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Structure–Activity Relationships of 1,3-Dialkylxanthine Derivatives at Rat A₃ Adenosine Receptors

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Abstract

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 A₃ adenosine receptors stably expressed in CHO cells using the new radioligand [¹²⁵I]AB-MECA (*N*⁶-(4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide), and at rat brain A₁ and A_{2a} receptors using [³H]PIA and [³H]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 A₃ receptors, although as previously observed an anionic group tended to diminish potency at A₁ and A_{2a} receptors. The rat A₃ receptor affinity was not highly dependent on the distance of a carboxylate group from the xanthine pharmacophore. 2-Thio vs 2-oxo substitution favored A₃ potency, and 8-alkyl vs 8-aryl substitution favored A₃ selectivity, although few derivatives were truly selective for rat A₃ receptors. 1,3-Dimethyl-8-(3-carboxypropyl)-2-thioxanthine was 7-fold selective for A₃ vs A_{2a} receptors. 1,3,7-Trimethyl-8-(*trans*-2-carboxyvinyl)xanthine was somewhat selective for A₃ vs A₁ receptors. For 8-aryl xanthines affinity at A₃ receptors was enhanced by 1,3-dialkyl substituents, in the order dibutyl > dipropyl > diallyl.

Introduction

Three major classes of adenosine receptors (A₁, A₂, and A₃) have been characterized pharmacologically^{1,4} and through cloning.^{2,3,6} A₁ 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} A₂ 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 A₃ 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 A₃ receptors are coupled to both inhibition of adenylate cyclase and activation of protein kinase C as second messengers. The rat A₃ 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 A₃ receptor was recently shown

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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 A₃ receptors is associated with cardiovascular actions. Fozard and Carruthers³⁴ have attributed a xanthine-insensitive component of the hypotensive effects of adenosine agonists in the rat to A₃ receptor activation. Activation of A₃ receptors has been suggested by Downey and colleagues¹¹ to be involved in the cardioprotective effect of preconditioning by adenosine agonists. The occurrence of A₃ receptors in the brain^{6,9,10} and testes⁷ 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 A₃ receptors.^{12,13} We recently reported⁹ that an N⁶- and 5'-substituted adenosine derivative, N⁶-(3-iodobenzyl)-5'-(*N*-methylcarbamoyl)adenosine (IB-MECA), is 50-fold selective for rat brain A₃ 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 A₁ and A₂ receptors only weakly displaced the binding of the radioligand [¹²⁵I]APNEA (N⁶-[2-(4-amino-3-iodophenyl)-ethyl]adenosine) from cloned rat A₃ 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 A₃ receptors, but generally with less affinity than at A₁ and A₂ receptors in a variety of species.¹ The human A₃ receptor was recently cloned¹⁵ and found to resemble the sheep A₃ 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 A₃ receptors is apparent, perhaps even suggesting multiple subtypes of A₃ receptors. Xanthines that are generally A₃-selective across species are needed as pharmacological and biochemical probes to define more clearly the physiological role, distribution, and regulation of A₃ adenosine receptors.

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 A₁, A_{2a}, and A₃ adenosine receptors. At A₃ receptors affinity was measured in CHO cells stably expressing cloned rat brain A₃ receptors.⁶ The radioligand used for binding to A₃ receptors was the recently reported [¹²⁵I]AB-MECA (N⁶-(4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide).¹⁶ Affinity at A₁ receptors was measured in rat cortical membranes using [³H]-(*R*)-N⁶-(phenylisopropyl)adenosine¹⁷ ([³H]PIA) and at A_{2a} affinity in rat striatal membranes using [³H]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-formamido-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 NaIO₄ 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 A₃ affinity (2-fold) and had no effect on A₁ affinity, whereas A₂ 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 A₃ affinity, but somewhat enhanced A₁ 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 A₁ or A_{2a} adenosine receptors,^{1,22} respectively, remained selective vs rat A₃ receptors. Compound **9** was nearly inactive at A₃ receptors. Among a related series of 8-styrylxanthines that showed selectivity for A_{2a} receptors²² was a water soluble succinylamino derivative, **10**. Compound **10** very weakly displaced [¹²⁵I]AB-MECA binding in transfected CHO cells (*K_i* value 189 μM). An attempt to improve the A₃ 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 A₃ receptors were minimal, the selectivity for A_{2a} vs A₁ receptors was maintained. Selectivity ratios (A_{2a} vs A₁) 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 A₁ receptors but not very potent,²⁴ was found to be selective also for rat A₃ vs A₁ receptors. Lengthening the 1,3-dialkyl substituent, as in the corresponding dibutyl derivative, **16**, greatly enhanced affinity at A₁ receptors, and at A₃ 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 A₁, A_{2a}, and A₃ receptors. The methyl ester, **15**, of the N⁷-H analogue was more potent at A₁ and A_{2a} receptors than the corresponding carboxylic acid, **14**, as expected²⁹ for a neutral vs an anionic 8-functionalized chain.

A series of 8-alkyl carboxylic acids (**17–19**) indicated that optimal affinity at A₃ relative to A₁ and A_{2a} receptors was observed for the 8-(3-carboxypropyl) derivative, **18**, although none of these xanthines were truly selective for rat A₃ receptors.

