reactive chain is attached to the aryl ring at the para-position. This reactive chain in turn is coupled covalently to a variety of sterically expansive groups, including peptides, spectroscopic probes [11], and other reporter groups, such as biotin for subsequent avidin complexation [11].

Among 8-alkyl substituted xanthine antagonists, the cyclopentyl and cyclohexyl groups have been found to produce high affinity and extremely high A_1 selectivity [12–14]. For example, 8-cyclopentyl-1,3-dipropylxanthine (CPX, Fig. 1), having a K_i value at A_1-receptors of 1.2 nM [12], has been developed as a tritiated radioligand [13].

We now have examined the structure–activity relationships of 8-aryl and 8-cycloalkyl substituted xanthines in two membrane assay systems: (i) the competitive binding of an adenosine analog to A_1-adenosine receptors, and (ii) the antagonism of activation of adenylate cyclase by an adenosine analog at A_2-receptors. Additional areas of structural flexibility and new determinants of potency and selectivity of xanthines at adenosine receptors were identified.

EXPERIMENTAL PROCEDURES

Synthesis

8-Cyclopentyl-1,3-dipropylxanthine, CPX, **1**, was synthesized as described [14]. Compounds **2**, **3** and **5**

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¶ Abbreviations: XAC, xanthine amine congener, 8-[4-[2 - aminoethylaminocarbonylmethyloxy]phenyl] - 1,3 - dipropylxanthine; XCC, xanthine carboxylic congener, 8-[4 - [carboxymethyloxy]phenyl] - 1,3 - dipropylxanthine; XCC-OEt, 1,3-di-*n*-propyl-8-(*p*-ethyloxycarbonylaminoethylaminocarbonylmethyloxyphenyl)xanthine (XCC ethyl ester); CPX, 8-cyclopentyl-1,3-dipropylxanthine; PIA, N^6-phenylisopropyladenosine; NECA, 5′-*N*-ethylcarboxamidoadenosine; EDAC, dimethylaminopropylethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; and TFA, trifluoroacetic acid.

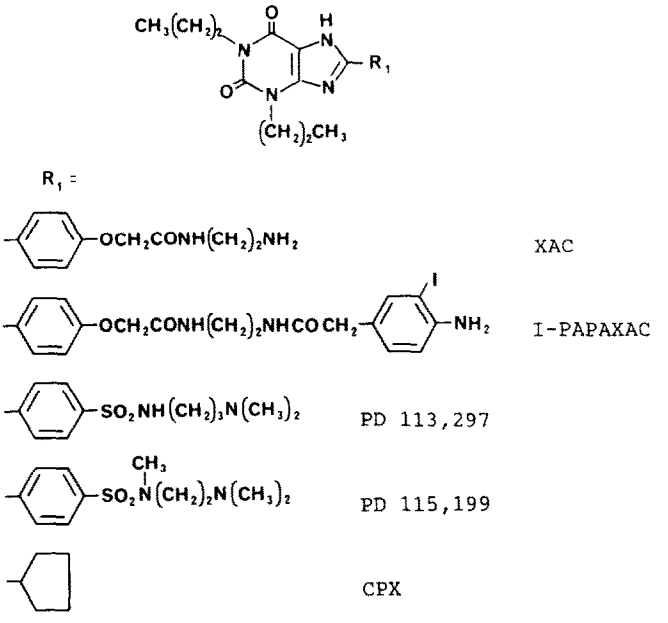


Fig. 1. Structures of 8-substituted 1,3-dialkylxanthine derivatives which are high affinity adenosine antagonists.

were synthesized from 5,6-diamino-1,3-dialkyluracil by condensation with the appropriate carboxylic acid followed by based-catalyzed ring closure or, alternatively, by condensation with an aldehyde followed by oxidative ring closure (similar procedures in Ref. 3). 1-Cyclopentenecarboxylic acid was obtained from Alfa Products (Danvers, MA), and tetrahydrobenzaldehyde was obtained from Aldrich Chemical (Milwaukee, WI). 3-Cyclopentenecarboxylic acid was synthesized by the methods of Murdock and others [15, 16]. XCC (8-[4-[carboxymethyloxy]-phenyl]-1,3-dipropylxanthine), the ethyl ester of XCC, and XAC were synthesized as described [4]. Bromoacetic anhydride was obtained from Pfaltz & Bauer (Waterbury, CT). Amino acid and peptide derivatives of XCC and XAC were synthesized in the manner previously described [10], using the water soluble 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) in dimethylformamide (DMF). Tyrosyl and para-nitrophenylalanyl intermediates were obtained from the Chemical Dynamics Corp. (South Plainfield, NJ). New compounds were characterized by 300 MHz proton NMR (unless noted, chemical shifts are in d_6-DMSO in ppm from TMS), chemical ionization mass spectroscopy (CIMS, NH_3, Finnigan 1015 spectrometer), and C, H, and N analysis. UV spectra were measured in methanol, and the results are expressed as peak wavelengths in nm with log ε values in parentheses.

