Structure–Activity Relationships of 1,3-Dialkylxanthine Derivatives at Rat A_3 Adenosine Receptors

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1,3-Dialkylxanthine analogues containing carboxylic acid and other charged groups on 8-position substituents were synthesized. These derivatives were examined for affinity in radioligand binding assays at rat brain A_3 adenosine receptors stably expressed in CHO cells using the new radioligand [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide), and at rat brain A_1 and A_{2a} receptors using [^{3}H]PIA and [^{3}H]CGS 21680, respectively. A synthetic strategy for introducing multiple carboxylate groups at the 8-position using iminodiacetic acid derivatives was explored. The presence of a sulfonate, a carboxylate, or multiple carboxylate groups did not result in a significant enhancement of affinity at rat A_3 receptors, although as previously observed an anionic group tended to diminish potency at A_1 and A_{2a} receptors. The rat A_3 receptor affinity was not highly dependent on the distance of a carboxylate group from the xanthine pharmacophore. 2-Thio vs 2-oxo substitution favored A_3 potency, and 8-alkyl vs 8-aryl substitution favored A_3 selectivity, although few derivatives were truly selective for rat A_3 receptors. 1,3-Dimethyl-8-(3-carboxypropyl)-2-thioxanthine was 7-fold selective for A_3 vs A_{2a} receptors. 1,3,7-Trimethyl-8-(*trans*-2-carboxyvinyl)xanthine was somewhat selective for A_3 vs A_1 receptors. For 8-arylxanthines affinity at A_3 receptors was enhanced by 1,3-dialkyl substituents, in the order dibutyl > dipropyl > diallyl.

Introduction

Three major classes of adenosine receptors (A_1, A_2, and A_3) have been characterized pharmacologically[1,4] and through cloning.[2,3,6] A_1 receptors are coupled to the inhibition of adenylate cyclase through G_i proteins[4,5] and have also been shown to couple to other second messenger systems, including inhibition or stimulation of phosphoinositol turnover and activation of ion channels.[1,4] A_2 receptors are further divided into two subtypes, A_{2a} and A_{2b}, at which adenosine agonists activate adenylate cyclase with high and low affinity, respectively. The A_3 receptor sequence was first identified in a rat testes cDNA library,[7] and this sequence, later cloned by homology to other G-protein coupled receptors from a rat brain cDNA library, was shown to correspond to a novel, functional adenosine receptor.[6] A_3 receptors are coupled to both inhibition of adenylate cyclase and activation of protein kinase C as second messengers. The rat A_3 receptor was unlike the previously characterized adenosine receptors in lack of either displacement of radioligand binding or functional antagonism by the usual high affinity xanthine ligands. The A_3 receptor was recently shown to be identical to an atypical adenosine receptor present in the RBL-2H3 rat mast cell line,[5,8] the activation of which promotes degranulation.

In addition to possible involvement in the inflammatory response, activation of A_3 receptors is associated with cardiovascular actions. Fozard and Carruthers[34] have attributed a xanthine-insensitive component of the hypotensive effects of adenosine agonists in the rat to A_3 receptor activation. Activation of A_3 receptors has been suggested by Downey and colleagues[11] to be involved in the cardioprotective effect of preconditioning by adenosine agonists. The occurrence of A_3 receptors in the brain[6,9,10] and testes[7] also suggests that it may be important in regulation of central nervous system (CNS) function and reproduction.

We have studied in detail the structure–activity relationships for adenosine derivatives as agonists at A_3 receptors.[12,13] We recently reported[9] that an N^6- and 5′-substituted adenosine derivative, N^6-(3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine (IB-MECA), is 50-fold selective for rat brain A_3 receptors in binding experiments and is also selective in *in vivo* behavioral experiments.

We have reported that xanthines that are potent antagonists at rat, rabbit, and human A_1 and A_2 receptors only weakly displaced the binding of the radioligand [^{125}I]APNEA (N^6-[2-(4-amino-3-iodophenyl)-ethyl]adenosine) from cloned rat A_3 receptors even at concentrations in the 10^{-4} M range.[12] Linden et al.[14] found that xanthines, particularly those containing anionic groups, bind appreciably to cloned sheep A_3 receptors, but generally with less affinity than at A_1 and A_2 receptors in a variety of species.[1] The human A_3 receptor was recently cloned[15] and found to resemble the sheep A_3 receptor, in the respect that many potent xanthines bind in the micromolar range. Thus, for xanthines as antagonists,[14] an unusually large species dependence of affinity at A_3 receptors is apparent, perhaps even suggesting multiple subtypes of A_3 receptors. Xanthines that are generally A_3-selective across species are needed as pharmacological and biochemical probes to define more clearly the physiological role, distribution, and regulation of A_3 adenosine receptors.

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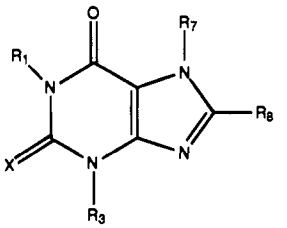
Table 1. Affinities of Xanthine Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_{2a}, and A_3 Receptors[a-d] with Ratios of Selectivity for A_1 or A_2 Receptors vs A_3 Receptors



compd	R_1, R_3[e]	R_8[e]	R_7	K_i (μM) or % inhibition[d] A_1[a]	A_{2a}[b]	A_3[c]	K_i A_3/K_i A_1	K_i A_3/K_i A_{2a}
1[f]	CH_3	H	H	8.5	25	23.1% (10^{-4})	>10	>4
2[f]	CH_3	H	CH_3	29	48	30.1% (10^{-4})	>3	>2
3[f]	CH_3	H	ϕCH_2	6.0	46	29.7% (10^{-4})	>10	>2
4[f]	$CH_3(CH_2)_3$	H	H	0.50	29.3	143	290	4.9
5[f]	$CH_3(CH_2)_5$	H	H	1.26	14.3% (10^{-5})	9.2% (10^{-5})	>10	
6[f]	ϕCH_2	H	H	2.0	3.6% (10^{-5})	20.3% (10^{-5})	>10	
7	1-$CH_3(CH_2)_3$ 3-ϕCH_2	H	H	0.668 ± 0.117	1.20 ± 0.33	74.2 ± 14.3	110	62
8	$CH_3(CH_2)_2$	cyclopentyl	H	0.00046 [28.2][h]	0.34 [0.054][h]	5.29 ± 0.79	12000	16
9	CH_3	CH=CH-*m*-ϕCl[e]	CH_3		0.00908 ± 0.00162[f]	4.2% (10^{-5})[f]		>500
10	CH_3	CH=CH-*m*-ϕNHCO$(CH_2)_2$COOH[e]	CH_3	20.5 ± 6.1[g] [35.1][h]	0.0129 ± 0.0026[g] [0.143][h]	189 ± 15[g]	9.2	15000
11	CH_3	CH=CH-*m*-ϕNHCO$(CH_2)_3$COOH[e]	CH_3	22.3 ± 7.6[g]	0.0099 ± 0.0013[g]	289 ± 15[g]	13	29000
12b	CH_3	CH=CH-*m*-ϕNHCO$(CH_2)_4$COOH[e]	CH_3	6.51 ± 1.81[g]	0.00722 ± 0.00205[g]	234 ± 8[g]	36	32000
13	Ch_3	CH=CHCOOH[e]	CH_3	3% (10^{-4})[i]	42[i]	130 ± 6.3	<1	3.1
14	$CH_3(CH_2)_3$	CH=CHCOOH[e]	H	3.37 ± 0.60	16.5 ± 1.3	73.7 ± 4.3	22	4.5
15	$CH_3(CH_2)_3$	CH=CHCOOCH$_3$[e]	CH_3	0.793 ± 0.137	5.85 ± 0.48	nd		
16	$CH_3(CH_2)_3$	CH=CHCOOH[e]	CH_3	5.91 ± 0.91	23.2 ± 4.7	127 ± 33	21	5.5
17	$CH_3(CH_2)_3$	$(CH_2)_2$COOH	H	7.11 ± 1.53	71.7 ± 12.9	129 ± 8.5	18	1.8
18	$CH_3(CH_2)_3$	$(CH_2)_3$COOH	H	14.2 ± 1.7	113 ± 20	133 ± 9.3	9.4	1.2
19	$CH_3(CH_2)_3$	$(CH_2)_4$COOH	H	5.27 ± 0.83	20	13% (3×10^{-5})		
20	CH_3	$(CH_2)_3$COOH	H	0% (10^{-4})	26 ± 5% (10^{-4})	93.4 ± 19.0	<1	–
21[j]	CH_3	$(CH_2)_3$COOH	H	5.70 ± 0.55	67.8 ± 4.0	9.36 ± 0.3	1.6	0.14

[a] Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in μM (n = 3–5). [b] Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± SEM in μM (n = 3–6). [c] Displacement of specific [^{125}I]AB-MECA binding, unless noted, in membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± SEM in μM (n = 3.5). [d] A percent value indicates the percent displacement of radioligand at the concentration (M) given in parentheses. [e] R_1 = R_3 and X = O, unless noted; a CH=CH group, when present in the R_8 substituent, is always *trans*. [f] Values from van Galen et al.[12] A_3 affinity vs [^{125}I]APNEA. [g] The assay was carried out in dark. [h] Values from Jacobson et al.[22] The binding assay was carried out under fluorescent room light. [i] Values from Jacobson et al.[24] [j] X = S (compound **21**). nd: not determined.

