# Structure-Activity Relationships of 8-Styrylxanthines as $A_2$-Selective Adenosine Antagonists[†,§]

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A series of substituted 8-styryl derivatives of 1,3,7-alkylxanthines was synthesized as potential $A_2$-selective adenosine receptor antagonists, and the potency at rat brain $A_1$- and $A_2$-receptors was studied in radioligand binding experiments. At the xanthine 7-position, only small hydrophobic substituents were tolerated in receptor binding. 7-Methyl analogues were roughly 1 order of magnitude more selective for $A_2$ versus $A_1$ receptors than the corresponding 7-H analogues. 1,3-Dimethylxanthine derivatives tended to be more selective for $A_2$-receptors than the corresponding 1,3-diallyl, diethyl, or dipropyl derivatives. Substitutions of the phenyl ring at the 3-(monosubstituted) and 3,5-(disubstituted) positions were favored. 1,3,7-Trimethyl-8-(3-chlorostyryl)xanthine was a moderately potent ($K_i$ vs [$^3$H]CGS 21680 was 54 nM) and highly $A_2$-selective (520-fold) adenosine antagonist. 1,3,7-Trimethyl-8-[3-[(3-carboxy-1-oxopropyl)amino]styryl]xanthine was highly $A_2$-selective (250-fold) and of enhanced water solubility (max 19 mM). 1,3-Dipropyl-7-methyl-8-(3,5-dimethoxystyryl)xanthine was a potent ($K_i$ = 24 nM) and very $A_2$-selective (110-fold) adenosine antagonist.

## Introduction

Adenosine acting through the $A_2$-receptor subtype elicits a range of physiological responses, including the inhibition of platelet aggregation[1] dilation of blood vessels,[2] erythropoietin production,[3] and depression of locomotor activity.[4] The second messenger system invariably associated with the $A_2$-receptor is the stimulation of adenylate cyclase, while the $A_1$-receptor has been found to be coupled to a number of second messenger systems, including inhibition of adenylate cyclase, inhibition of calcium entry, stimulation of potassium flux, and effects on phosphoinositide metabolism.[5]

All of the adenosine agonists yet reported are derivatives of adenosine, but many classes of adenosine antagonists are known.[5] The earliest examples of adenosine antagonists are the naturally-occurring xanthines, e.g. caffeine and theophylline, which are nonselective for adenosine receptor subtypes and have phosphodiesterase inhibitory effects as well.[6] While thousands of ligands selective for $A_1$ receptors have been synthesized, it is only recently that $A_2$-selective agonists and antagonists have been reported.[2,7-11] Certain 2-substituted adenosine[2] and adenosine-5'-carboxamide derivatives[8] are $A_2$-selective agonists. Several xanthine derivatives, most notably, simple caffeine derivatives bearing a 1-propargyl group (1, Figure 1),[6,7] and those doubly substituted by 7-methyl and 8-cycloalkyl groups (2),[10,11] have been reported to be $A_2$-selective in binding and/or adenylate cyclase experiments. A number of non-xanthine derivatives have been found to be weakly selective for $A_2$-receptors, e.g. the triazoloquinazoline CGS 15943 (3),[12] the triazoloquinoxaline CP66,713 (4),[13] and a series of imidazotriazinones, including M216765 (5).[14]

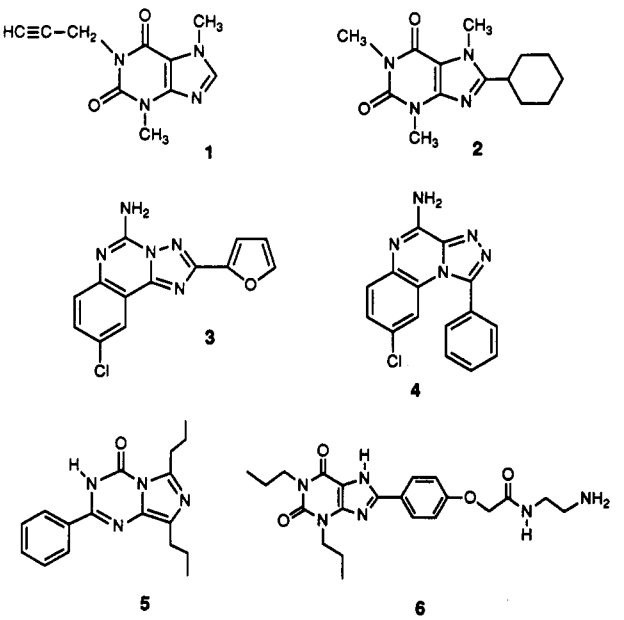


**Figure 1.** Xanthine and non-xanthine derivative having either high affinity or some selectivity for $A_2$-adenosine receptors.