The NMR spectrum of the intermediate 8-(3-cyclopentenyl)-1,3-dipropylxanthine, **3**, has the following resonances: 5.74 (s, 2H, C3-cyclopent.); 3.92 and 3.83, each (t, 2H, J = 7.2 Hz, CH_2N); 3.55 (m, 1H, Cl-cyclopent.); 2.7–2.8 (m, 4H, C2-cyclopent.); 1.67 and 1.54, each (m, 2H, Cβ-Pr); 0.85 (t, 6H, CH_3). The NMR spectrum of 8-(3-cyclohexenyl)-1,3-dipropylxanthine, **5**, has the following resonances: 5.72 (s, 2H, olefin); 3.93 and 3.83, each (t, 2H, J = 7 Hz, CH_2N); 2.95 (m, 1H, Cl-cyclohex.); 2.31 and 2.11, each (m, 2H, C2 and C6-cyclohex.); 2.0 and 1.8, each (m, 1H, C5-cyclohex.); 1.67 and 1.55, each (m, 2H, Cβ-Pr); 0.86 (t, 6H, CH_3).

8-*(3-Halocyclopentyl)*-1,3-*dipropylxanthines*, compounds **4a–c**. Compound **3** was treated with the corresponding halogen acid (for compounds **4a**, **b**, and **c**: 70% HF-pyridine (Aldrich) at 50° [17], 30% HBr in acetic acid at 50° [18], and KI in phosphoric acid at 80° [19]). Typical reaction times were 3–4 days. The products were purified by thin-layer chromatography on silica gel using a mixture of chloroform:methanol:acetic acid (96:2:2).

1,3-*Dialkyl*-8-*(p-amidosulfonylphenyl)xanthines*, compounds **8–15**. General procedure: 1 mmol of the starting 1,3-dialkyl-8-(*p*-sulfophenyl)-xanthine was refluxed in 10 ml of $POCl_3$ for 2 hr. The excess $POCl_3$ was removed by distillation in vacuum, and the residue was treated with ice to form a colorless solid. The precipitate (corresponding sulfonyl chloride) was collected by suction, dried over KOH, and, because of instability, was used without further purification. The xanthine sulfonyl chloride derivatives melted at 310° with decomposition and 285° (1,3-dimethyl and 1,3-dipropyl analogs, respectively) and gave acceptable (within 0.3%) C, H, and N analyses. UV spectrum of 1,3-dipropyl-8-(*p*-chlorosulfonylphenyl)xanthine showed peaks at 203 (4.43), 234 (4.21), and 342 (4.33) nm.

1,3-Dialkyl-8-(*p*-chlorosulfonylphenyl)xanthine (1 mmol) was suspended in 25 ml of ethanol, treated with 1 ml of the appropriate amine, and heated under reflux. Alternately, the reaction was carried out in 50 ml of chloroform at room temperature. After removal of solvent, the residue was recrystallized from H_2O/ethanol for compounds **8–15**. UV spec-

trum of 1,3-dipropyl-8-[4-[amino[ethyl[amino-[sulfonyl]]]]phenyl]xanthine, **9**, showed peaks at 203 (4.44), 247 (4.27), and 329 (4.36) nm.

Secondary or tertiary amine derivatives of XAC, compounds **21**, **23**, **25**, **28**, and **29**. To 0.45 g (1 mmol) of 1,3-di-*n*-propyl-8-[4-ethyloxycarbonyl-[amino-[ethyl[amino[carbonyl[methyloxy]-phenyl]]]]]-xanthine (XCC-OEt, Ref. 4) suspended in 10 ml of absolute ethanol was added 1 ml of the appropriate diamine (reactive amine is primary). The mixture was refluxed for 2 hr and evaporated to dryness. The residue was crystallized from H_2O/ethanol. Compound **21**, Nα-Me-XAC, was obtained exclusively upon aminolysis of XCC-OEt in *N*-methylethylenediamine. XAC, **20**, showed UV absorption peaks at 205 (4.44), 249 (4.31), and 315 (4.45) nm. N-Acetyl-XAC, **31** (and N,N-dimethyl-XAC, **24**, with nearly identical UV parameters), had peaks at 206 (4.49), 249 (4.32), and 315 (4.46) nm.

8-[2-Trimethylammonium[ethyl[aminocarbonylmethyloxyphenyl]]]-1,3-dipropylxanthine iodide, compound **27**. Compound **24** (31 mg 68 μmol) was suspended in 2 ml dimethylformamide and treated with 0.14 ml methyl iodide. Upon warming at 40° for 10 min a solution formed, and the reaction was judged complete by thin-layer chromatography (silica, $CHCH_3$:MeOH:HOAc, 10:10:1). Ester was added, and the product (36 mg, 98% yield) precipitated as a white solid. CIMS peaks as 471, 457, and 412. Proton NMR showed a singlet from the trimethylammonium group at 3.09 ppm.