The 1,3-dimethyl analogue of **18** was prepared, since smaller 1,3-dialkyl substituents seemed to favor A₃ selectivity (compare **13** vs **16**), although not A₃ affinity. Compound **20** was selective for A₃ vs A₁ 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 A₃ vs A_{2a} receptors. The A₃ receptor affinity for **21** vs **20** was enhanced by 10-fold.

The affinities of 8-aryl xanthines at adenosine receptors were compared (Table 2). Linden et al.¹⁴ reported that at sheep A₃ receptors both sulfonate- and carboxylate-containing 8-aryl xanthines 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 A₃ receptors. Both the *p*-carboxylate group, **23**,²⁵ and the *p*-acrylate group, **24** (BW1433),²⁶ were more favorable in A₃ affinity than the *p*-sulfonate group, **22**. The affinities of **22** and **23** at both A₁ and A_{2a} receptors were nearly identical. The *p*-acrylate group in **24** resulted in enhanced (9-fold) A₁ 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 A₃ 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 A₃ receptors, the affinities of additional carboxylate derivatives of 8-aryl xanthines were compared.

The xanthine amine congener, XAC, **31**,¹⁹ was highly selective for A₁ and A_{2a} receptors vs A₃ receptors. The K_i value of XAC (29 μM) was similar to values for compounds **22–24**, indicating that rat A₃ receptors, unlike A₁ 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 A₃ receptors in comparison to the parent XCC, **26**. The enhancement of affinity by the 3-(3-iodo-4-aminobenzyl) group at A₁ and A_{2a} receptors was only 2–3-fold. Nevertheless, the affinity of **27** at rat A₃ receptors was much less than at sheep A₃ receptors,¹⁴ and it was not A₃ selective.

Lengthening the 1,3-dialkyl substituents of XCC was found to increase affinity somewhat at A_{2a} and A₃ receptors, but A₁ affinity was unaffected. The corresponding 1,3-dibutylxanthine carboxylic congener, **30**, displayed a K_i value at A₃ receptors of 28 μM, although the compound was still highly A₁ 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 A₃ receptors and a 13-fold loss of affinity at A₁ receptors.

A 2-thio vs 2-oxo substitution²⁸ enhanced A₃ affinity for 8-aryl xanthines as well as for 8-alkyl xanthines. The A₃ affinity of 2-thioXCC, **28** (K_i value of 6.8 μM), was 11-fold greater than the affinity of XCC, **26**.

The A₃ 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 A₁ and A₃ receptors and 5-fold more potent at A_{2a} receptors. Succinyl XAC, **32**,³⁰ although definitely not A₃ selective, was nearly equipotent to XAC at A₃ receptors and considerably less potent than XAC at A₁ and A_{2a} receptors, inconsistent with a proposed role of an anionic group in enhancing A₃ affinity as noted for sheep A₃ receptors.¹⁴ The A₁, A_{2a}, and A₃ 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 A₃ receptors. The affinity at A₁ 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 A₃ agonist-elicited adenylate cyclase inhibition in A₃-transfected CHO cells.¹² We examined the antagonist properties of several carboxylic acid derivatives in a functional assay at A₃

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 preincubation) 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 A₃ receptors. There is an uncommonly large species dependence of the affinity of xanthines at A₃ receptors. Very few of the compounds studied were actually A₃ selective in the rat. Compound **24** was 710-fold more potent at sheep A₃ receptors¹⁴ than at rat A₃ receptors. Compound **27** was 400-fold more potent at sheep A₃ receptors¹⁴ than at rat A₃ receptors. Certain xanthines were also found to be moderately potent in displacing radioligand from human A₃ receptors.¹⁵ Thus, it is to be expected that some of these xanthine derivatives are potentially selective for A₃ receptors in sheep and human tissue and possibly in other species.

A reported lead for enhancing A₃ affinity at sheep and human A₃ 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 A₃ receptors, although as previously observed²⁹ an anionic group tended to diminish potency at A₁ and A_{2a} receptors.

One may speculate on which amino acid residues on the A₃ receptor may interact with carboxylate group in sheep and human A₃ receptors. Presumably the residue should be a positively charged residue that is conserved between sheep and human A₃ receptor sequences but is absent in A₁ or A_{2a} receptors (across species) and in rat A₃ 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 Arg¹⁷³ residue (human) occurring approximately at the boundary of the fifth transmembrane helix and the second extracellular loop. The corresponding residues are Gly¹⁷⁵ in the rat A₃ receptor and Ser¹⁷⁶/Pro¹⁷³ in the human A₁/A_{2a} receptors. Site-directed mutagenesis and molecular modeling will be essential tools in locating the ligand binding site on the A₃ receptor and determining if Arg¹⁷³ plays a role. We recently proposed a molecular model for binding of nucleosides to A₃ 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 A₃ receptors.

Several of the more potent derivatives in this study (compounds **21** and **24**) were too weak to functionally antagonize the effects of an A₃ agonist. Compound **27**, BWA 522, was recently used as a weak antagonist *in vivo* at rat A₃ receptors.³¹ Our findings suggest that although considerable affinity for A₃ receptors is present (μM), it is still somewhat selective (32-fold) for A₁ receptors.