It would be highly desirable to have a general, high affinity $A_2$-antagonist probe for receptor characterization. At this time, several 8-phenylxanthines, such as XAC, 6, may serve as radioligands at striatal $A_2$-receptors, but this high-affinity binding is limited to certain species in which XAC is not $A_1$-selective.[15] Shimada et al.[11] have reported that 1,3,7-alkylxanthine derivatives substituted with 8-styryl groups, particularly 3,4-dimethoxy and 3,4,5-trimethoxy, act as selective $A_2$-antagonists. In this study

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† Abbreviations: t-Boc, *tert*-butyloxycarbonyl; CGS 21680, 4-[2-[[6-amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl]-9*H*-purin-2-yl]amino]-ethyl]benzenepropanoic acid, DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; NECA, *N*-ethyladenosin-5'-uronamide; PIA, (*R*)-$N^6$-(2-phenylisopropyl)adenosine; Tris, tris(hydroxymethyl)aminomethane.

§ Dedicated to Prof. Murray Goodman on the occasion of his 65th birthday.

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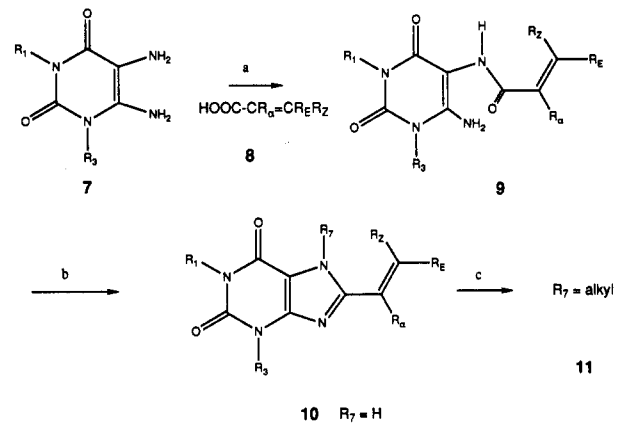



**Figure 2.** Synthesis of 8-styrylxanthine derivatives. Reagents: (a) EDAC, DMAP, imidazole; (b) NaOH, 80 °C; alkyl bromide, 50 °C.

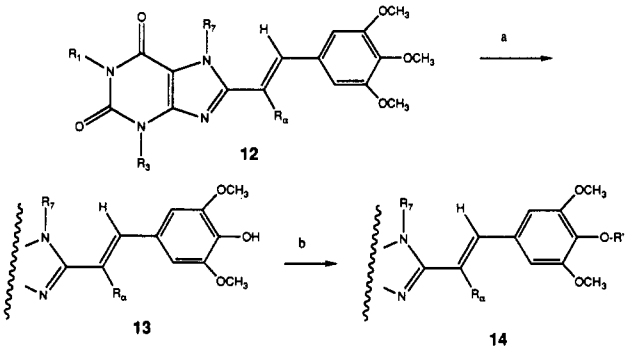



**Figure 3.** Synthesis of 8-(4-hydroxy-3,5-dimethoxystyryl)-xanthines and derivatives. Reagents: (a) $C_6H_5SNa$, 160 °C; (b) R′-Br.

we have further explored the structure–activity relationships of 8-styrylxanthines at adenosine receptors.

## Results

**Chemistry.** 8-Styrylxanthine derivatives, substituted at the 1-, 3-, and 7-xanthine positions and at the α- and phenyl positions of the styryl moiety were synthesized according to the routes shown in Figures 2 and 3. The structures of compounds **15–56** and the affinities in radioligand binding assays at rat brain $A_1$- and $A_2$-receptors are given in Table I. Small alkyl substituents at the 1- and 3-positions were identical and varied from methyl to propyl. Substituents at the 7-position varied from H to 2-phenylethyl. A number of related xanthines (not 8-styryl) were prepared for comparison (Table II). The physical characteristics of the xanthine derivatives are summarized in Table III.