(8-[2-Hydroxyethyl[amino[carbonyl[methyl[oxyphenyl]]]]]-1,3-dipropylxanthine), compound **40**. XCC-OEt (63 mg, 0.15 mmol) was dissolved in 3 ml of ethanolamine and warmed (50°) for 1 hr. The volume was reduced *in vacuo*. The product crystallized slowly upon addition of methanol/ether/petroleum ether, to give the pure product (59 mg, 90% yield). The NMR, mass spectra (CI), and elemental analyses were consistent with the assigned structure.

8-[2-Thioethyl[aminocarbonylmethyloxophenyl]]-1,3-dipropylxanthine, compound **45**. To 0.11 g (0.27 mmol) of XCC-OEt was added 2-aminoethanethiol (Aldrich, 0.55 g, 7 mmol) and 0.2 ml DMF. The mixture was heated until it liquified and then for an additional 10 min. Upon cooling, the solid residue was recrystallized from DMF containing sodium borohydride (40 mg) and water. Yield 94 mg (76% yield). C, H, N analysis.

ε-(p-Bromomethylbenzoyl)-D-lysyl-XAC trifluoroacetate, compound **57**. α-(*t*-Butyloxycarbonyl)-ε-(benzyloxycarbonyl)-D-lysyl-XAC [10] was hydrogenated over palladium black in DMF. The pure product, α-(*t*-butyloxycarbonyl)-D-lysyl-XAC, was isolated by ether precipitation in 54% yield and was coupled to α-bromotoluic acid using the EDAC/*N*-hydroxy-succinimide preactivation. The product was isolated by addition of water (82% yield) and treated with trifluoroacetic acid at room temperature for 10 min. The acid was evaporated, and the residue was recrystallized from MeOH/ether to give compound **57** in 62% yield.

Biochemical assays

Stock solutions of xanthines in the millimolar concentration range in dimethyl sulfoxide were prepared and stored frozen. Solutions were warmed to 50° prior to dilution in aqueous medium. Inhibition of binding of 1 nM [^{3}H]N^6-phenylisopropyladenosine to A_1-adenosine receptors in rat cerebral cortex membranes was assayed as described [7]. Inhibition of binding by a range of concentrations of xanthines was assessed in triplicate in at least three separate experiments. The IC_{50} values were converted to K_i values using a K_D value for [^{3}H]PIA of 1.0 nM and the Cheng–Prusoff equation [20]. Inhibition of *N*-ethylcarboxamidoadenosine stimulation of adenylate cyclase in membranes from rat PC12 cells was used to measure A_2-receptor activity [21]. The EC_{50} values for *N*-ethylcarboxamidoadenosine were obtained from concentration–response curves in the absence or presence of xanthine in three experiments. K_B-values were then calculated using the Schild equation [22].

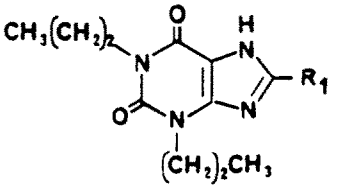
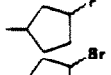
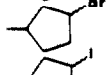
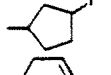
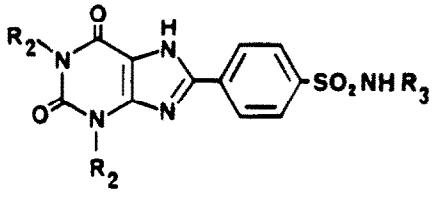
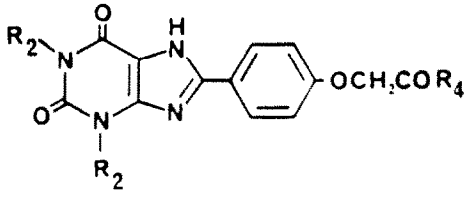
RESULTS AND DISCUSSION

A variety of new 8-alkyl and 8-aryl xanthine analogs were prepared (Table 1). The rationale for this set of analogs included mainly the introduction of halogen atoms for potential radioligands (compounds **4**, **30**, **32**, **36**, **43**, **44**, and **54**), probing effects of substitution of xanthine alkylamine derivatives (compounds **8–15**, **19–30**, **52–54**, **56**, **58**), probing effects of chirality and conformational restriction (compounds **2**, **3**, **5**, **16**, and **17**), and modification directed towards chemically irreversible inhibition of adenosine receptors and ligands for immobilization (compounds **30**, **32–34**, **36**, **40**, **42**, **45**, **47–54**, **56**, and **58**).