In conclusion, the development of A₃ receptor antagonists of general applicability across species remains a challenge. A₃ 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 A₁ antagonists of diverse structure indicated negligible affinity at rat A₃ 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 (NH₃) 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 purification. 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%): ¹H NMR (DMSO-*d*₆) δ 1.41–1.47 (m, 6 H, 2 $\times$ CH₃), 1.61–1.68 (m, 4 H, 2 $\times$ CH₂), 1.74–1.79 (m, 2 H, CH₂), 1.82–1.85 (m, 2 H, CH₂), 3.84 (t, *J* = 7.3 Hz, 2 H, N-CH₂), 3.98 (t, *J* = 7.3 Hz, 2 H, N-CH₂), 8.10 (s, 1 H, H-8), 13.7 (br s, 1 H, exchanged with D₂O, 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 MgSO₄, filtered, and concentrated to give a brown solid which was purified on silica gel column chromatography (CHCl₃–MeOH, 50:1 $\rightarrow$ 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: ¹H NMR (DMSO-*d*₆) δ 0.89 (t, *J* = 7.3 Hz, 3 H, CH₃), 1.23–1.68 (m, 4 H, CH₂CH₂), 3.88, 4.00 [2 $\times$ t, *J* = 7.3 Hz, 2 H (2.2:1), N-CH₂], 5.08, 5.18 [2 $\times$ s, 2 H (1:2.4), N-CH₂Ph], 7.20–7.35 (m, 5 H, Ph), 8.02, 8.04 [2 $\times$ 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**: ^1H 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, $\text{N}-\text{CH}_2$), 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-(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: ^1H NMR ($\text{DMSO}-d_6$) 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)xanthine²² (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: ^1H NMR ($\text{DMSO}-d_6$) δ 1.60 (br s, 4 H, $2 \times \text{CH}_2$), 2.35 (br s, 4 H, $2 \times \text{CH}_2$), 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 $^\circ\text{C}$ 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%): ^1H NMR ($\text{DMSO}-d_6$) δ 1.59 (m, 4 H, $2 \times \text{CH}_2$), 2.25 and 2.34 (m, 4 H, $2 \times \text{CH}_2$), 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%): ^1H NMR ($\text{DMSO}-d_6$) δ 0.87–0.93 (m, 6 H, $2 \times \text{CH}_3$), 1.26–1.34 (m, 4 H, $2 \times \text{CH}_2$), 1.51–1.66 (m, 4 H, $2 \times \text{CH}_2$), 3.88 (pseudo t, J = 7.4 and 7.2 Hz, 2 H, $\text{N}-\text{CH}_2$), 4.00 (pseudo t, J = 7.2 and 7.0 Hz, 2 H, $\text{N}-\text{CH}_2$), 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 $^\circ\text{C}$ for 30 min, ice water was added to precipitate the product (77 mg, 91%): ^1H NMR ($\text{DMSO}-d_6$) δ 0.87–0.93 (m, 6 H, $2 \times \text{CH}_3$), 1.25–1.34 (m, 4 H, $2 \times \text{CH}_2$), 1.49–1.66 (m, 4 H, $2 \times \text{CH}_2$), 3.34 (s, 3 H, COOCH_3), 3.87 (pseudo t, J = 7.4 and 6.9 Hz, 2 H, $\text{N}-\text{CH}_2$), 3.99 (pseudo t, J =

7.2 and 6.9 Hz, 2 H, N-CH₂), 4.04 (s, 3 H, N₇-CH₃), 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%): ¹H NMR (DMSO-*d*₆) δ 0.90 (m, 6 H, 2 × CH₃), 1.30 (m, 4 H, 2 × CH₂), 1.54 (m, 4 H, 2 × CH₂), 1.66 (m, 4 H, -CH₂CH₂), 3.87 (t, 2 H, N-CH₂), 4.00 (t, 2 H, CH₂), 6.76 and 7.54 (each: d, J = 15.8 Hz, 1 H, vinyl).

4-(1,3-Dibutylxanth-8-yl)propionic Acid (17)

An aliquot (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): ¹H NMR (DMSO-*d*₆) δ 0.89 (m, 6 H, 2 × CH₃), 1.27 (m, 4 H, 2 × CH₂), 1.50 and 1.63 (each: m, 2 H, CH₂), 2.71 (pseudo t, J = 7.7 and 6.8 Hz, 2 H, CH₂), 2.91 (pseudo t, J = 7.2 and 7.0 Hz, 2 H, CH₂), 3.86 (pseudo t, J = 7.4 and 6.9 Hz, 2 H, N-CH₂), 3.95 (t, J = 7.2 Hz, 3 H, CH₂).