A *trans*-cinnamic acid, **8** (Figure 2), was condensed with a 1,3-dialkyl-5,6-diaminouracil, **7**. The resulting amide, **9**, was cyclized under strongly basic conditions to give the xanthine derivative **10**. The intermediate cinnamic acids were obtained from commercial sources or prepared as described in the Experimental Section. After formation of the 7-H xanthine derivative **10**, methylation using methyl iodide at 50 °C was carried out. The *trans* orientation of the 8-styryl group was verified for each of the derivatives based on the proton–proton coupling constants of the olefinic protons (typically ≥15 Hz). In the case of compound **16** (α-fluoro), the fluorine–proton coupling constant was found to be 37 Hz, consistent with the *trans* configuration.[16]

Synthesis of hydroxyl ring-substituted 8-styrylxanthines was attempted by the usual route (Figure 2), starting with the 3- or 4-hydroxycinnamic acid. The intermediate amide was formed in low yield, and cyclization provided the desired xanthine in only very low yield (e.g. **18**). Carrying out the sequence with hydroxyl protection in order to obtain a free hydroxyl group in the para position of the final product was attempted, but proved unsatisfactory. Acetyl ester and *p*-methoxybenzyl ether derivatives formed the amide intermediate **9**, but the cyclization step in 4 N NaOH failed. The attempted deprotection of monomethoxy derivatives in the series using sodium thiophenolate, trimethylsilyl iodide, or nitrogen bases at high temperature was unsuccessful. It was however possible to selectively demethylate the *p*-methoxy of 8-(3,4,5-trimethoxy)styrylxanthines, **12** (Figure 3), using sodium thiophenolate in DMF at 160 °C. The position of the free hydroxyl group (4) in **13** was determined by proton NMR. This hydroxyl group could be readily acylated or alkylated (in some cases carried out in situ following the deprotection reaction) to provide **14**.

Aryl amino substituents were obtained via Zn/HOAc reduction of the corresponding nitro derivative (e.g. **21**) or in the case of tertiary aniline (e.g. **30a**), by direct incorporation of the corresponding cinnamic acid. The N-7 position of **30a** was selectively alkylated using methyl iodide at 50 °C to provide **30b**. Catalytic hydrogenation of the nitrostyryl derivative **21** afforded the saturated aniline analogue **57**.

**Biological Evaluation. Binding assay.** The analogs were tested in a radioligand binding assay for affinity at adenosine receptors in rat brain membranes. The compounds were assayed for affinity at rat $A_1$ cortical receptors using [$^3$H]-$N^6$-(2-phenylisopropyl)adenosine[17] and at rat striatal $A_{2a}$ receptors using [$^3$H]CGS 21680 (Tables I and II).[8] $K_i$ values of nearly $10^{-8}$ M at $A_2$-receptors and selectivities of hundreds of fold were achieved.

Several sites on the xanthine molecule have been varied. The N-1 and N-3 positions have been substituted with small alkyl groups ($C_1$–$C_3$). In all cases, substituents at the 1- and 3-positions are identical. The greatest effect of elongating NMe to NPr groups at these positions was a substantial increase in $A_1$-affinity, thus diminishing $A_2$-selectivity. A 1,3-diethyl-7-methylxanthine, **45b**, was nearly as $A_2$-selective (44-fold) as the 1,3-dimethyl analogue, **44b**, which was 70-fold selective. The corresponding diallyl analogue, **46** (reported previously[11] to be >6700 $A_2$-selective), was only 13-fold selective in rat brain in this study.

The N-7 position is either unsubstituted or substituted with groups as large as 2-phenylethyl (compound **44f**). Only small, hydrophobic groups (including ethyl and propargyl) at this position were tolerated in binding to either receptor. The 7-methyl analogues were found to exhibit the greatest degree of $A_2$-selectivity. Figures 4 and 5 show correlations of affinity for the 7-H to 7-Me modification, which generally results in decreased $A_1$-affinity and increased $A_2$-affinity. In general, among 8-styrylxanthine derivatives, the 1,3,7-trimethylxanthines were $A_2$-selective by factors between 10- and 500-fold, whereas the corresponding 1,3-dimethylxanthines were generally $A_2$-selective by factors of only 2–6-fold. The 7-(hydroxyethyl) and phenylethyl substituents (**45d** and **45f**) were nearly inactive, in addition to having less favorable aqueous solubility. In the 1,3-dipropyl series (Figure 5), each 7-H analogue was relatively nonselective. The selectivity of the 1,3-dipropyl-7-methyl-8-styrylxan-

thines (resulting from decreased $A_1$-affinity upon methylation) was highly dependent on the styryl substitution.