8-Cyclopentyl and 8-cyclohexyl derivatives of 1,3-dialkylxanthines are potent and highly A_1-selective adenosine antagonists [6, 12–14]. To investigate further this class of xanthines, six new 8-cycloalkyl analogs were synthesized. Compounds **2**, **3**, and **5** possess an olefinic group. This unsaturation was introduced as a site for subsequent addition reactions and also to investigate the effect of rigidity in the alkyl rings. The olefinic modifications markedly diminished potency at A_1-receptors, but had little effect on potency at A_2-receptors (Table 2). It is not clear why more rigid monoolefinic rings were less potent in comparison to 8-phenyl analogs. Subsequent hydrohalogenation of compound **3** in acidic medium produced the three racemic halogenated analogs, **4a–c**, in which the halogen atom occupies the most distal position. These halogen substitutions of the cyclopentyl ring similarly diminished potency at A_1-receptors; thus, these are not promising candidates for radiolabeled analogs.

8-Aryl sulfonic acid and 8-aryl sulfonamide xanthine derivatives have been synthesized [3, 5] in efforts to enhance water solubility, while retaining the high potency conferred by the 8-aryl substituent. We further examined potency and selectivity of various sulfonamide derivatives. Compounds **6–15**, similar to those analogs already reported, contain amine functionalized chains, and methyl or propyl substitutions at the 1- and 3-positions. Two primary amine derivatives, **8** and **9**, which may serve as functionalized congeners analogous to XAC, also

Table 1. Synthesis and characterization of xanthine derivatives

Compound	Structure		Method*	Yield	M.p. (°C)
					
Cycloalkyl derivatives (R_2 = Pr)					
1			A	70	162–163
2			A	51	183–188
3			A	36	198–199
4a			B	4	—
4b			B	40	—
4c			B	12	—
5			C	40	178–180
					
Sulfonamide derivatives					
	R_3 =	R_2 =			
6	H	Me	A	90	>350
7	H	Pr	A	79	307
8	$(CH_2)_2NH_2$	Me	D	80	278
9	$(CH_2)_2NH_2$	Pr	D	63	235
10	$(CH_2)_2N(CH_3)_2$	Me	D	96	281
11	$(CH_2)_2N(CH_3)_2$	Pr	D	64	265
12	$(CH_2)_3N(CH_3)_2$	Me	D	78	235
13	$(CH_2)_3N(CH_3)_2$	Pr	D	65	238
14	$(CH_2)_3N(C_2H_5)_2$	Me	D	62	222
15	$(CH_2)_3N(C_2H_5)_2$	Pr	D	63	210
					
Derivatives of XCC					
	R_4 =				
16	$NHCH(CH_3)CH_2C_6H_5$	(*R*-config)	F	73	245–248
17	$NHCH(CH_3)CH_2C_6H_5$	(*S*-config)	F	94	247–251
18	$NH(CH_2)_2NH_2$	(R_2 = Et)	E	92	233–235
19	$NCH_3(CH_2)_2N(CH_3)_2$	(R_2 = Et)	F	75	—
20	$NH(CH_2)_2NH_2$		E	65	220–230
21	$NH(CH_2)_2NHCH_3$		E	82	195–197
22	$NCH_3(CH_2)_2NH_2$		M	90	—

(R_2 = Pr unless noted)

Table 1 (*continued*)

	R_4 =			
23	$NCH_3(CH_2)_2NHCH_3$	F	30	—
24	$NH(CH_2)_2N(CH_3)_2$	E	87	229
25	$NCH_3(CH_2)_2N(CH_3)_2$	F	60	168–169
26	$NH(CH_2)_2N(C_2H_5)_2$	E	90	212
27	$NH(CH_2)_2N(CH_3)_3^+ I^-$	G†	89	288–290
28	$NH(CH_2)_2NH(CH_2)_2NH_2$	E	74	158–162
29	$NH(CH_2)_2NH(CH_2)_2OH$	E	86	185–185.5
30	$NH(CH_2)_2NH(CH_2)_2F$	G	55	—
31	$NH(CH_2)_2NHCOCH_3$	H	84	302
32	$NH(CH_2)_2NHCOCH_2Br$	H	93	275–279
33	$NH(CH_2)_2NHCO(CH_2)_2COOH$	H	80	210–212
34	$NH(CH_2)_2NHCO(CH_2)_4COCH_3$	I	33	236–239
35	$NH(CH_2)_2NHCOCH_2$-(thiophene)	I	73	270–274
36	$NH(CH_2)_2NHCO$-(C$_6$H$_4$)-CH_2Br	I	51	277–280
37	$NH(CH_2)_2NHSO_2$-(C$_6$H$_4$)-CH_3	J	90	211–216
38	$NH(CH_2)_2NHCO_2CH_2CH_3$	J	72	265–269
39a	$NH(CH_2)_2NHCO_2CH_2$-(C$_6$H$_5$)	J	84	137–138
39b	$NCH_3(CH_2)_2NHCO_2CH_2$-(C$_6$H$_5$)	F	62	200
Hydroxy congener and derivatives				
40	$NH(CH_2)_2OH$	E	90	283–284
41	$NHCH_2CH(OCH_3)_2$	F	57	238–239
42	$NHCH_2CHO$	M	76	203–206
43	$NH(CH_2)_2F$	F	78	256–258
44	$NH(CH_2)_2Br$	F	15	238–240
45	$NH(CH_2)_2SH$	E†	76	292
46	$NH(CH_2)_2OCOCH_3$	H	63	220–222
Amino acid and peptide conjugates				
47	-D-Phe(NO_2)-OBzl	F	97	230–234
48	-D-Phe(NO_2)-OH	K	100	273–277
49	-D-Phe(NH_2)-OH	L	64	250–256
50	Boc-D-Phe(NO_2)-$NH(CH_2)_2NH$-	I	66	197–199
51	Boc-D-Phe(NH_2)-$NH(CH_2)_2NH$-	L	94	201–205
52	TFA.H-D-Phe(NO_2)-$NH(CH_2)_2NH$-	M	90	165–169
53	TFA.H-D-Phe(NH_2)-$NH(CH_2)_2NH$-	L	54	233–237
54	TFA.H-D-Lys(CO-(C$_6$H$_4$)-CH_2Br)-$NH(CH_2)_2NH$-	M†	90	—
55	Cbz-L-Tyr-Gly-$NH(CH_2)_2NH$-	I	75	195–198
56	HBr.H-L-Tyr-Gly-$NH(CH_2)_2NH$-	M	94	177–182
57	Ac-L-Tyr(OAc)-Gly-$NH(CH_2)_2NH$-	H	80	amorph. solid
58	HBr.H-L-Tyr-L-Val-$NH(CH_2)_2NH$-	M	94	248–252