4-(1,3-Dibutylxanth-8-yl)butyric acid (18)

was prepared by a similar procedure using glutaric anhydride: ¹H NMR (DMSO-*d*₆) δ 0.89 (pseudo t, J = 7.3 and 7.2 Hz, 6 H, 2 × CH₃), 1.24–1.31 (m, 4 H, 2 × CH₂), 1.50 and 1.62 (each: m, 2 H, CH₂), 1.90 (m, 2 H, aliphatic CH₂), 2.27 (t, J = 7.3 Hz, 2 H, CH₂), 2.71 (pseudo t, J = 7.4 and 7.3 Hz, 2 H, CH₂), 3.86 (t, J = 7.3 Hz, 2 H, N-CH₂), 3.96 (t, J = 7.3 and 6.8 Hz, 2 H, CH₂). 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 NH₄OH, filtering, neutralizing with formic acid, and crystallizing with water: ¹H NMR (DMSO-*d*₆) δ 0.89 (m, 6 H, 2 × CH₃), 1.28 (m, 4 H, 2 × CH₂), 1.51 (m, 4 H, 2 × CH₂), 1.65 (m, 4 H, CH₂CH₂), 2.23 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, CH₂), 2.28 (pseudo t, J = 7.3 and 7.2 Hz, 2 H, CH₂), 3.86 (pseudo t, J = 7.5 and 7.2 Hz, 2 H, N-CH₂), 3.96 (pseudo t, J = 7.2 and 7.1 Hz, 2 H, CH₂), 11.98 (s, 1 H, exchanged with D₂O, COOH), 13.14 (s, 1 H, exchanged with D₂O, NH).

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, $\text{CH}_2\text{CH}_2\text{CH}_2$), 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 , COOH), 13.20 (s, 1 H, exchanged with D_2O , NH).

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, $\text{CH}_2\text{CH}_2\text{-CH}_2$), 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 $\times$ s, 2 $\times$ 3 H, 2 $\times$ CH_3), 12.10 (s, 1 H, exchanged with D_2O , COOH), 13.60 (s, 1 H, exchanged with D_2O , NH).

[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 $\times$ CH_3), 1.24–1.36 (sextet, $J = 7.5$ Hz, 4 H, 2 $\times$ CH_2), 1.45–1.58 (m, 4 H, 2 $\times$ 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 , COOH). Anal. Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_4\text{O}_5$: 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, 7.0 mmol) 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 $\times$ CH_3), 1.26–1.36 (sextet, $J = 7.5$ Hz, 4 H, 2 $\times$ 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 , COOH), 13.61 (s, 1 H, exchanged with D_2O , NH).

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 $\times$ CH_3), 1.60 and 1.72 (each: m, 2 H, 2 $\times$ 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 (pseudo 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 μ mol) 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, $2 \times \text{CH}_3$), 1.58 and 1.74 (each: m, 2 H, CH_2), 3.21 and 3.29 (m, 8 H, COCH_2 , $\text{CH}_2\text{-COOH}$, 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 μ mol) was dissolved in DMF and treated with succinic anhydride (10 mg, 100 μ mol) and diisopropylethylamine (10 μ L) 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, $2 \times \text{CH}_3$), 1.57 and 1.74 (each: m, 2 H, CH_2), 2.42 [m, 4 H, $\text{HO}_2\text{C}(\text{CH}_2)_2\text{CO}$], 3.23 and 3.59 (m, 4 H, $2 \times \text{NCH}_2$), 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, $R_f = 0.75$ ($\text{CHCl}_3\text{-MeOH}$, 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 ($2 \times$ br s, $2 \times$ 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 $90.1\text{--}92.4^\circ\text{C}$; ^1H NMR (DMSO- d_6) δ 1.72–1.93 (m, 6 H, $2 \times \text{CH}_3$), 1.18–1.33 (m, 4 H, $2 \times \text{CH}_2$), 1.39–1.52 (m, 4 H, $2 \times \text{CH}_2$), 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; ^1H 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 (9.3 g, 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; ^1H NMR (DMSO- d_6) δ 3.31 (s, 3 H, CH_3), 3.52 (s, 3 H, CH_3), 5.03 (s, 1 H, $\text{CH}=\text{C}$), 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; ^1H NMR (CDCl_3) δ 0.97–1.01 (m, 6 H, $2 \times \text{CH}_3$), 1.36–1.49 (m, 4 H, $2 \times \text{CH}_2$), 1.62–1.74 (m, 4 H, $2 \times \text{CH}_2$), 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 $\text{C}_{12}\text{H}_{20}\text{N}_4\text{O}_3$: 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-amino-1-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; ^1H 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 ($2 \times$ s, 2 H, $\text{N-CH}_2\text{Ph}$), 7.22–7.40 (m, 5 H, Ph), 9.70, 13.20 ($2 \times$ 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; ^1H NMR (DMSO- d_6) δ 3.71, 3.74 ($2 \times$ s, 2×3 H, $2 \times \text{CH}_3$), 9.11, 12.88 ($2 \times$ br s, s $\times$ 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; ^1H 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-NH $_3$) showed a peak at 373 corresponding to $M + 1 + \text{NH}_3$. Anal. Calcd for $\text{C}_{19}\text{H}_{17}\text{N}_1\text{O}_6$: 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 $\text{C}_{19}\text{H}_{15}\text{NO}_5 \cdot 0.8\text{H}_2\text{O}$: 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 [^3H]PIA was from Amersham (Arlington Heights, IL), and [^3H]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 receptor⁶ were grown in F-12 medium containing 10% FBS and penicillin/streptomycin (100 units/mL and 100 $\mu\text{g}/\text{mL}$, 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 $\times$ 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 43000g 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 μ M NECA. K_i values were calculated according to Cheng–Prusoff,³² assuming a K_d for [¹²⁵I]AB-MECA of 1.48 nM.²⁸