Results

Xanthine analogues modified with alkyl substituents at 1-, 3-, and 7-positions and with carboxylic acid groups on the 8-position substituent were synthesized (Schemes 1–4) and tested in radioligand binding assays (Tables 1 and 2) for affinity at rat brain A_1, A_{2a}, and A_3 adenosine receptors. At A_3 receptors affinity was measured in CHO cells stably expressing cloned rat brain A_3 receptors.[6] The radioligand used for binding to A_3 receptors was the recently reported [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide).[16] Affinity at A_1 receptors was measured in rat cortical membranes using [^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine[17] ([^{3}H]PIA) and at A_{2a} affinity in rat striatal membranes using [^{3}H]CGS 21680.[18]

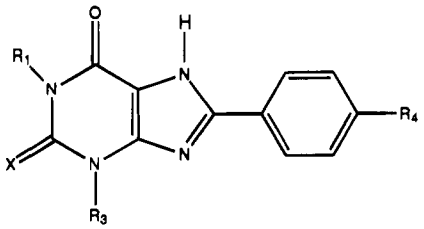
Synthesis of 1,3-dibutylxanthine, **4**, was performed by the classical method starting from *N*,*N*′-dibutylurea, **36a** (Scheme 1). 6-Amino-1,3-dibutyluracil, **38a**, was synthesized by condensation of *N*,*N*′-dibutylurea, **36a**, and cyanoacetic acid in the presence of acetic anhydride followed by base-catalyzed cyclization.[33] Nitrosation and Pd/C-catalyzed hydrogenation gave 5,6-diamino-1,3-dibutyluracil, **40a**, which without purification was then condensed with formic acid in the presence of EDAC to yield 6-amino-5-formamido-1,3-dibutyluracil. 1,3-Dibutylxanthine, **4**, was obtained by cyclization of 6-amino-5-formamino-1,3-dibutyluracil under basic conditions. Similarly, 3-benzyl-1-butylxanthine, **7**, was prepared starting from *N*-benzyl-*N*′-butylurea.

8-Substituted xanthines were synthesized through the condensation of 5,6-diaminouracils, **40**, with various anhydrides (Scheme 2) or an aldehyde, **45** (Scheme 3), followed by cyclization either in base or by the $NaIO_4$ method,[20] respectively. Detailed characterization of the compounds synthesized is listed in Table 3.

8-Unsubstituted xanthines in which 1-, 3-, and 7-substituents were modified were compared (Table 1). A comparison of 1,3-dibutylxanthine, **4**, and the corresponding 3-benzyl analogue, **7**, indicated that for these simple xanthines, the presence of a bulky 3-position substituent only slightly increased A_3 affinity (2-fold) and had no effect on A_1 affinity, whereas A_2 affinity was dramatically increased (24-fold). Limited aqueous solubility of the dibenzyl analogue, **6**, prevented a precise comparison with 1,3-dibutylxanthine. A benzyl vs methyl group at the 7-position did not have a major effect on A_{2a} or A_3 affinity, but somewhat enhanced A_1 affinity (5-fold).

8-Cyclopentyl-1,3-dipropylxanthine (CPX), **8**, and 8-(3-chlorostyryl)caffeine (CSC), **9**,[21] two xanthines found previously to be highly selective for either A_1 or A_{2a} adenosine receptors,[1,22] respectively, remained selective

Table 2. Affinities of Xanthine Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_{2a}, and A_3 Receptors[a-d] with Ratios of Selectivity for A_1 or A_2 Receptors vs A_3 Receptors



compd	R_1, R_3[e]	R_4[e]	K_i (μM) or % inhibition[d] A_1[a]	A_{2a}[b]	A_3[c]	$K_i A_3/K_i A_1$	$K_i A_3/K_i A_{2a}$
22[g]	$CH_3(CH_2)_2$	SO_3H	0.14	0.79	90.1 ± 10.9	640	110
23	$CH_3(CH_2)_2$	COOH	0.20[f]	0.637 ± 0.085, 0.32[f]	45.1 ± 2.5	230	71
24	$CH_3(CH_2)_2$	CH=CHCOOH[e]	0.015[f]	0.80[f]	15.0 ± 1.7	1000	19
25	CH_2=$CHCH_2$	OCH_2COOH	0.756 ± 0.147	4.29 ± 0.57	173 ± 18	230	40
26	$CH_3(CH_2)_2$	OCH_2COOH	0.058[k]	2.20 [k]	75.7 ± 6.5	1300	34
27	1-$CH_3(CH_2)_2$ 3-CH_2(3-I-4-$NH_2\phi$)	OCH_2COOH	0.037[f,h]	0.7[f]	1.17 ± 0.18	32	2
28[i]	$CH_3(CH_2)_2$	OCH_2COOH	0.0538[g]	0.226 ± 0.011	6.77 ± 0.011	126	30
29	$CH_3(CH_2)_2$	$OCH_2CONHCH_2COOH$	0.073[i]	0.455 ± 0.086	58.5 ± 5.9	800	130
30	$CH_3(CH_2)_3$	OCH_2COOH	0.0431 ± 0.0099	0.874 ± 0.107	27.5 ± 2.5	640	31
31	$CH_3(CH_2)_2$	$OCH_2CONH(CH_2)_2NH_2$	0.0012[j]	0.063	29.0 ± 7.0	24000	460
32	$CH_3(CH_2)_2$	$OCH_2CONH(CH_2)_2NHCO(CH_2)_2COOH$	0.078[k]	0.252 ± 0.041	40.6 ± 1.7	520	210
33	$CH_3(CH_2)_2$	OCH_2CO-$NH(CH_2)_2NHCOOCH_2$ N(Fmoc)CH_2COOH	0.0191 ± 0.0068	0.33 ± 0.10	21.7 ± 0.8	1100	66
34	$CH_3(CH_2)_2$	$OCH_2CONH(CH_2)_2NHCOCH_2NHCH_2COOH$	0.0360 ± 0.0101	0.157 ± 0.026	98.0 ± 6.8	2700	620
35	$CH_3(CH_2)_2$	$OCH_2CONH(CH_2)_2NHCOCH_2N(CH_2COOH)$ $COCH_2CH_2COOH$	2.86 ± 1.00	9.22 ± 1.11	99.9 ± 12.2	35	1

[a] Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in μM (n = 3–5). [b] Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± SEM in μM (n = 3–6). [c] Displacement of specific [^{125}I]AB-MECA binding, unless noted, in membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± SEM in μM (n = 3–5). [d] A percent value indicates the percent displacement of radioligand at the concentration (M) given in parentheses. [e] R_1 = R_3 and X = O, unless noted; a CH=CH group, when present in the R_4 substituent, is always *trans*. [f] Values from Linden et al.[26,27] or Shamim et al.[25] A_2 values represent K_B for inhibition of NECA stimulated adenylate cyclase platelet membranes. [g] A_1/A_{2a} affinity from ref 25. [h] A_1 affinity measured in binding assay vs [^{125}I]AB-MECA binding in rat brain membranes. [i] X = S (compound **28**). [j] Values from Jacobson et al.[29] A_1 affinity vs [^{3}H]CHA. [k] Values from Jacobson et al.[28,30] A_{2a} affinity vs [^{3}H]NECA. nd: not determined.

vs rat A_3 receptors. Compound **9** was nearly inactive at A_3 receptors. Among a related series of 8-styrylxanthines that showed selectivity for A_{2a} receptors[22] was a water soluble succinylamino derivative, **10**. Compound **10** very weakly displaced [^{125}I]AB-MECA binding in transfected CHO cells (K_i value 189 μM). An attempt to improve the A_3 affinity by lengthening the alkyl carboxylate chain by one and two methylenes, e.g., 11 and **12b**, was unsuccessful.

The susceptibility of 8-styrylxanthines to photoisomerization has been noted;[23] thus binding assays of affinity of compounds **9**–**12b** were carried out in the dark. Although the affinities at A_3 receptors were minimal, the selectivity for A_{2a} vs A_1 receptors was maintained. Selectivity ratios (A_{2a} vs A_1) when the assays were performed in the dark were 1590 (**10**), 2300 (**11**), and 900 (**12b**).

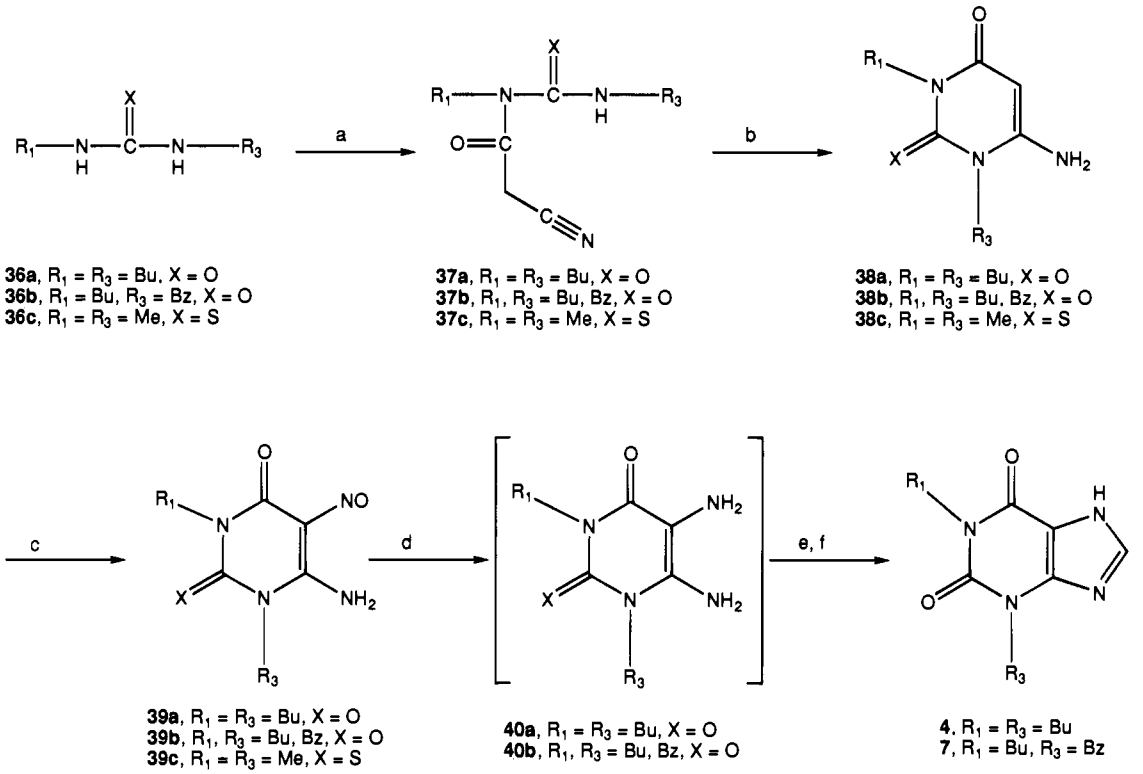
A caffeine 8-acrylic acid derivative, **13**, 1,3,7-trimethyl-8-(*trans*-2-carboxyvinyl)xanthine, which was reported to be moderately selective for rat A_{2a} vs A_1 receptors but not very potent,[24] was found to be selective also for rat A_3 vs A_1 receptors. Lengthening the 1,3-dialkyl substituent, as in the corresponding dibutyl derivative, **16**, greatly enhanced affinity at A_1 receptors, and at A_3 receptors affinity was unchanged, resulting in the loss of selectivity. The presence of a hydrogen at the 7-position, in **14**, slightly enhanced affinity relative to **16** in parallel at A_1, A_{2a}, and A_3 receptors. The methyl ester, **15**, of the N_7-H analogue was more potent at A_1 and A_{2a} receptors than the corresponding carboxylic acid, **14**, as expected[29] for a neutral vs an anionic 8-functionalized chain.