* Key to synthetic procedures:
(A) step 1: 1,3-dialkyl-5,6-diaminouracil + R_1COOH + carbodiimide [3], step 2: aqueous NaOH.
(B) olefin + HX [18].
(C) step 1: 1,3-dialkyl-5,6-diaminouracil + R_1CHO [4], step 2; $FeCl_3$ or $NaIO_4$.
(D) $ArSO_2Cl$ + amine [3].
(E) XCC ethyl ester + amine (neat or in EtOH) [4].
(F) XCC + amine + carbodiimide [10].
(G) alkylation of xanthine amine.
(H) acylation of xanthine amine using anhydride [4].
(I) XAC + acid + carbodiimide [10].
(J) XAC + alkyl chloroformate.
(K) aqueous NaOH.
(L) H_2/Pd [23].
(M) strong acid, TFA or 30% HBr/acetic acid [10], anhydrous except for compound **42**.
† Refer to text.

Table 2. Potencies of xanthine derivatives at adenosine A_1- and at A_2-receptors

Compound	K_i, A_1-receptors*	K_B, A_2-receptors†	$K_B(A_2)/K_i(A_1)$
1	0.9 ± 0.1	250 ± 100	280
2	525 ± 48	942	1.8
3	45 ± 9	470 ± 93	10
4a	42 ± 15	—	—
4b	19 ± 5.6	—	—
4c	57.5 ± 13	—	—
5	10.1 ± 0.6	276 ± 74	27
6	227 ± 20	150 ± 30	0.66
7	8.5 ± 1.1	18 ± 2.2	0.78
8	164 ± 14.4	303 ± 39	1.9
9	4.4 ± 0.7	43 ± 10	12
10	211 ± 12	260 ± 9.8	1.2
11	4.05 ± 0.38	23 ± 0.7	6.9
12	280	223 ± 13	0.80
13	8.0 ± 1.6	27 ± 5.4	4.2
14	267 ± 21	165 ± 11	0.62
15	5.6 ± 0.7	32 ± 3.7	6.3
16	8.88 ± 0.44	108 ± 15	12
17	8.72 ± 0.66	37 ± 4.9	4.2
18	12	83 ± 21	6.9
19	1.5	—	—
20	1.2 ± 0.5	83	69
21	15.1 ± 1.6	9.3 ± 2.1	0.62
22	2.62 ± 0.17	21.7 ± 1.4	8.3
23	14.3 ± 1.9	37.8	2.6
24	2.8 ± 0.19	12 ± 2.1	4.3
25	0.93 ± 0.03	18.2	20
26	7.5 ± 0.42	27 ± 2	3.6
27	25.1 ± 0.7	58 ± 9	2.3
28	3.53 ± 0.57	37 ± 2.4	10
29	1.16 ± 0.07	—	—
30	4.5	—	—
31	26 ± 5.3	107 ± 22	4.1
32	17.6 ± 1.5	—	—
33	78 ± 6.7	—	—
34	44 ± 9	—	—
35	7.26 ± 0.36	—	—
36	11.5 ± 1.5	—	—
37	72 ± 7.2	—	—
38	27 ± 1.9	—	—
39a	6.5 ± 1.1	—	—
39b	7.5 ± 1.1	—	—
40	10.2 ± 0.8	41 ± 10	4.0
41	16 ± 1.0	—	—
42	266 ± 40	—	—
43	23.4 ± 2.6	—	—
44	5.18 ± 0.47	—	—
45	16.2 ± 1.0	76 ± 4.3	4.7
46	10.3 ± 0.6	—	—
47	37 ± 5.3	—	—
48	101 ± 13	—	—
49	99 ± 4.6	—	—
50	64 ± 5.5	—	—
51	32 ± 1.2	—	—
52	6.5 ± 0.5	—	—
53	2.85 ± 0.20	55 ± 5	19
54	6.5	—	—
55	17 ± 1.2	—	—
56	3.68 ± 0.34	—	—
57	44 ± 6.2	—	—
58	2.47 ± 0.09	—	—