Binding of [³H]PIA to A₁ receptors from rat brain membranes and of [³H]CGS 21680 to A₂ receptors from rat striatal membranes was performed as described previously.^{17,18} Rat cerebral cortical membranes and striatal membranes were prepared¹⁸ 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 IC₅₀ of each compound, were used. IC₅₀ 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 value¹² of 1.0 and 15.5 nM for [³H]PIA and [³H]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	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
EDAC	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
Fmoc	9-fluorenylmethyloxycarbonyl
NECA	5'-(<i>N</i> -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

References

1. Jacobson KA, van Galen PJM, Williams M. Perspective, Adenosine receptors: pharmacology, structure-activity relationships and therapeutic potential. *J. Med. Chem.* 1992; 35:407–422. [PubMed: 1738138]
2. Libert F, Schiffmann SN, Lefort A, Parmentier M, Gerard C, Dumont JE, Vanderhaeghen J-J, Vassart G. The orphan receptor cDNA RDC7 encodes an A1 adenosine receptor. *EMBO J.* 1991; 10:1677–1682. [PubMed: 1646713]
3. Maenhaut C, Sande Jv, Libert F, Abramowicz M, Parmentier M, Vanderhaeghen JJ, Dumont JE, Vassart G, Schiffmann S. RDC8 codes for an adenosine A2 receptor with physiological constitutive activity. *Biochem. Biophys. Res. Commun.* 1990; 173:1169–1178. [PubMed: 2125216]
4. Stiles GL. Adenosine receptors. *J. Biol. Chem.* 1992; 267:6451–6454. [PubMed: 1551861]
5. Ali H, Cunha-Melo JR, Saul WF, Beavan MF. Activation of phospholipase C via adenosine receptors provides synergistic signals for secretion in antigen stimulated RBL-2H3 cells. *J. Biol. Chem.* 1990; 265:745–753. [PubMed: 2295618]

6. Zhou QY, Li CY, Olah ME, Johnson RA, Stiles GL, Civelli O. Molecular cloning and characterization of an adenosine receptor - the A₃ adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* 1992; 89:7432–7436. [PubMed: 1323836]
7. Meyerhof W, Müller-Brechlin R, Richter D. Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermiogenesis. *FEBS Lett.* 1991; 284:155–160. [PubMed: 1647979]
8. Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃AR is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J. Biol. Chem.* 1993; 268:168871–166890.
9. Jacobson KA, Nikodijevi O, Shi D, Gallo-Rodriguez C, Olah ME, Stiles GL, Daly JW. A role for central A₃-adenosine receptors: Mediation of behavioral depressant effects. *FEBS Lett.* 1993; 336:57–60. [PubMed: 8262217]
10. von Lubitz DKJE, Lin RC-S, Popik P, Carter MF, Jacobson KA. Adenosine A₃ receptor stimulation and cerebral ischemia. *Eur. J. Pharmacol.* in press.
11. Liu GS, Richards RA, Olsson RA, Mullane K, Walsh RS, Downey JM. Evidence that the adenosine A₃ receptor can mediate preconditioning's protection in isolated rabbit hearts. *Cardiovasc. Res.* 1994; 28:1057–1061. [PubMed: 7954592]
12. (a) van Galen PJM, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, IJzerman AP, Stiles GL, Jacobson KA. A binding site model and structure-activity relationships for the rat A₃ adenosine receptor. *Mol. Pharmacol.* 1994; 45:1101–1111. [PubMed: 8022403] van Bergen, A.; van Galen, PJM.; Stiles, GL.; Jacobson, KA. A₃ receptors: Structure activity relationships and molecular modeling. ACS 206th National Meeting; August 1993; Chicago, IL. Abstract MEDI217.
13. Gallo-Rodriguez C, Ji X-D, Melman N, Siegman BD, Sanders LH, Orlina J, Pu Q-L, Olah ME, van Galen PJM, Stiles GL, Jacobson KA. Structure-activity relationships of N⁶-benzyladenosine-5'-uronamides as A₃-selective adenosine agonists. *J. Med. Chem.* 1994; 37:636–646. [PubMed: 8126704]
14. Linden J, Taylor HE, Robeva AS, Tucker AL, Stehle JH, Rivkees SA, Fink JS, Reppert SM. Molecular cloning and functional expression of a sheep A₃ adenosine receptor with widespread tissue distribution. *Mol. Pharmacol.* 1993; 44:524–532. [PubMed: 8396714]
15. Salvatore CA, Jacobson MA, Taylor HE, Linden J, Johnson RG. Molecular cloning and characterization of the human A₃ adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* 1993; 90:10365–10369. [PubMed: 8234299]
16. Olah ME, Gallo-Rodriguez C, Jacobson KA, Stiles GL. [¹²⁵I]AB-MECA, a high affinity radioligand for the rat A₃ adenosine receptor. *Mol. Pharmacol.* 1994; 45:978–982. [PubMed: 8190112]
17. Schwabe U, Trost T. Characterization of adenosine receptors in rat brain by (–)[³H]N⁶-phenylisopropyladenosine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1980; 313:179–187.
18. Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills MA, Williams M. [³H]CGS 21680, a selective A₂ adenosine receptor agonist directly labels A₂ receptors in rat brain. *J. Pharmacol. Exp. Ther.* 1989; 251:888–893. [PubMed: 2600819]
19. Jacobson KA, Kirk KL, Padgett WL, Daly JW. Functionalized congeners of 1,3-dialylxanthines: preparation of analogues with high affinity for adenosine receptors. *J. Med. Chem.* 1986; 28:1334–1340. [PubMed: 2993622]
20. Jacobson KA, Ukena D, Daly JW, Kirk KL. Synthesis of tritiated functionalized congeners of 1,3-dipropylxanthine having high affinity at adenosine receptors. *J. Lab. Comp. Radiopharm.* 1985; 23(5):519–526.
21. Jacobson KA, Nikodijevi O, Padgett W, Gallo-Rodriguez C, Maillard M, Daly JW. 8-(3-Chlorostyryl)caffeine is a selective A₂-adenosine antagonist *in vitro* and *in vivo*. *FEBS Lett.* 1993; 323:141–144. [PubMed: 8495727]
22. Jacobson KA, Gallo-Rodriguez C, Melman N, Fischer B, Maillard M, van Bergen A, van Gallen PJM, Karton Y. Structure-activity relationships of 8-styrylxanthines as A₂-selective adenosine antagonists. *J. Med. Chem.* 1993; 36:1333–1342. [PubMed: 8496902]