A series of 8-alkyl carboxylic acids (**17**–**19**) indicated that optimal affinity at A_3 relative to A_1 and A_{2a} receptors was observed for the 8-(3-carboxypropyl) derivative, **18**, although none of these xanthines were truly selective for rat A_3 receptors.

The 1,3-dimethyl analogue of **18** was prepared, since smaller 1,3-dialkyl substituents seemed to favor A_3 selectivity (compare **13** vs **16**), although not A_3 affinity. Compound **20** was selective for A_3 vs A_1 receptors, although not very potent. Inclusion of the 2-thio modification in **21**, 1,3-dimethyl-8-(carboxypropyl)-2-thioxanthine, resulted in enhanced affinity at all adenosine receptors subtypes and increased selectivity for A_3 vs A_{2a} receptors. The A_3 receptor affinity for **21** vs **20** was enhanced by 10-fold.

The affinities of 8-arylxanthines at adenosine receptors were compared (Table 2). Linden et al.[14] reported that at sheep A_3 receptors both sulfonate- and carboxylate-containing 8-arylxanthines were among the most potent antagonists, many of which had K_i values in the submicromolar range. Thus we compared the affinities of a number of anionic derivatives of 1,3-dipropyl-8-phenylxanthine at rat A_3 receptors. Both the *p*-carboxylate group, **23**,[25] and the *p*-acrylate group, **24** (BW1433),[26] were more favorable in A_3 affinity than the *p*-sulfonate group, **22**. The affinities of **22** and **23** at both A_1 and A_{2a} receptors were nearly identical. The *p*-acrylate group in **24** resulted in enhanced (9-fold) A_1

Scheme 1. Synthesis of 1,3-Dialkylxanthines[a]



36a, $R_1 = R_3 = Bu$, X = O
36b, $R_1 = Bu$, $R_3 = Bz$, X = O
36c, $R_1 = R_3 = Me$, X = S

37a, $R_1 = R_3 = Bu$, X = O
37b, R_1, $R_3 = Bu$, Bz, X = O
37c, $R_1 = R_3 = Me$, X = S

38a, $R_1 = R_3 = Bu$, X = O
38b, R_1, $R_3 = Bu$, Bz, X = O
38c, $R_1 = R_3 = Me$, X = S

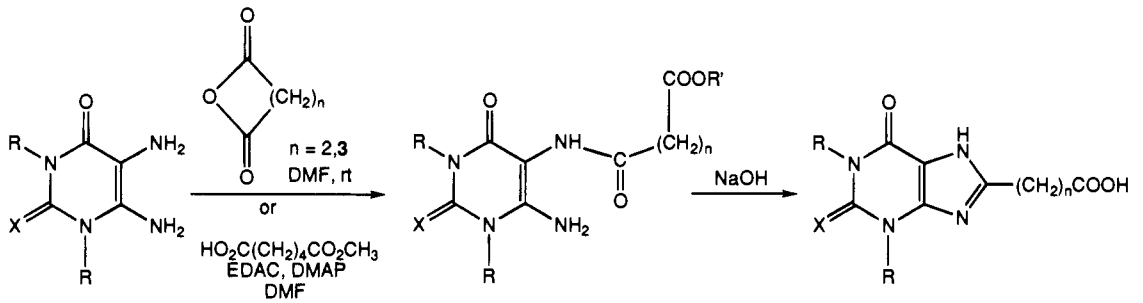
39a, $R_1 = R_3 = Bu$, X = O
39b, R_1, $R_3 = Bu$, Bz, X = O
39c, $R_1 = R_3 = Me$, X = S

40a, $R_1 = R_3 = Bu$, X = O
40b, R_1, $R_3 = Bu$, Bz, X = O

4, $R_1 = R_3 = Bu$
7, $R_1 = Bu$, $R_3 = Bz$

[a] Reagents: (a) $NCCH_2CO_2H$, Ac_2O; (b) 2 N NaOH; (c) $NaNO_2$, HCl, AcOH, H_2O; (d) H_2, Pd/C, MeOH; (e) HCO_2H, EDAC, DMF; (f) 2 N NaOH, reflux.

Scheme 2. Synthesis of 8-(Carboxyalkyl)-Substituted Xanthines



40a, R = CH_3, X = O
40b, R = CH_3, X = S
40c, R = $(CH_2)_3CH_3$, X = O

41, R = $(CH_2)_3CH_3$, X = O, n = 2, R = H
42, R = $(CH_2)_3CH_3$, X = O, n = 3, R = H
43, R = $(CH_2)_3CH_3$, X = O, n = 4, R = CH_3
44, R = CH_3, X = S, n = 3, R = H

17, R = $(CH_2)_3CH_3$, X = O, n = 2
18, R = $(CH_2)_3CH_3$, X = O, n = 3
19, R = $(CH_2)_3CH_3$, X = O, n = 4
20, R = CH_3, X = O, n = 3
21, R = CH_3, X = S, n = 3

affinity, but the affinities of **22**, **23**, and **24** at A_{2a} receptors were identical. A xanthine carboxylic congener, XCC, **26**,[19] containing a *p*-[(carboxymethyl)oxy] group was slightly less potent at A_3 receptors than the corresponding 8-(4-carboxyphenyl)-1,3-dipropylxanthine, **23**.[25] On the basis of the finding that a carboxylate group is more desirable than a sulfonate group at rat A_3 receptors, the affinities of additional carboxylate derivatives of 8-arylxanthines were compared.

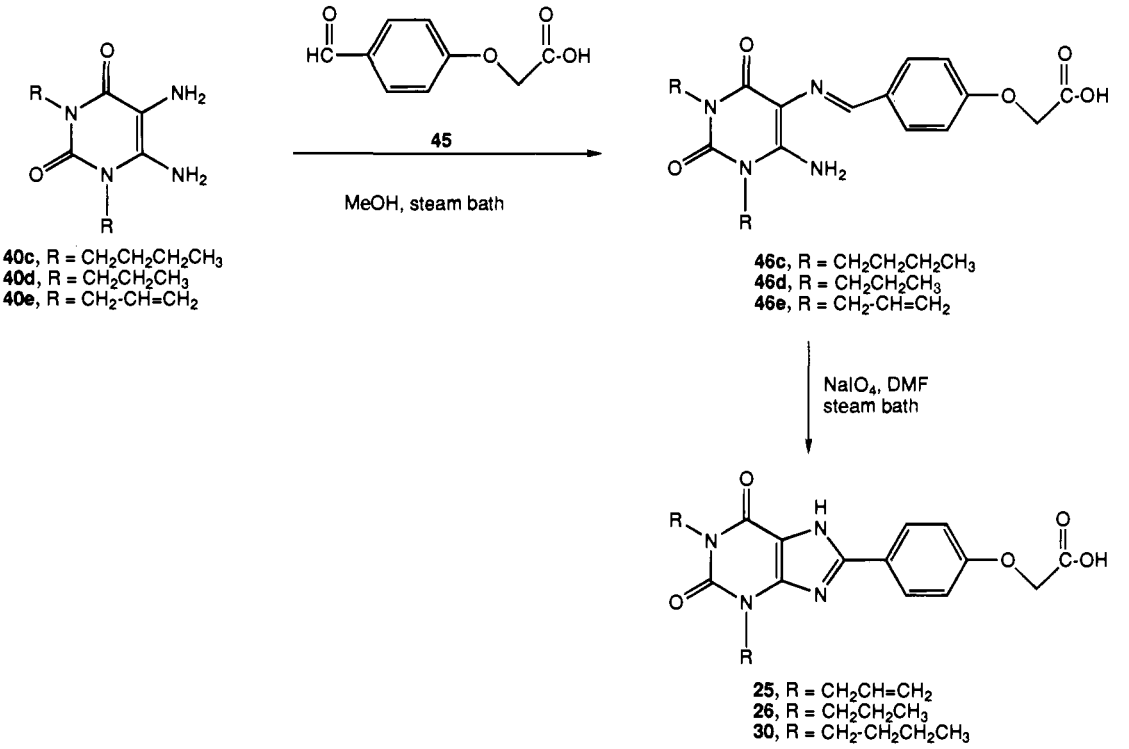
The xanthine amine congener, XAC, **31**,[19] was highly selective for A_1 and A_{2a} receptors vs A_3 receptors. The K_i value of XAC (29 μM) was similar to values for compounds **22**–**24**, indicating that rat A_3 receptors, unlike A_1 and A_{2a} receptors, tolerate equally a distal carboxylate group and a distal amino group.