* Inhibition of [^{3}H]PIA binding to rat cerebral cortex membranes. Each K_i value was from a single experiment with triplicate determinations or average of three ± SEM.

† Antagonism of NECA-induced stimulation of adenylate cyclase activity in rat PC12 membranes, three experiments.

were prepared. Substitution of the primary amine with methyl groups had little effect on potency at A_1-receptors (Table 2). 1,3-Dipropyl analogs were between 35- and 52-fold more potent at A_1-receptors than the corresponding 1,3-dimethyl analogs. This modification had a less pronounced effect of roughly an order of magnitude on potency at A_2-receptors. A 1,3-dipropylxanthine tertiary amine derivative, **11**, showed nearly 60-fold A_1-selectivity, while two 1,3-dimethylxanthine sulfonamide derivatives, **6** and **14**, were slightly A_2-selective. Lengthening the terminal amine substituents from methyl to ethyl had little effect at either receptor subtype, except for the sulfonamide compound **14**, which had a nearly 3-fold enhancement at A_2-receptors. XAC, **20**, was moderately A_1-selective (69-fold), as found previously [4].

A pair of enantiomeric amides of XCC and *R*- and *S*-amphetamine* (compounds **16** and **17**) showed a slight stereoselectivity at A_2-receptors only (3-fold in preference to the *S*-isomer).

XAC, **20**, and CPX, **1**, constitute a useful pair for the definition of adenosine receptors. At A_1-receptors, XAC and CPX are close in potency [12]; at A_2-receptors, as indicated here and in other studies [8, 21], XAC was consistently more potent than CPX.

Many new derivatives of XAC (compound **20**) were prepared in order to investigate further structure–activity relationships for this class of xanthines. These derivatives contain various terminal primary (**18**, **22**), secondary (**21**, **23**), tertiary (**19**, **24–26**), or quaternary (**27**) amines. Several 1,3-diethylxanthines are included in this series of derivatives, since ethyl substituents were found to confer high potency in the human platelet A_2-receptor assay [24]. Similarly, at the A_2-receptor of rat PC12 cells, 1,3-diethyl and 1,3-dipropyl derivatives of XAC, compounds **18** and **20**, were equal in potency (Table 2). Substitution of either nitrogen of the ethylene diamine moiety of XAC by small alkyl groups, as in compounds **21–27**, enhanced potency at A_2-receptors. Nα-Methyl- and Nα-dimethyl-XAC, **21** and **24** respectively, were particularly potent at A_2-receptors (K_B values roughly 10^{-8} M). Thus, the addition of a single methyl group to XAC at the terminal amine position raised potency at A_2-receptors 9-fold and lowered potency at A_1-receptors by a factor of 13. XAC, **20**, and Nα-methyl-XAC, **21**, were reversed in receptor subtype selectivities and were very similar in physicochemical properties. This pair of xanthines may prove to be useful for the definition of adenosine receptor subtypes in other pharmacological or physiological systems. Furthermore, Nα-methyl-XAC may be useful as a tritiated radioligand for A_2-adenosine receptors. An amido N-methyl substitutent did not have a major effect on potency; thus, the amide N–H group of XAC is not required for high A_1 potency. A trimethyl-XAC derivative, **25**, was particularly potent at A_1-receptors.

The quaternary amine derivative, **27**, was synthesized by selective alkylation of a tertiary amine, **24**. This result emphasizes the relatively low nucleophilicity of the 7-position nitrogen (here unreactive towards methyl iodide at room temperature) in 8-aryl xanthines. The loss of potency upon quaternization of **24** may be a result of steric factors.

Terminal N-aminoethyl-, N-hydroxyethyl-, and N-fluoroethyl-derivatives of XAC, compounds **28–30** were extremely potent at A_1-receptors.