23. Nonaka Y, Shimada J, Nonaka H, Koike N, Aoki N, Kobayashi H, Kase H, Yamaguchi K, Suzuki F. Photoisomerization of a potent and selective adenosine A₂ antagonist, (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine. *J. Med. Chem.* 1993; 36:3731–3733. [PubMed: 8246243]
24. Jacobson KA, Shi D, Gallo-Rodriguez C, Manning M, Müller C, Daly JW, Neumeyer JL, Kiriasis L, Pfeleiderer W. Effect of trifluoromethyl - and other substituents on activity of xanthines at adenosine receptors. *J. Med. Chem.* 1993; 36:3937–3946. [PubMed: 8254622]
25. Shamim MT, Ukena D, Padgett WL, Hong O, Daly JW. 8-Aryl- and 8-cycloalkyl-1,3-dipropylxanthines: further potent and selective antagonists for A₁-adenosine receptors. *J. Med. Chem.* 1988; 31:613–617. [PubMed: 3346878]
26. Linden J. Structure and function of A₁-adenosine receptors. *FASEB. J.* 1991; 5:2668–2676. [PubMed: 1916091]
27. Linden J, Patel A, Earl CQ, Craig RH, Daluge SM. ¹²⁵I-labeled 8-phenylxanthine derivatives: antagonist radioligands for adenosine A₁ receptors. *J. Med. Chem.* 1988; 31:745–751. [PubMed: 3351850]
28. Jacobson KA, Kiriasis L, Barone S, Bradbury BJ, Kammula U, Campagne JM, Secunda S, Daly JW, Neumeyer JL, Pfeleiderer W. Sulfur-containing 1,3-dialkylxanthine derivatives as selective antagonists at A₁-adenosine receptors. *J. Med. Chem.* 1989; 32:1873–1879. [PubMed: 2754711]
29. Jacobson KA, Kirk KL, Padgett WL, Daly JW. A functionalized congener approach to adenosine receptor antagonists: amino acid conjugates of 1,3-dipropylxanthine. *Mol. Pharmacol.* 1986; 29:126–133. [PubMed: 3005825]
30. Jacobson KA, de la Cruz R, Schulick R, Kiriasis L, Padgett W, Pfeleiderer W, Kirk KL, Neumeyer JL, Daly JW. 8-Substituted xanthines as antagonists at A₁- and A₂-adenosine receptors. *Biochem. Pharmacol.* 1988; 37:3653–3661. [PubMed: 3178879]
31. Fozard JR, Hannon JP. BW-A522 blocks adenosine A₃ receptor-mediated hypotensive responses in the rat. *Eur. J. Pharmacol.* 1994; 252:R5–R6. [PubMed: 8157050]
32. Cheng YC, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC₅₀) of an enzyme reaction. *Biochem. Pharmacol.* 1973; 22:3099–3108. [PubMed: 4202581]
33. Papesch V, Schroeder EF. Synthesis of 1-mono- and 1,3-disubstituted 6-aminouracils. Diuretic activity. *J. Org. Chem.* 1951; 16:1879–1890.
34. Fozard JR, Carruthers AM. Adenosine A₃ receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat. *Br. J. Pharmacol.* 1993; 109:3–5. [PubMed: 8495245]

Scheme 1. Synthesis of 1,3-Dialkylxanthines^a^a Reagents: (a) $\text{NCCH}_2\text{CO}_2\text{H}$, 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.