The iodinated xanthine derivative, **27**, also known as I-ABOPX or BWA 577,[27] had 65-fold enhanced affinity at A_3 receptors in comparison to the parent XCC, **26**. The enhancement of affinity by the 3-(3-iodo-4-aminobenzyl) group at A_1 and A_{2a} receptors was only 2–3-fold. Nevertheless, the affinity of **27** at rat A_3 receptors was much less than at sheep A_3 receptors,[14] and it was not A_3 selective.

Lengthening the 1,3-dialkyl substituents of XCC was found to increase affinity somewhat at A_{2a} and A_3 receptors, but A_1 affinity was unaffected. The corresponding 1,3-dibutylxanthine carboxylic congener, **30**, displayed a K_i value at A_3 receptors of 28 μM, although the compound was still highly A_1 selective. Introduction of unsaturation in the 1,3-dialkyl groups, **25**, vs the corresponding 1,3-dipropyl analogue, **26**, resulted in a 2-fold loss of affinity at A_{2a} and A_3 receptors and a 13-fold loss of affinity at A_1 receptors.

A 2-thio vs 2-oxo substitution[28] enhanced A_3 affinity

Scheme 3. Synthesis of 8-[[(Carboxymethyl)oxy]phenyl]-Substituted Xanthines



40c, R = $CH_2CH_2CH_2CH_3$
40d, R = $CH_2CH_2CH_3$
40e, R = CH_2-CH=CH_2

46c, R = $CH_2CH_2CH_2CH_3$
46d, R = $CH_2CH_2CH_3$
46e, R = CH_2-CH=CH_2

25, R = CH_2CH=CH_2
26, R = $CH_2CH_2CH_3$
30, R = CH_2-$CH_2CH_2CH_3$

Table 3. Characterization of Xanthine Derivatives

compd	method[a]	% yield[b]	mp (°C)	MS[c]	formula	analysis
4	A	75	165.5–169	EI: 164 (M^+)	$C_{13}H_{20}N_4O_2$	C, H, N
7	A	68	137–140	EI: 298 (M^+)	$C_{16}H_{18}N_4O_2$	C, H, N
11	B	60	170–172	FAB: 426 ($M^+ + 1$)	$C_{11}H_{14}N_4O_4$	*f*
12a[d]	C	57	192–194	FAB: 454 ($M^+ + 1$)	$C_{23}H_{27}N_5O_5$	C, H, N[f]
12b	C	66[e]	159–160	FAB: 440 ($M^+ + 1$)	$C_{22}H_{25}N_5O_5 \cdot 0.5DMF$	C, H, N[f]
14	B	32	260–263	335, 352	$C_{16}H_{22}N_4O_4 \cdot 0.5DMF$	C, H, N
15	C	91	129–130	363	$C_{17}H_{24}N_4O_4$	C, H, N
16	C	66	225–230	349	$C_{17}H_{24}N_4O_4 \cdot 0.5DMF$	C, H, N
17	B	45	229–230	337 ($M^+ + 1$), 354 ($M^+ + 18$)	$C_{16}H_{24}N_4O_4$	C, H, N
18	B	39	189–191	348, 365	$C_{17}H_{26}N_4O_4$	C, H, N
19	B	19	225 dec	365, 382	$C_{18}J_{28}N_4O_4 \cdot 0.15NH_4OH$	C, H, N
20	B	56	239–240	267 ($M^+ + 1$)	$C_{11}H_{14}N_4O_4$	C, H, N
21	B	48	245–247 dec	283 ($M^+ + 1$), 300 ($M^+ + 18$)	$C_{11}H_{14}N_4O_3S_1 \cdot 1.18H_2O$	C, H, N
30	D	57	325–330 dec	415 ($M^+ + 1$)	$C_{21}H_{26}N_4O_5 \cdot 0.5NH_4OH$	C, H, N
33	D	78	172–173		$C_{40}H_{43}N_7O_9 \cdot 1.5H_2O$	C, H, N
34	D	100	215–220 dec	544	$C_{25}H_{33}N_7O_7 \cdot 1.5H_2O$	C, H, N
35	D	70	204–207 dec	FAB: 644 ($M^+ + 1$)	$C_{23}H_{33}N_8O_{10}$	*f*

[a] Methods: (A) synthesis as in Scheme 1; (B) synthesis as in Scheme 2; (C) refer to text; (D) refer to Scheme 3. [b] Percent yield calculated from 1,3-dialkyl-6-amino-5-nitrosouracil. [c] CI, unless noted. [d] Methyl ester of compound **12b**. [e] Saponification step. [f] High-resolution mass, measured (ppm from calculated), in FAB mode: **11**, 426.1778 (+0.1); **12a**, 454.2091 (+0.2); **12b**, 440.1944 (+2.2); **35**, 644.2686 (+0.8).

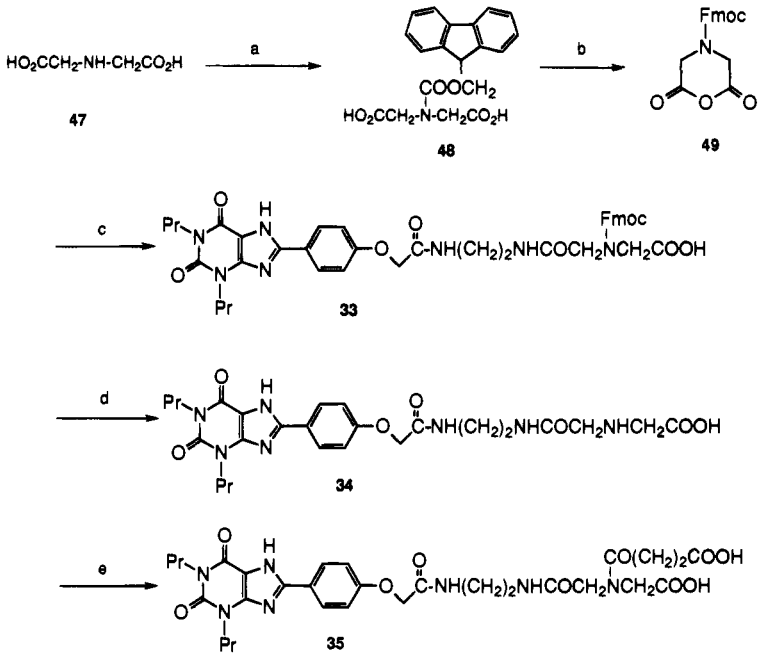
for 8-arylxanthines as well as for 8-alkylxanthines. The A_3 affinity of 2-thioXCC, **28** (K_i value of 6.8 μM), was 11-fold greater than the affinity of XCC, **26**.

The A_3 affinity was not highly dependent on the distance of the carboxylate group from the xanthine pharmacophore. A glycine conjugate of XCC, **29**,[29] was equipotent to the parent carboxylate **26** at A_1 and A_3 receptors and 5-fold more potent at A_{2a} receptors. Succinyl XAC, **32**,[30] although definitely not A_3 selective, was nearly equipotent to XAC at A_3 receptors and considerably less potent than XAC at A_1 and A_{2a} receptors, inconsistent with a proposed role of an anionic group in enhancing A_3 affinity as noted for sheep A_3 receptors.[14] The A_1, A_{2a}, and A_3 receptor affinities of this succinyl derivative, **32**, were nearly the same as for the slightly shorter glycine conjugate, **29**, and thus similar to the considerably shorter XCC, **26**.

Introduction of multiple carboxylate groups on the 8-aryl substituent was accomplished by attaching a derivative of iminodiacetic acid to XAC followed by succinylation of the secondary amine (Scheme 4). However, the dicarboxylate, **35**, did not display enhanced affinity at A_3 receptors. The affinity at A_1 and A_{2a} receptors of **35** vs the monoanionic and Zwitterionic intermediates, **33** and **34**, respectively, was greatly diminished.

We have shown that theophylline lacks functional antagonistic properties vs A_3 agonist-elicited adenylate cyclase inhibition in A_3-transfected CHO cells.[12] We examined the antagonist properties of several carboxylic acid derivatives in a functional assay at A_3 receptors, since these derivatives were more potent in binding than theophylline. Adenylate cyclase was inhibited by N^6-benzyl-NECA in the concentration range of 10^{-9}–10^{-4} M in transfected CHO cells (maximal degree of inhibition was 40%).[12a] Compounds **21** or **24** at concentrations as high as 20 μM (or 100 μM during a preincuba-

Scheme 4. Synthesis of an Intermediate for Carboxylate Derivatization of Xanthines[a]



[a] Regeants: (a) Fmoc-Cl; (b) trifluoracetic acid; (c) XAC; (d) Et_2NH; (e) succinic anhydride.

tion) showed no significant effect on potency or maximal effect of agonist inhibition of adenylate cyclase.

Discussion

In this study xanthine derivatives were examined for selectivity for rat A_3 receptors. There is an uncommonly large species dependence of the affinity of xanthines at A_3 receptors. Very few of the compounds studied were actually A_3 selective in the rat. Compound **24** was 710-fold more potent at sheep A_3 receptors[14] than at rat A_3 receptors. Compound **27** was 400-fold more potent at sheep A_3 receptors[14] than at rat A_3 receptors. Certain xanthines were also found to be moderately potent in displacing radioligand from human A_3 receptors.[15] Thus, it is to be expected that some of these xanthine derivatives are potentially selective for A_3 receptors in sheep and human tissue and possibly in other species.

A reported lead for enhancing A_3 affinity at sheep and human A_3 receptors was an anionic group on the 8-substituent.[14,15] In this study the presence of a sulfonate, a carboxylate, or multiple carboxylate groups did not result in a significant enhancement of affinity at rat A_3 receptors, although as previously observed[29] an anionic group tended to diminish potency at A_1 and A_{2a} receptors.