Derivatives of XAC containing terminal N-acyl groups having alkyl, **31–34**, or aryl, **35–37**, substituents are included. Electrophilic bromoacetyl, as in **32**, and benzyl bromide, as in **36** and **54**, groups designed for possible irreversible labeling of receptors, were incorporated. The thiophene derivative, **35**, permits the facile introduction of mercury [25] as a potential electron opaque label. Three urethane derivatives, **38** and **39a** and **b**, of XAC are included. The N-ethyloxycarbonyl blocking group on certain biogenic amines has been reported to be enzymatically removed in the brain [26]. Thus, the use of potentially bioreversible blocking groups, as in **38**, may be generally applicable to xanthines as the basis for a drug delivery scheme *in vivo*. The benzyloxycarbonyl group present in **39** was readily removed by hydrogenolysis, and thus this derivative served as a synthetic intermediate, for example, for selective alkylation of the N-7 position, followed by hydrogenolysis of the benzyloxycarbonyl protecting group.

In addition to the carboxylic acid congener (XCC) and amine congener (XAC) previously reported, we now introduce hydroxy-, **40**, aldehyde-, **42**, and thiol-functionalized, **45**, congeners. These analogs were synthesized for comparison with the particularly potent XAC, which they resemble structurally. As with XAC, the hydroxy- and thiol-analogs would have the ability to form a hydrogen bond as a donor with the receptor, but they do not carry a positive charge at physiological pH. The aldehyde group is able to serve as a hydrogen bond acceptor. The lower potency of **40**, **45**, and particularly **42** was consistent with our hypothesis that an electrostatic, rather than a hydrogen bonding, interaction of XAC at the receptor site produces the observed nanomolar potency. As observed previously, chain terminal carboxylic acid derivatives of xanthines, such as **48** and **49**, were of low potency in A_1-receptor binding.

We reported previously [10] that the 8-aryl xanthine functionalized congeners may be coupled to amino acids and oligopeptides, either totally synthetic in sequence or resembling a native peptide hormone [27], with the retention of high affinity of binding of the xanthine portion of the molecule at adenosine receptors. We report here additional, highly potent amino acid and peptide conjugates, **50–62**. *p*-Nitrophenylalanine has been used as one of the amino acid residues for two reasons. First, aryl nitro groups are known to be photoreactive and to bind covalently to receptor proteins upon irradiation [28]. Second, the nitro group is a convenient synthetic intermediate for *p*-aminophenylalanine. Similar aryl amino groups have been used previously for the introduction of radioiodine into high affinity ligands [23, 29] and for photoaffinity labeling both via azides directly generated from the amino group or by the use of photoactivatable cross-linking reagents. The xanthine aryl amine ^{125}I-

* *R*- and *S*-PIA, for which a stereoselectivity exists at A_1-receptors, are synthesized from amphetamine and 6-chloropurine riboside.