40a, R = CH₃, X = O

40b, R = CH₃, X = S

40c, R = (CH₂)₃CH₃, X = O

41, R = (CH₂)₃CH₃, X = O, n = 2, R = H

42, R = (CH₂)₃CH₃, X = O, n = 3, R = H

43, R = (CH₂)₃CH₃, X = O, n = 4, R = CH₃

44, R = CH₃, X = S, n = 3, R = H

17, R = (CH₂)₃CH₃, X = O, n = 2

18, R = (CH₂)₃CH₃, X = O, n = 3

19, R = (CH₂)₃CH₃, X = O, n = 4

20, R = CH₃, X = O, n = 3

21, R = CH₃, X = S, n = 3

Scheme 2.

Synthesis of 8-(Carboxyalkyl)-Substituted Xanthines

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

Scheme 4.

Synthesis of an Intermediate for Carboxylate Derivatization of Xanthines^a^a Reagents: (a) Fmoc-Cl; (b) trifluoroacetic acid; (c) XAC; (d) Et₂NH; (e) succinic anhydride.

Table 1

Affinities of Xanthine Derivatives in Radioligand Binding Assays at Rat Brain A₁, A_{2a}, and A₃ Receptors^{a-d} with Ratios of Selectivity for A₁ or A₂ Receptors vs A₃ Receptors

compd	R ₁ , R ₃ ^e	R ₈ ^e	R ₇	K _i (μM) or % inhibition ^d				K _i A ₃ / K _i A ₁	K _i A ₃ / K _i A _{2a}
				A ₁ ^a	A _{2a} ^b	A ₃ ^c			
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	0.34	5.29 ± 0.79		12000	16
9	CH ₃	CH=CH- <i>m</i> -φCl ^e	CH ₃	[28.2] ^h	0.00908 ± 0.00162 ^f	4.2% (10 ⁻⁵) ^f		>500	
					[0.054] ^h				
10	CH ₃	CH=CH- <i>m</i> -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- <i>m</i> -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- <i>m</i> -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

compd	R ₁ , R ₃ ^e	R ₈ ^e	R ₇	K _i (μM) or % inhibition ^d			K _i A ₃ / K _i A ₁	K _i A ₃ / K _i A _{2a}
				A ₁ ^a	A _{2a} ^b	A ₃ ^c		
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	CH ₃	(CH ₂) ₃ COOH	H	5.70 ± 0.55	67.8 ± 4.0	9.36 ± 0.3	1.6	0.14

^aDisplacement of specific [³H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in μM (n = 3–5).

^bDisplacement of specific [³H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± SEM in μM (n = 3–6).

^cDisplacement of specific [¹²⁵I]AB-MECA binding, unless noted, in membranes of CHO cells stably transfected with the rat A₃-cDNA, expressed as K_i ± SEM in μM (n = 3.5).

^dA percent value indicates the percent displacement of radioligand at the concentration (M) given in parentheses.

^eR₁ = R₃ and X = O, unless noted; a CH=CH group, when present in the R₈ substituent, is always *trans*.

^fV_{max} values from van Galen et al.¹² A₃ affinity vs [¹²⁵I]APNEA.

^gThe assay was carried out in dark.

^hV_{max} values from Jacobson et al.²². The binding assay was carried out under fluorescent room light.

ⁱV_{max} values from Jacobson et al.²⁴

^jX = S (compound **21**). nd: not determined.

Table 2

Affinities of Xanthine Derivatives in Radioligand Binding Assays at Rat Brain A₁, A_{2a}, and A₃ Receptors^{a-d} with Ratios of Selectivity for A₁ or A₂ Receptors vs A₃ Receptors

compd	R ₁ , R ₃ ^e	R ₄ ^e	A ₁ ^a	K _i (μM) or % inhibition ^d			K _i A ₃ /K _i A ₁	K _i A ₃ /K _i A _{2a}
				A _{2a} ^b	A ₃ ^c			
22 ^g	CH ₃ (CH ₂) ₂	SO ₃ H	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 ₂ COOH	0.756 ± 0.147	4.29 ± 0.57	173 ± 18		230	40
26	CH ₃ (CH ₂) ₂	OCH ₂ COOH	0.058 ^k	2.20 ^k	75.7 ± 6.5		1300	34
27	1-CH ₃ (CH ₂) ₂ 3-CH ₂ (3-14-NH ₂) ₄	OCH ₂ COOH	0.037 ^{l,h}	0.7 ^f	1.17 ± 0.18		32	2
28 ^j	CH ₃ (CH ₂) ₂	OCH ₂ COOH	0.0538 ^g	0.226 ± 0.011	6.77 ± 0.011		126	30
29	CH ₃ (CH ₂) ₂	OCH ₂ CONHCH ₂ COOH	0.073 ^j	0.455 ± 0.086	58.5 ± 5.9		800	130
30	CH ₃ (CH ₂) ₃	OCH ₂ COOH	0.0431 ± 0.0099	0.874 ± 0.107	27.5 ± 2.5		640	31
31	CH ₃ (CH ₂) ₂	OCH ₂ CONH(CH ₂) ₂ NH ₂	0.0012 ^j	0.063	29.0 ± 7.0		24000	460
32	CH ₃ (CH ₂) ₂	OCH ₂ CONH(CH ₂) ₂ NHCO(CH ₂) ₂ COOH	0.078 ^k	0.252 ± 0.041	40.6 ± 1.7		520	210
33	CH ₃ (CH ₂) ₂	OCH ₂ CO-NH(CH ₂) ₂ NHCOOCH ₂ N(Fmoc)CH ₂ COOH	0.0191 ± 0.0068	0.33 ± 0.10	21.7 ± 0.8		1100	66
34	CH ₃ (CH ₂) ₂	OCH ₂ CONH(CH ₂) ₂ NHCOCH ₂ NHCH ₂ COOH	0.0360 ± 0.0101	0.157 ± 0.026	98.0 ± 6.8		2700	620
35	CH ₃ (CH ₂) ₂	OCH ₂ CONH(CH ₂) ₂ NHCOCH ₂ N(CH ₂ COOH) COCH ₂ CH ₂ COOH	2.86 ± 1.00	9.22 ± 1.11	99.9 ± 12.2		35	1