One may speculate on which amino acid residues on the A_3 receptor may interact with carboxylate group in sheep and human A_3 receptors. Presumably the residue should be a positively charged residue that is conserved between sheep and human A_3 receptor sequences but is absent in A_1 or A_{2a} receptors (across species) and in rat A_3 receptors. In the vicinity of the transmembrane region, which is certainly but perhaps not exclusively involved in ligand binding,[12] one residue which satisfies these conditions is the Arg^{173} residue (human) occurring approximately at the boundary of the fifth transmembrane helix and the second extracellular loop. The corresponding residues are Gly^{175} in the rat A_3 receptor and Ser^{176}/Pro^{173} in the human A_1/A_{2a} receptors. Site-directed mutagenesis and molecular modeling will be essential tools in locating the ligand binding site on the A_3 receptor and determining if Arg^{173} plays a role. We recently proposed a molecular model for binding of nucleosides to A_3 receptors based on the presence of a conserved His residue in the seventh transmembrane helix as a ribose-anchoring site.[12] We demonstrated that 1,3-dialkylxanthine-7-ribosides displayed greatly enhanced affinity vs the parent xanthines at rat A_3 receptors.

Several of the more potent derivatives in this study (compounds **21** and **24**) were too weak to functionally antagonize the effects of an A_3 agonist. Compound **27**, BWA 522, was recently used as a weak antagonist *in vivo* at rat A_3 receptors.[31] Our findings suggest that although considerable affinity for A_3 receptors is present (μM), it is still somewhat selective (32-fold) for A_1 receptors.

In conclusion, the development of A_3 receptor antagonists of general applicability across species remains a challenge. A_3 receptor antagonists may prove useful as antiinflammatory agents. This study has identified several leads, such as 2-thio and 8-(3-carboxypropyl)-xanthines modifications. It is possible also that non-xanthine antagonists will also provide leads, although a screen of six non-xanthine A_1 antagonists of diverse structure indicated negligible affinity at rat A_3 receptors.[12]

Experimental Section

Chemistry. New compounds were characterized (and resonances assigned) by 300 MHz proton nuclear magnetic resonance spectroscopy using a Varian GEMINI-300 FT-NMR spectrometer. Unless noted, chemical shifts are expressed as ppm downfield from tetramethylsilane. Synthetic intermediates were characterized by chemical ionization mass spectrometry (NH_3) on a JEOL SX102 mass spectrometer. In the EI mode accurate mass was determined using a VG7070F mass spectrometer. C, H, and N analyses were carried out by Atlantic Microlabs (Norcross, GA), and $\pm 0.4\%$ was acceptable. All xanthine derivatives were judged to be homogeneous using thin layer chromatography (silica, 0.25 mm, glass backed, Alltech Assoc., Deerfield, IL) following final purifica-

tion. XAC, CPX, CSC, SPX, and 2-chloroadenosine were obtained from Research Biochemicals International (Natick, MA). Analytical TLC plates and silica gel (230–400 mesh) were purchased from VWR (Bridgeport, NJ). The following compounds were kindly provided by the sources stated: **23** (Dr. John Daly, NIDDK), **24** (Dr. Susan Daluge, Burroughs Wellcome), **27** (Dr. Joel Linden, University of Virginia, Charlottesville VA), **28** (Dr. John Neumeyer, Research Biochemicals International). Compound **13** was prepared as described in ref 24. Compound **25** was prepared as described in ref 20. Compound **26** was prepared as described in ref 19. Compound **29** was prepared as described in ref 29. Compound **32** was prepared as described in ref 30.

1,3-Di-*n*-butylxanthine (4). A mixture of compound **39a** (3.1 g, 11.6 mmol) and 5% Pd/C in dry DMF (30 mL) was hydrogenated at 50 psi until it become colorless solution (ca. 2 h). The catalyst was filtered off through Celite pad, and the filtrate was mixed with 88% formic acid (15 mL) and then was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.1 g, 5.74 mmol) at 0 °C under nitrogen. The violet solution was stirred for 3 h at room temperature. DMF and formic acid were removed by rotary evaporation, and the solid residue was mixed with 2 N aqueous sodium hydroxide (50 mL, 100 mmol). The reaction mixture was heated for 1 h at reflux. The yellow solution was cooled and neutralized with 6 N hydrochloric acid to pH 3. The solid was collected by filtration, washed with water, and dried to give compound **4** (2.3 g, 75%): ^{1}H NMR (DMSO-d_6) δ 1.41–1.47 (m, 6 H, 2 × CH_3), 1.61–1.68 (m, 4 H, 2 × CH_2), 1.74–1.79 (m, 2 H, CH_2), 1.82–1.85 (m, 2 H, CH_2), 3.84 (t, J = 7.3 Hz, 2 H, N-CH_2), 3.98 (t, J = 7.3 Hz, 2 H, N-CH_2), 8.10 (s, 1 H, H-8), 13.7 (br s, 1 H, exchanged with D_2O, NH).

3-Benzyl-1-butylxanthine (7). A solution of 6-amino-1-benzyl-3-butyl-5-nitrosouracil and 6-amino-3-benzyl-1-butyl-5-nitrosouracil (**39b**, 10 g, 33 mmol) in DMF (100 mL) was hydrogenated using 5% Pd/C (0.5 g) catalyst until the pink color disappear to give a mixture of 5,6-diaminouracil derivatives. After removal of catalyst by filtration through Celite pad, the filtrate was mixed with 88% formic acid (25 mL) and then 1-[3-(dimethylamino)propyl]-1-ethylcarbodiimide (3.5 g, 18 mmol). The reaction mixture was stirred for 2.5 h at room temperature. It was concentrated to dryness, and 2 N NaOH (100 mL) was added. The mixture was refluxed for 1 h, cooled, neutralized with 6 N HCl, and extracted with ethyl acetate (200 mL). The organic layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to give a brown solid which was purified on silica gel column chromatography ($CHCl_3$–MeOH, 50:1 → 20:1) to give a mixture of 1-benzyl-3-butylxanthine and 3-benzyl-1-butylxanthine (6.76 g, 68.5%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.3 Hz, 3 H, CH_3), 1.23–1.68 (m, 4 H, CH_2CH_2), 3.88, 4.00 [2 × t, J = 7.3 Hz, 2 H (2.2:1), N-CH_2], 5.08, 5.18 [2 × s, 2 H (1:2.4), N-CH_2Ph], 7.20–7.35 (m, 5 H, Ph), 8.02, 8.04 [2 × s, 1 H (2.4:1), H-8, 13.60 (br s, 1 H, NH). In order to separate two isomers, the solid was recrystallized in ethyl acetate to yield compound **7**: ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J = 7.3 Hz, 3 H, CH_3), 1.40 (sextet, J = 7.5 Hz, 2 H, CH_2), 1.65 (m, 2 H, CH_2), 4.07 (pseudo t, J = 7.6 and 7.4 Hz, N-CH_2), 5.33 (s, 2 H, NCH_2Ph), 7.28–7.53 (m, 5 H, Ph), 7.77 (s, 1 H, H-8), 11.85 (br s, 1 H, exchanged with D_2O, NH).

1,3,7-Trimethyl-8-[3-[[(3-carboxypropyl)carbonyl]amino]styryl]xanthine (11) was prepared by acylation of the corresponding arylamine using glutaric anhydride by a procedure similar to that reported for compound **10** in ref 22: ^{1}H NMR (DMSO-d_6) δ 1.83 (m, 2 H, CH_2), 2.28 and 2.38 (each: m, 2 H, $COCH_2$), 3.23 (s, 3 H, N_3-CH_3), 3.47 (s, 3 H, N_1-CH_3), 4.03 (s, 3 H, N_7-CH_3), 7.2–7.6 (m, 3 H, Ar), 7.24 and 7.61 (each: d, J = 15.8 Hz, 1 H, vinyl), 7.90 (m, 1 H, Ar).

1,3,7-Trimethyl-8-[3-[[[4-(methyloxycarbonyl)butyl]carbonyl]amino]styryl]xanthine (12a). 1,3,7-Trimethyl-8-(3-aminostyryl)xanthine[22] (33.1 mg, 106 μmol), adipic acid monomethyl ester (38 mg, 0.24 mmol), and EDAC (47 mg, 0.24 mmol) were suspended in DMF (1 mL) and sonicated for 5 min. The mixture was treated with DMAP (3 mg), and sonicated for 30 min. Upon addition of ice water, a fine yellow precipitate (27 mg, 57%) formed: ^{1}H NMR (DMSO-d_6) δ 1.60 (br s, 4 H, 2 × CH_2), 2.35 (br s, 4 H, 2 × CH_2), 3.23 (s, 3 H, N_3-CH_3), 3.47 (s, 3 H, N_1-CH_3), 3.59 (s, 3 H, $COOCH_3$), 4.03 (s, 3 H, N_7-CH_3), 7.21–7.89 (m, 3 H, Ar), 7.24 and 7.61 (each: d, J = 15.8 Hz, 1 H, vinyl), 9.98 (m, 1 H, Ar).

1,3,7-Trimethyl-8-[3-[[(4-carboxybutyl)carbonyl]amino]styryl]xanthine (12b). Compound **12a** (14 mg, 45 μmol) was dissolved in methanol (1 mL) and treated with 0.2 mL of 1 N NaOH. The solution was heated to 50 °C and cooled. The reaction mixture was acidified with 4 N hydrochloric acid, and the precipitate was collected and dried to give compound **12b** (12.7 mg, 66%): ^{1}H NMR (DMSO-d_6) δ 1.59 (m, 4 H, 2 × CH_2), 2.25 and 2.34 (m, 4 H, 2 × CH_2), 3.23 (s, 3 H, N_3-CH_3), 3.47 (s, 3 H, N_1-CH_3), 4.03 (s, 3 H, N_7-CH_3), 7.21–7.89 (m, 3 H, Ar), 7.24 and 7.61 (each: d, J = 15.8 Hz, 1 H, vinyl), 9.98 (m, 1 H, Ar).