Supplementary information:
Elemental Analyses

Compound	Formula	Calculated %C	Calculated %H	Calculated %N	Found %C	Found %H	Found %N
2	$C_{16}H_{22}N_4O_2 \cdot H_2O$	59.98	7.55	17.49	59.98	7.57	17.56
3	$C_{16}H_{22}H_4O_2$	63.55	7.33	18.53	63.34	7.41	18.47
5	$C_{17}H_{24}H_4O_2$	64.53	7.65	17.71	64.72	7.70	17.59
8	$C_{15}H_{18}N_6O_4S \cdot 0.5H_2O$	46.50	4.94	21.69	46.88	4.88	21.45
9	$C_{19}H_{26}N_6O_4S \cdot 0.5H_2O$	51.45	6.14	18.95	51.88	6.16	18.59
10	$C_{17}H_{22}N_6O_4S \cdot 0.25H_2O$	49.68	5.52	20.45	49.73	5.47	20.06
11	$C_{21}H_{30}N_6O_4S$	54.53	6.54	18.17	54.28	6.44	17.95
12	$C_{18}H_{24}N_6O_4S \cdot HCl$	47.40	5.40	18.40	47.55	5.36	17.86
13	$C_{22}H_{32}N_6O_4S$	55.44	6.77	17.63	55.05	6.47	17.51
14	$C_{20}H_{28}N_6O_4S$	53.55	6.29	18.73	53.79	6.29	18.66
15	$C_{24}H_{36}N_6O_4S \cdot 0.5H_2O$	56.12	7.26	16.36	56.57	7.33	16.27
18	$C_{19}H_{24}N_6O_4 \cdot DMF \cdot H_2O$	53.76	6.77	19.95	54.09	6.25	18.97
20	$C_{21}H_{28}N_6O_4$	58.86	6.57	19.61	58.77	6.64	19.06
21	$C_{22}H_{30}N_6O_4 \cdot 0.5H_2O$	58.52	6.92	18.61	58.60	6.91	18.61
24	$C_{23}H_{32}N_6O_4$	60.51	7.06	18.41	60.08	7.05	18.11
25	$C_{24}H_{34}N_6O_4 \cdot 1.5H_2O$	57.93	7.50	16.89	57.98	7.07	16.86
26	$C_{25}H_{30}N_6O_4$	61.96	7.49	17.34	62.56	7.78	16.77
27	$C_{24}H_{35}N_6O_4I \cdot 0.5H_2O$	47.45	5.97	13.83	47.47	5.83	13.75
31	$C_{23}H_{30}N_6O_3$	58.71	6.43	17.86	58.52	6.26	17.93
32	$C_{23}H_{29}N_6O_3Br \cdot H_2O$	48.68	5.51	14.81	48.64	5.55	14.78
33	$C_{25}H_{32}N_6O_7 \cdot 1.5H_2O$	54.05	6.35	15.13	54.01	5.97	14.97
34	$C_{28}H_{38}N_6O_6$	60.63	6.91	15.15	60.54	6.94	15.10
35	$C_{27}H_{32}N_6O_5S \cdot H_2O$	56.83	6.01	14.73	56.98	5.76	14.84
37	$C_{28}H_{34}N_6O_6S \cdot O.5H_2O$	56.84	5.96	14.20	56.86	5.93	14.12
38	$C_{24}H_{32}N_6O_6 \cdot 0.75H_2O$	56.08	6.57	16.35	56.12	6.50	16.69
39a	$C_{29}H_{34}N_6O_6 \cdot 0.5H_2O$	60.93	6.17	14.70	60.82	6.21	14.74
40	$C_{21}H_{27}N_5O_5 \cdot 1.5DMF \cdot 0.5H_2O$	55.55	6.89	16.06	55.71	6.73	16.46
41	$C_{23}H_{31}N_5O_6$	58.34	6.60	14.79	58.41	6.65	14.76
43	$C_{21}H_{26}N_5O_4F \cdot 0.5H_2O$	57.26	6.18	15.90	57.36	6.27	16.03
45	$C_{21}H_{27}N_5O_4S \cdot 0.75H_2O$	54.95	6.26	15.26	55.02	6.18	14.99
46	$C_{23}H_{29}N_5O_6 \cdot 2H_2O$	54.43	6.55	13.80	54.57	6.20	13.84
47	$C_{35}H_{36}N_6O_8 \cdot 0.5H_2O$	62.03	5.50	12.40	62.06	5.53	12.48
48	$C_{28}H_{30}N_6O_8 \cdot 3H_2O$	53.16	5.74	13.28	53.97	5.97	14.97
49	$C_{28}H_{32}N_6O_6 \cdot 0.75H_2O$	59.83	6.01	14.95	60.27	6.07	14.21
50	$C_{35}H_{44}N_8O_9 \cdot H_2O$	56.90	6.28	15.17	56.60	6.33	15.10
52	$C_{30}H_{36}N_8O_7 \cdot 2TFA$	48.12	4.51	13.20	48.02	4.82	13.94
53	$C_{32}H_{39}N_8O_7F_3 \cdot 4H_2O$	49.48	6.10	14.43	49.50	5.87	14.39
55	$C_{40}H_{46}N_8O_9 \cdot H_2O$	59.99	6.04	13.99	60.14	6.05	14.26
56	$C_{32}H_{41}N_8O_7Br \cdot 1.5H_2O$	50.80	5.86	14.81	50.68	5.86	14.76
57	$C_{36}H_{44}N_8O_9 \cdot 4H_2O$	53.72	6.51	13.92	52.24	5.84	13.71
58	$C_{35}H_{46}N_8O_7 \cdot 2HBr \cdot 2H_2O$	48.28	5.79	12.87	47.82	5.98	12.57

PAPA-XAC (Fig. 1) has been cross-linked cleanly to the A_1-adenosine receptor by the action of ultraviolet light [23]. A dipeptide "carrier", consisting of L-Tyr-Gly, and several related analogs were included. Urethane- and other acyl-blocked forms were examined. Amino acid, **52–54**, and peptide derivatives, **56** and **58**, of XAC having free amino termini were particularly potent at A_1-receptors.

In summary, several promising new xanthine ligands were identified. Nα-Methyl XAC and XAC were of divergent selectivities for adenosine receptor subtypes and taken together may be useful for the definition of adenosine receptors. Nα-Methyl XAC and a sulfonamide derivative, **14**, were slightly A_2-selective antagonists *in vitro*. A wide variety of N-alkyl and N-acyl substitutions of XAC were possible, some of which enhanced A_2 potency (e.g. **21**, **24–26**) and some of which preserved or enhanced nanomolar A_1 potency (e.g. **25** and **29**). Chemically reactive bromo derivatives were prepared as potential affinity labels for adenosine receptors. New functionalized congeners containing aldehyde, thiol, and hydroxyl groups for covalent coupling to carriers were introduced. Dipeptide conjugates of XAC were most potent in the free amino form. Amino acid conjugates bearing aryl amino groups for potential radioiodination were prepared. The high potency and selectivity of 8-cycloalkyl xanthine derivatives as A_1-adenosine antagonists did not tolerate distal halogen substitutions or olefinic substitutions of the ring.

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