^a Displacement of specific [³H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in μM (n = 3–5).

- b* Displacement of specific [³H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as $K_I \pm \text{SEM}$ in μM ($n = 3-6$).
- c* Displacement of specific [¹²⁵I]AB-MECA binding, unless noted, in membranes of CHO cells stably transfected with the rat A₃-cDNA, expressed as $K_I \pm \text{SEM}$ in μM ($n = 3-5$).
- d* *A* percent value indicates the percent displacement of radioligand at the concentration (M) given in parentheses.
- e* R₁ = R₃ and X = 0, unless noted; a CH=CH group, when present in the R₄ substituent, is always *trans*.
- f* Values from Linden et al.^{26,27} or Shamim et al.²⁵. A₂ values represent K_B for inhibition of NECA stimulated adenylylate cyclase platelet membranes.
- g* A₁/A_{2a} affinity from ref 25.
- h* A₁ affinity measured in binding assay vs [¹²⁵I]AB-MECA binding in rat brain membranes.
- i* X = S (compound **28**).
- j* Values from Jacobson et al.²⁹. A₁ affinity vs [³H]CHA.
- k* Values from Jacobson et al.^{28,30}. A_{2a} affinity vs [³H]NECA. nd: not determined.

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 ₄ O ₂	C, H, N
7	A	68	137–140	EI: 298 (M ⁺)	C ₁₆ H ₁₈ N ₄ O ₂	C, H, N
11	B	60	170–172	FAB: 426 (M ⁺ + 1)	C ₁₁ H ₁₄ N ₄ O ₄	<i>f</i>
12a^d	C	57	192–194	FAB: 454 (M ⁺ + 1)	C ₂₃ H ₂₇ N ₅ O ₅	C, H, N ^f
12b	C	66 ^e	159–160	FAB: 440 (M ⁺ + 1)	C ₂₂ H ₂₅ N ₅ O ₅ •0.5DMF	C, H, N ^f
14	B	32	260–263	335, 352	C ₁₆ H ₂₂ N ₄ O ₄ •0.5DMF	C, H, N
15	C	91	129–130	363	C ₁₇ H ₂₄ N ₄ O ₄	C, H, N
16	C	66	225–230	349	C ₁₇ H ₂₄ N ₄ O ₄ •0.5DMF	C, H, N
17	B	45	229–230	337 (M ⁺ + 1), 354 (M ⁺ + 18)	C ₁₆ H ₂₄ N ₄ O ₄	C, H, N
18	B	39	189–191	348, 365	C ₁₇ H ₂₆ N ₄ O ₄	C, H, N
19	B	19	225 dec	365, 382	C ₁₈ J ₂₈ N ₄ O ₄ •0.15NH ₄ OH	C, H, N
20	B	56	239–240	267 (M ⁺ + 1)	C ₁₁ H ₁₄ N ₄ O ₄	C, H, N
21	B	48	245–247 dec	283 (M ⁺ + 1), 300 (M ⁺ + 18)	C ₁₁ H ₁₄ N ₄ O ₅ S•1.18H ₂ O	C, H, N
30	D	57	325–330 dec	415 (M ⁺ + 1)	C ₂₁ H ₂₆ N ₄ O ₅ •0.5NH ₄ OH	C, H, N
33	D	78	172–173		C ₄₀ H ₄₃ N ₇ O ₉ •1.5H ₂ O	C, H, N
34	D	100	215–220 dec	544	C ₂₅ H ₃₃ N ₇ O ₇ •1.5H ₂ O	C, H, N
35	D	70	204–207 dec	FAB: 644 (M ⁺ + 1)	C ₂₃ H ₃₃ N ₈ O ₁₀	<i>f</i>

^aMethods: (A) synthesis as in Scheme 1; (B) synthesis as in Scheme 2; (C) refer to text; (D) refer to Scheme 3.

^bPercent yield calculated from 1,3-dialkyl-6-amino-5-nitrosouracil.

^cCI, unless noted.

^dMethyl ester of compound **12b**.

^eSaponification step.

^fHigh-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).