1,3-Dibutyl-8-(*trans*-2-carboxyvinyl)xanthine (14). A solution of **39a** (1.95 g, 7.28 mmol) and 5% Pd/C (200 mg) in dry DMF (30 mL) was hydrogenated for 3 h. The catalyst was removed by filtration through Celite, and the filtrate was divided for synthesis of several compounds. An aliquot of the filtrate (12 mL) was mixed with monomethyl fumarate (0.258 g, 1.79 mmol), DMAP (50 mg, 0.41 mmol), imidazole (100 mg, 1.47 mmol), and EDAC (0.4 g, 1.73 mmol) and sonicated for 3 h. DMF was removed by rotary evaporation, and the residue was mixed with 2 N NaOH (40 mL). After the reaction mixture was heated for 1 h, it was cooled, filtered, and neutralized with 6 N HCl. The solid was collected by filtration and dried to give compound **14** (0.17 g, 32%): ^{1}H NMR (DMSO-d_6) δ 0.87–0.93 (m, 6 H, 2 × CH_3), 1.26–1.34 (m, 4 H, 2 × CH_2), 1.51–1.66 (m, 4 H, 2 × CH_2), 3.88 (pseudo t, J = 7.4 and 7.2 Hz, 2 H, N-CH_2), 4.00 (pseudo t, J = 7.2 and 7.0 Hz, 2 H, N-CH_2), 6.76 and 7.29 (each: d, J = 15.9 Hz, 1 H, vinyl).

1,3-Dibutyl-7-methyl-8-[*trans*-2-(methyloxycarbonyl)vinyl]xanthine (15). Compound **14** (78 mg, 0.23 mmol) was dissolved in DMF (2 mL) and treated with methyl iodide (0.1 mL, 1.6 mmol) and potassium carbonate (0.1 g). After heating at 50 °C for 30 min, ice water was added to precipitate the product (77 mg, 91%): ^{1}H NMR (DMSO-d_6) δ 0.87–0.93 (m, 6 H, 2 × CH_3), 1.25–1.34 (m, 4 H, 2 × CH_2), 1.49–1.66 (m, 4 H, 2 × CH_2), 3.34 (s, 3 H, $COOCH_3$), 3.87 (pseudo t, J = 7.4 and 6.9 Hz, 2 H, N-CH_2), 3.99 (pseudo t, J = 7.2 and 6.9 Hz, 2 H, N-CH_2), 4.04 (s, 3 H, N_7-CH_3), 6.80 and 7.62 (each: d, J = 15.5 Hz, 1 H, vinyl).

1,3-Dibutyl-7-methyl-8-(*trans*-2-carboxyvinyl)xanthine (16). Compound **15** (66 mg, 0.18 mmol) was dissolved in DMF (2 mL) and treated with 1 mL of 1 N NaOH. After warming to 40 °C for 10 min, the solution was acidified with HCl. The precipitate was collected and dried (42 mg, 66%): ^{1}H NMR (DMSO-d_6) δ 0.90 (m, 6 H, 2 × CH_3), 1.30 (m, 4 H, 2 × CH_2), 1.54 (m, 4 H, 2 × CH_2), 1.66 (m, 4 H, -CH_2CH_2), 3.87 (t, 2 H, N-CH_2), 4.00 (t, 2 H, CH_2), 6.76 and 7.54 (each: d, J = 15.8 Hz, 1 H, vinyl).

4-(1,3-Dibutylxanth-8-yl)propionic Acid (17). An aliqout (7 mL) of the solution of hydrogenated compound **39a** (see procedure for **14**) was treated with succinic anhydride (0.19 g, 0.19 mmol) overnight. Half saturated NaCl was added, and the slowly forming insoluble residue was separated by decantation. This residue was dissolved in a 1:1 mixture of methanol and 4 N NaOH and heated to reflux for 30 min. Upon acidification, a precipitate formed, which was collected and dried (159 mg, 45% overall): ^{1}H NMR (DMSO-d_6) δ 0.89 (m, 6 H, 2 × CH_3), 1.27 (m, 4 H, 2 × CH_2), 1.50 and 1.63 (each: m, 2 H, CH_2), 2.71 (pseudo t, J = 7.7 and 6.8 Hz, 2 H, CH_2), 2.91 (pseudo t, J = 7.2 and 7.0 Hz, 2 H, CH_2), 3.86 (pseudo t, J = 7.4 and 6.9 Hz, 2 H, N-CH_2), 3.95 (t, J = 7.2 Hz, 3 H, CH_2).

4-(1,3-Dibutylxanth-8-yl)butyric acid (18) was prepared by a similar procedure using glutaric anhydride: ^{1}H NMR (DMSO-d_6) δ 0.89 (pseudo t, J = 7.3 and 7.2 Hz, 6 H, 2 × CH_3), 1.24–1.31 (m, 4 H, 2 × CH_2), 1.50 and 1.62 (each: m, 2 H, CH_2), 1.90 (m, 2 H, aliphatic CH_2), 2.27 (t, J = 7.3 Hz, 2 H, CH_2), 2.71 (pseudo t, J = 7.4 and 7.3 Hz, 2 H, CH_2), 3.86 (t, J = 7.3 Hz, 2 H, N-CH_2), 3.96 (t, J = 7.3 and 6.8 Hz, 2 H, CH_2). MS (CI) 368, 351, 333.

4-(1,3-Dibutylxanth-8-yl)pentanoic Acid (19). A solution of **39a** (0.31 g, 1.15 mmol) and 5% Pd/C (26 mg) in dry DMF (20 mL) was hydrogenated for 15 h. After filtration of

Pd/C through a Celite pad, the filtrate treated with DMAP (37.6 mg, 0.03 mmol) and EDAC (332 mg, 1.73 mmol) for 3.3 h at room temperature. DMF was removed by rotary evaporation, and the residue was mixed with 2 N NaOH (20 mL). After the reaction mixture was heated for 2 h, it was cooled, filtered, and neutralized with 6 N HCl. The solid was collected by filtration and dried to give compound **19** (31.5 mg, 13%). Analytical sample was prepared by dissolving **19** in DMF/concentrated NH_4OH, filtering, neutralizing with formic acid, and crystallizing with water: ^1H NMR (DMSO-d_6) δ 0.89 (m, 6 H, 2 × CH_3), 1.28 (m, 4 H, 2 × CH_2), 1.51 (m, 4 H, 2 × CH_2), 1.65 (m, 4 H, CH_2CH_2), 2.23 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, CH_2), 2.28 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, CH_2), 3.86 (pseudo t, J = 7.5 and 7.2 Hz, 2 H, N-CH_2), 3.96 (pseudo t, J = 7.2 and 7.1 Hz, 2 H, CH_2), 11.98 (s, 1 H, exchanged with D_2O, COO*H*), 13.14 (s, 1 H, exchanged with D_2O, N*H*).

General Procedure for Scheme 2. 4-(1,3-Dimethylxanth-8-yl)butyric Acid (20). Glutaric anhydride (0.5 g, 4.38 mmol) was added to a red solution of 5,6-diamino-1,3-dimethyluracil (0.5 g, 2.94 mmol, purchased from Aldrich Chemical Co.) in anhydrous DMF (10 mL), and the reaction mixture was stirred at room temperature for 17 h. The yellow solution was concentrated to dryness to give a yellow solid, which was mixed with 2 N NaOH (15 mL). After heating for 1.5 h at 75 °C, the yellow solution was cooled and neutralized with 6 N HCl. The solid was collected by suction and washed with small quantity of water and dried to give compound **20** (0.436 g, 56%) as a colorless solid. ^1H NMR (DMSO-d_6) δ 1.94 (sextet, J = 7.4 Hz, 2 H, $CH_2CH_2CH_2$), 2.27 (t, J = 7.3 Hz, 2 H, CH_2), 2.72 (t, J = 7.5 Hz, 2 H, CH_2), 3.33, 3.42 (each: s, 3H, CH_3), 12.10 (s, 1 H, exchanged with D_2O, COO*H*), 13.20 (s, 1 H, exchanged with D_2O, N*H*).

4-(1,3-Dimethyl-2-thioxanth-8-yl)butyric Acid (21). A solution of 6-amino-1,3-dimethyl-5-nitroso-2-thiouracil (**39c**, 0.5 g, 2.5 mmol) in anhydrous DMF (100 mL) was hydrogenated for 2 days in the presence of 5% Pd/C (0.1 g), and the catalyst was removed by filtration through Celite pad. The filtrate was treated with glutaric anhydride (0.43 g, 3.77 mmol) for 18 h at room temperature. After DMF was removed *in vacuo*, the solid was dissolved in 2 N NaOH (16 mL), heated for 1.5 h at 80 °C, and neutralized with 6 N HCl. The solid formed was filtered and dried to give **21** (0.337 g, 48%): ^1H NMR (DMSO-d_6) δ 1.94 (quintet, J = 7.3 Hz, 2 H, CH_2CH_2-CH_2), 2.29 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, CH_2), 2.78 (t, J = 7.5 Hz, 2 H, CH_2), 3.68, 3.87 (2 × s, 2 × 3 H, 2 × CH_3), 12.10 (s, 1 H, exchanged with D_2O, COO*H*), 13.60 (s, 1 H, exchanged with D_2O, N*H*).

[4-(1,3-Dibutylxanth-8-yl)phenoxy]acetic Acid (30). A solution of 6-amino-1,3-dibutyl-5-nitrosouracil (**39a**, 2.68 g, 10 mmol) in methanol (27 mL) was hydrogenated in the presence of 5% Pd/C (0.2 g). Catalyst was removed by suction through Celite pad, and the filtrate was mixed with (4-formylphenoxy)-acetic acid (1.8 g, 10 mmol). The reaction mixture was stirred for 1 h on a stream of steam bath. It was cooled, and the solid was collected by suction and dried to give [4-[[(6-amino-1,3-dibutyluracil-5-yl)imino]methyl]phenoxy]acetic acid (**46c**, 3.24 g, 78%): ^1H NMR (DMSO-d_6) δ 0.86–0.93 (m, 5 H, 2 × CH_3), 1.24–1.36 (sextet, J = 7.5 Hz, 4 H, 2 × CH_2), 1.45–1.58 (m, 4 H, 2 × CH_2), 3.80 (pseudo t, J = 7.5 and 6.9 Hz, 2 H, N-CH_2), 3.95 (pseudo t, J = 7.5 and 7.2 Hz, 2 H, N-CH_2), 4.72 (s, 2 H, CH_2COOH), 6.93 (d, J = 8.6 Hz, 2 H, Ph), 7.28 (s, 2 H, exchanged with D_2O, NH_2), 7.32 (d, J = 8.7 Hz, 2 H, Ph), 9.67 (s, 1 H, N=CH), 12.99 (s, 1 H, exchanged with D_2O, COO*H*). Anal. Calcd for $C_{21}H_{27}N_4O_5$: C, 60.71; H, 6.55; N, 13.49. Found: C, 60.60; H, 6.72; N, 13.45.

To a solution of **46c** (2.9 g, 7.0mmol) in DMF (50 mL) was added sodium periodate (1.5 g, 7 mmol) in water (8 mL), and the mixture became clear immediately. While the reaction mixture was stirring for 3 h, a solid formed and it was filtered (1.1 g), and the filtrate was concentrated to dryness. The residue was triturated with ether–methanol, and the solid was filtered by suction (2.4 g). The combined solid was recrystallized from 4 N NaOH–AcOH to yield compound **30** (1.66 g, 58%) as a colorless solid. Analytical sample of **30** was prepared by dissolving in DMF/concentrated NH_4OH and acidifying with formic acid. The solid was filtered and dried *in vacuo* overnight: ^1H NMR (DMSO-d_6) δ 0.87–0.94 (m, 5 H, 2 × CH_3), 1.26–1.36 (sextet, J = 7.5 Hz, 4 H, 2 × CH_2), 1.49–1.59 (m, 2 H, CH_2), 1.65–1.74 (m, 2 H, CH_2), 3.89 (pseudo t, J = 7.5 and 7.1 Hz, 2 H, N-CH_2), 4.05 (pseudo t, J = 7.1 and 6.9 Hz, 2 H, N-CH_2), 4.77 (s, 2 H, CH_2COOH), 7.04 (d, J = 9.3 Hz, 2 H, Ph), 8.04 (d, J = 8.6 Hz, 2 H, Ph), 13.02 (s, 1 H, exchanged with D_2O, COO*H*), 13.61 (s, 1 H, exchanged with D_2O, N*H*).

Compound 33. Compound **49** (33 mg, 0.10 mmol) was dissolved in 4 mL of an equivolume mixture of dimethylformamide and ethyl acetate. XAC (compound **31**, 36 mg, 83 μmol) was added, and the mixture was warmed (50 °C) for 1 hour. The volume was reduced by half by evaporation, and sodium bisulfate (1 M) was added. The precipitate was collected and dried *in vacuo* to give 50 mg (78% yield) of product **33**. This could be recrystallized from ethyl acetate/petroleum ether to give pure **33**: ^1H NMR (DMSO-d_6) δ 0.85–0.92 (m, 6 H, 2 × CH_3), 1 60 and 1.72 (each: m, 2 H, 2 × CH_2), 3.41 (m, 2 H), 3.86 (pseudo t J = 7.4 and 7.2 Hz, 4 H), 4.00 (s, 4 H), 4.21 (s, 4 H), 4.53 (d, J = 8.7 Hz, 2 H), 7.08 (pseudo t, J = 8.3 and 7.7 Hz, 2 H), 7.29 (psudo t, J = 7.4 and 6.8 Hz, 2 H), 7.40 (pseudo t, J = 7.3 and 6.9 Hz, 2 H), 7.60 (dd, J = 7.3 and 3.9 Hz, 2 H), 7.87 (d, J = 6.8 Hz, 2 H), 8.07 (m, 2 H), 8.21 and 8.23 (each: br s, 1 H).

8-[4-[[[[2-[[[[(Carboxymethyl)amino]methyl]carbonyl]-amino]ethyl]amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (34). Compound 33 (12.1 mg, 15.8 μmol) was dissolved in DMF (0.5 mL), treated with diethylamine (0.2 mL), and stirred for 1 h. The solvent was evaporated, and the residue was washed with ether and dried *in vacuo*: ^1H NMR (DMSO-d_6) δ 0.88 (m, 6 H, 2 × CH_3), 1 58 and 1.74 (each: m, 2 H, CH_2), 3.21 and 3.29 (m, 8 H, $COCH_2$, CH_2-COOH, and $NHCH_2$), 3.87 (pseudo t, J = 8.3 and 6.4 Hz, 2 H, N-CH_2), 4.02 (t, J = 7.3 and 6.2 Hz, 2 H, N-CH_2), 4.55 (m, 2 H, OCH_2CO), 7.10 and 8.08 (each: d, J = 8.8 Hz, 1 H, vinyl H), 8.11 and 8.23 (each: br s, 1 H, NH).

8-[4-[[[[[2-[[[[(3-Carboxypropanoyl)(carboxymethyl)-amino]methyl]carbonyl]amino]ethyl]amino]carbonyl]-methyl]oxy]phenyl]-1,3-dipropylxanthine (35). Compound **34** (5.4 mg, 10 μmol) was dissolved in DMF and treated with succinic anhydride (10 mg, 100 μmol) and diisopropylethylamine (10 μL) and sonicated for 1 h. Dilute HCl was added to acidify and the solvent was evaporated. Upon addition of methanol and ether a precipitate formed. The precipitate was collected and dried *in vacuo*: ^1H NMR (DMSO-d_6) δ 0.89 (m, 6 H, 2 × CH_3), 1 57 and 1.74 (each: m, 2 H, CH_2), 2.42 [m, 4 H, $HO_2C(CH_2)_2CO$], 3.23 and 3.59 (m, 4 H, 2 × NCH_2), 3.87 and 4.00 (m, 8 H, $COCH_2$, CH_2COOH, NCH_2), 4.55 (m, 2 H, OCH_2CO), 7.11 and 8.08 (each: d, J = 9.0 Hz, 1 H, vinyl H), 8.23 and 8.38 (each: br s, 1 H, NH).

***N*-Benzyl-*N'*-butylurea (36b).** A solution of butyl isocyanate (10.1 mL, 97.6 mmol) in chloroform (10 mL) was added dropwise to a solution of benzylamine (10 mL, 91.6 mmol) in chloroform (10 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature, and solvent was removed *in vacuo* to give *N*-benzyl-*N'*-butylurea [**36b**, R_f = 0.75 ($CHCl_3$–MeOH, 8.5:1.5), 18.2 g, 96%] as a colorless solid: mp 98 °C; MS 206 (M^+); ^1H NMR (DMSO-d_6) δ 0.85 (pseudo t, J = 7.1 and 6.9 Hz, 3 H, CH_3), 1.2–1.4 (m, 4 H, CH_2CH_2), 2.98 (m, 2 H, NH-CH_2), 4.2 (d, J = 5.0 Hz, 2 H, CH_2Ph), 5.85, 6.26 (2 × br s, 2 × 1 H, exchanged with D_2O, NH), 7.18–7.25 (m, 5 H, Ph).

1,3-Di-*n*-butyl-6-aminouracil (38a). *N,N*-Di-*n*-butylurea (**36a**, 34.45 g, 0.2 mol), cyanoacetic acid (19.56 g, 0.23 mol), and acetic anhydride (81.66 mL, 0.8 mol) was heated at 80 °C for 2 h under nitrogen, and the reaction mixture was concentrated to dryness to give crude compound **37a**. The crude mixture was dissolved in methanol (100 mL), and 4 N sodium hydroxide (100 mL) was added. The reaction mixture was stirred for 30 min with cooling, and the solid formed was filtered and dried to give compound **38a** (47.04 g, 99.5%): mp 90.1–92.4 °C; ^1H NMR (DMSO-d_6) δ 1.72-1.93 (m, 6 H, 2 × CH_3), 1.18–1.33 (m, 4 H, 2 × CH_2), 1.39–1.52 (m, 4 H, 2 × CH_2), 3.70 (t, 2 H, N-CH_2), 3.76 (t, 2 H, N-CH_2), 4.64 (s, 1 H, H-4), 6.77 (br s, exchangeable with D_2O, 2 H, NH_2).

6-Amino-1-benzyl-3-butyluracil and 6-Amino-3-benzyl-1-butyluracil (38b). A mixture of *N*-benzyl-*N'*-butylurea (**36b**, 18 g, 87.3 mmol), cyanoacetic acid (8.2 g, 96.1 mmol),

and acetic anhydride (30 mL) was heated for 2 h at 80 °C under nitrogen. After the solution was cooled, it was concentrated to dryness to give **37b** [R_f = 0.5 (Hx–EtOAc, 3:1)]. It was dissolved in methanol (20 mL), and 20% NaOH (10 mL) was added. The reaction mixture was stirred for 0.7 h at 0 °C and for 10 min at room temperature. It was concentrated to half volume, and the resulting yellow solid was filtered and dried to give a mixture of 6-amino-1-benzyl-3-butyluracil and 6-amino-3-benzyl-1-butyluracil [**38b**, R_f = 0.22 (Hx–EtOAc, 1:3), 23.5 g, 98%]: mp 100–103 °C; ^{1}H NMR (DMSO-d_6) δ 0.81–0.90 (m, 3 H, CH_3), 1.17–1.62 (m, 4 H, CH_2CH_2), 3.69–3.80 (m, 2 H, N-CH_2), 4.70 (s, 1 H, H-4), 5.07 (s, 2 H, CH_2Ph), 6.80 (br s, 2 H, exchanged with D_2O, NH_2), 7.15–7.38 (m, 5 H, Ph).

6-Amino-1,3-dimethyl-2-thiouracil (38c). A mixture of dimethylthiourea (purchased from Aldrich Chemical Co., 10.4 g, 0.1 mol), cyanoacetic acid (9.3g, 0.11 mol), and acetic anhydride (40 mL) was heated for 2 h at 80 °C under nitrogen. The dark solution was concentrated *in vacuo*, and methanol (45 mL) and 4 N NaOH (45 mL) were added. After the reaction mixture was stirred for 10 min, the solid was collected by suction and dried for 0.5 h at 60 °C to give compound **38c** as a slightly yellow solid (9.02 g, 53%): mp 277–279 °C; ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 3 H, CH_3), 3.52 (s, 3 H, CH_3), 5.03 (s, 1 H, C*H*=C), 7.02 (br s, 2 H, exchanged with D_2O, NH_2).

6-Amino-1,3-di-*n*-butyl-5-nitrosouracil (39a). To a mixture of compound **38a** (27 g, 112.8 mmol), glacial acetic acid (10 mL), and 6 N hydrochloric acid (18.8 mL) in water (500 mL) was added dropwise a solution of sodium nitrite (7.78 g, 112.8 mmol) in water (30 mL) on ice bath. The reaction mixture was stirred for 30 min. The violet solid was collected by filtration, washed with water (2 × 20 mL), and dried for 2 days at 60 °C in a vacuum oven to give compound **39a** (24.99 g, 82.6%): mp 199–205 °C; ^{1}H NMR ($CDCl_3$) δ 0.97–1.01 (m, 6 H, 2 × CH_3), 1.36–1.49 (m, 4 H, 2 × CH_2), 1.62–1.74 (m, 4 H, 2 × CH_2), 3.94 (t, J = 7.8 Hz, 2 H, N-CH_2), 4.08 (t, J = 7.5 Hz, 2 H, N-CH_2), 6.46 (br s, 2 H, NH_2). Anal. Calcd for $C_{12}H_{20}N_4O_3$: C, 53.72; H, 7.51; N, 20.88. Found: C, 54.37; H, 7.67; N, 20.65.

6-Amino-1-benzyl-3-butyl-5-nitrosouracil and 6-Amino-3-benzyl-1-butyl-5-nitrosouracil (39b). A mixture of 6-amino1-benzyl-3-butyluracil and 6-amino-3-benzyl-1-butyluracil (**38b**, 23.5 g, 86 mmol) was nitrosated to give a mixture of 6-amino-1-benzyl-3-butyl-5-nitrosouracil and 6-amino-3-benzyl-1-butyl-5-nitrosouracil (**39b**, 25.5 g, 98%) as a pink solid using the same method for 6-amino-1,3-dibutyl-5-nitrosouracil (**39a**): mp 178–9 °C; ^{1}H NMR (DMSO-d_6) δ 0.87–0.98 (m, 3 H, CH_3), 1.22–1.60 (m, 4 H, CH_2CH_2), 3.80, 3.92 (t, J = 7.8 Hz, 2 H, N-CH_2), 5.40, 5.60 (2 × s, 2 H, N-CH_2Ph), 7.22–7.40 (m, 5 H, Ph), 9.70, 13.20 (2 × br s, 2 H, exchanged with D_2O, NH_2).

6-Amino-1,3-dimethyl-5-nitroso-2-thiouracil (39c). A solution of sodium nitrite (3.63 g, 52.6 mmol) in water (15 mL) was added to a suspension of 6-amino-1,3-dimethyl-2-thiouracil (**38c**, 9 g, 52.6 mmol) in water (850 mL) and the 6 N HCl (8.8 mL) was added dropwise. The reaction mixture was stirred well for 20 min, and the blue solid was collected by suction and dried to give compound **39c** (10.12 g, 96%): mp 228 °C; ^{1}H NMR (DMSO-d_6) δ 3.71, 3.74 (2 × s, 2 × 3 H, 2 × CH_3), 9.11, 12.88 (2 × br s, s × 1H, exchanged with D_2O, NH_2).

Fmoc-iminodiacetic Acid (48). Iminodiacetic acid (**47**, 2.19 g, 16.5 mmol, Sigma Chemical Co., St. Louis, MO) was dissolved in a mixture of 1 M sodium bicarbonate (40 mL) and acetone (30 mL). A solution of 9-fluorenylmethyl chloroformate (2.0 g, 7.7 mmol) in acetone (10 mL) was added to the above solution. After 6 h, the mixture was diluted with saline, acidified with 1 N HCl, and extracted with ethyl acetate. Drying and evaporation of the organic layer afforded 2.38 g (87%) of **48**: mp 213–215.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.99 and 4.07 (each: s, 2 H, CH_2), 4.24 (s, 3 H), 7.32 and 7.42 (each: pseudo t, J = 7.5 and 7.3 Hz, 2 H, Ph), 7.63 and 7.90 (each: d, J = 7.5 Hz, 2 H, Ph), 12.75 and 12.77 (each: br s, 1 H, COOH); the mass spectrum (CI-NH3) showed a peak at 373 corresponding to M + 1 + NH_3. Anal. Calcd for $C_{19}H_{17}N_1O_6$: C, 64.22; H, 4.82; N, 3.94. Found C, 64.15; H, 4.83; N, 3.90.

4-Fmoc-morpholine-2,6-dione (49). Fmoc-iminodiacetic acid, compound **48** (0.40 g, 1.13 mmol), was suspended in 30 mL of ethyl acetate, treated with 1 mL of trifluoroacetic anhydride, and stirred for 1 h. Evaporation of most of the solvent, addition of petroleum ether, and evaporation resulted in a solid residue which was dried *in vacuo* at room temperature. The yield was 0.37 g (97%); mp 180–185 °C. This compound tended to hydrolyze upon long term storage. Anal. Calcd for $C_{19}H_{15}NO_5 \cdot 0.8H_2O$: C, 64.88; H, 4.76; N, 3.98. Found: C, 64.58; H, 4.77; N, 3.90.

Biological Methods. Materials. F-12 (Ham's) medium, fetal bovine serum (FBS), and penicillin/streptomycin were from Gibco BRL (Gaithersburg, MD). [^{125}I]AB-MECA was prepared as described.[16] [^{3}H]PIA was from Amersham (Arlington Heights, IL), and [^{3}H]CGS 21680 was from DuPont NEN (Boston, MA). Adenosine deaminase (ADA) was from Boehringer Mannheim (Indianapolis, IN). Composition of lysis buffer: 10 mM Tris/5 mM EDTA, pH 7.4 at 5 °C. 50/10/1 buffer: 50 mM Tris; 10 mM $MgCl_2$; 1 mM EDTA, pH 8.26 at 5 °C. All other materials were from standard local sources and of the highest grade commercially available.

Cell Culture and Membrane Preparation. CHO cells stably expressing the A_3 receptor[6] were grown in F-12 medium containing 10% FBS and penicillin/streptomycin (100 units/mL and 100 μg/mL, respectively) at 37 °C in a 5% CO_2 atmosphere. When cells had reached confluence, they were washed twice with Dulbecco's phosphate buffer solution before dislodging after addition of 3 mL of trypsin–EDTA. For the final passage cells were grown in 150 × 50 mm tissue culture dishes. Cells were washed twice with 10 mL of lysis buffer. After addition of 5 mL of lysis buffer, cells were mechanically scraped and homogenized in an ice-cold Dounce homogenizer (20 strokes by hand). The suspension was centrifuged at 43000*g* for 10 min. The pellet was resuspended in the minimum volume of ice-cold 50/10/1 buffer required for the binding assay and homogenized in a Dounce homogenizer. Typically, 6–8 175-cm^2 flasks were used for a 48-tube assay. Adenosine deaminase was added to a final concentration of 3 units/mL, and the suspension was incubated at 37 °C for 15 min; the membrane suspension was subsequently kept on ice until use. When large batches (ca. 100 flasks) were processed, homogenization was performed with a Polytron (Brinkman, Luzern, Switzerland), and further workup was as described above. The preparation was stored at −70 °C and retained its [^{125}I]AB-MECA binding properties for at least 1 month.

Receptor Binding. Binding of [^{125}I]AB-MECA to CHO cells stably transfected with the A_3 receptor clone was performed essentially as described.[16] Assays were performed in 50/10/1 buffer in glass tubes and contained 100 μL of the membrane suspension, 50 μL of [^{125}I]AB-MECA (final concentration 0.3 nM), and 50 μL of inhibitor. Inhibitors were routinely dissolved in DMSO, while the final DMSO concentration never exceeded 1%. Incubations were carried out in duplicate for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 200 μM NECA. K_i values were calculated according to Cheng–Prusoff,[32] assuming a K_d for [^{125}I]AB-MECA of 1.48 nM.[28]

Binding of [^{3}H]PIA to A_1 receptors from rat brain membranes and of [^{3}H]CGS 21680 to A_2 receptors from rat striatal membranes was performed as described previously.[17,18] Rat cerebral cortical membranes and striatal membranes were prepared[18] and treated with adenosine deaminase (2 units/mL) for 30 min at 37 °C prior to storage at −70 °C. Solid samples of the adenosine derivatives were dissolved in DMSO and stored in the dark at −20 °C. The stock solutions were diluted with DMSO to a concentration of ≤0.1 mM prior to adding to the aqueous medium. The final concentration of DMSO in the assay medium was generally 2%, and adenosine deaminase was not added.

At least six different concentrations spanning 3 orders of magnitude, adjusted appropriately for the IC_{50} of each compound, were used. IC_{50} values, computer-generated using a nonlinear regression formula on the InPlot program (Graph-

pad, San Diego, CA), were converted to apparent K_i values using K_d values[12] of 1.0 and 15.5 nM for [^{3}H]PIA and [^{3}H]CGS 21680 binding, respectively, and the Cheng–Prusoff equation.[32]

Adenylate cyclase in transfected CHO cell membranes was measured as previously described.[6,12]

Abbreviations: SPX, 1,3-dipropyl-8-(4-sulfophenyl)xanthine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; Fmoc, 9-fluorenylmethyloxycarbonyl; NECA, 5′-(*N*-ethylcarboxamido)adenosine; Tris, tris(hydroxymethyl)-aminomethane; XAC, 8-[4-[[[[(2-aminoethyl)amino]carbonyl]-methyl]oxy]phenyl]-1,3-dipropylxanthine; XCC, 8-[4-[(carboxymethyl)oxy]phenyl]-1,3-dipropylxanthine.

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