The effects of substitution of the 8-styryl group could be compared within the 1,3-dimethyl series and within the 1,3,7-trimethyl series. The unsubstituted styryl analogue **15a** (7-H) was nonselective, but was moderately selective (41-fold) following methylation (**15b**). Fluorine substitution in the $\alpha$-position resulted in diminished potency at both $A_1$- (3-fold) and $A_2$-receptors (7-fold). Monomethoxy substitution of the phenyl ring (compounds **17**, **19**, and **29**) resulted in selectivity of 18–63-fold in the 7-Me series, but did not result in significant $A_2$-selectivity in the 7-H series. Compound **19b**, the meta derivative, was the most potent and selective monomethoxy derivative, with a $K_i$ value of 85 nM at $A_2$-receptors. The analogue bearing a 3-hydroxystyryl group in the 7-H series, **18**, was equipotent with the methoxy compound **19a** at $A_2$-receptors and more potent at $A_1$-receptors.

The $A_2$-potency of 1,3,7-trimethylxanthines having a variety of styryl 3-position substituents varied in the order: acetylamino > chloro, amino > fluoro, methoxy > H > trifluoromethyl > nitro. Although the 3-chloro derivative (**28**, $K_i$ value of 54 nM) was slightly less potent than the 3-acetylamino derivative (**23**, $K_i$ value of 39 nM, 240-fold selective) it was more selective (520-fold). It was equipotent to the amino derivative **22b**, but considerably more selective. Very bulky substituents at the 3-position (urethanes **25** and **26**) reduced potency at $A_2$-receptors roughly 20-fold, but moderate $A_2$-selectivity remained. A water-solubilizing 3-[(3-carboxy-1-oxopropyl)amino] group (**24**) resulted in decreased potency (134 nM) but high selectivity (250-fold).

For comparison to the methoxy group at the styryl 4-position, a highly electron-donating group, e.g. dimethylamino, was incorporated and resulted in greatly diminished potency at both receptors. Only the 7-Me form, **30b**, displayed $A_2$-selectivity.

Dimethoxy substitutions at various positions of the phenyl ring were compared, and substantial differences were observed. The order of both potency and selectivity was 3,5 > 3,4 > 2,3. In the 1,3,7-trimethyl series, 3,5-dimethoxy or 3,5-difluoro substituents (**33b** and **34b**, respectively) resulted in >200-fold selectivity.

In the 1,3-dipropyl-7-methyl series, $A_2$-selectivity was generally merely 5–19-fold, with only one exception (**53b**). The 3-chlorostyryl analogue **51b**, analogous to the most selective agent in the 1,3,7-trimethyl series, was only 14-fold selective. 1,3-Dipropyl-7-methyl-8-(3,5-dimethoxystyryl)xanthine, **53b**, proved to be a potent ($K_i$ vs [$^3$H]CGS 21680 was 24 nM) and $A_2$-selective (110-fold) adenosine antagonist, i.e. 5-fold more selective than the corresponding 3,4-dimethoxy analogue, **52b**. Compound **52b** was prepared by Shimada et al. [KF17837][11] and was reported to be 190-fold selective, versus 19-fold in this study.

High selectivities were also observed among 1,3,7-trimethylxanthines that were trisubstituted on the phenyl ring. 1,3,7-Trimethyl-8-(3,4,5-trimethoxy)styrylxanthine, **44b**, was 70-fold $A_2$-selective in binding in the rat brain (vs >5600-fold reported by Shimada et al.[11]). The corresponding 1,3-dimethyl analogue **44a**was only 6-fold $A_2$-selective. In general, the order of both potency and selectivity for trisubstituted phenyl substituents was 3,4,5 > 2,3,4 > 2,4,5. Among 3,4,5-substituted analogues there was considerable substitution of the 4-methoxy group tolerated at $A_2$-receptors. The moderately selective 3,5-dimethoxy-4-hydroxy analogue, **35**, was acylated (**36**) and alkylated (**37**, **38**), resulting in enhanced $A_2$-selectivity and potency. The 4-acetoxy-3,5-dimethoxy analogue, **36**, was 93-fold $A_2$-selective. Functional groups that also tended to increase water solubility, such as alkylamines (**38** and **40**), were included. These amino derivatives may serve as functionalized congeners[20] since it appears that long chain extension is possible without disrupting receptor binding. Moderately potent and selective acylated derivatives (**39**, **41**, and **42**) were prepared from the amine functionalized congeners. Butyl versus *trans*-butenylamine congeners were compared to examine the effect of altering conformational flexibility at this distal site. No major differences in potency or selectivity between butyl (e.g. **38**) and butenyl (e.g. **40**) analogues were found.

In an attempt to account for the discrepancy in $K_i$ values between the present study and Shimada et al.[11], the effects of varying concentrations of DMSO in the assay medium were examined. DMSO was needed because of the limited aqueous solubility (in the range of $10^{-5}$ M) of most of the 8-styrylxanthines tested. To avoid precipitation associated with serial aqueous dilutions, the only point at which xanthine dissolved in DMSO was added to aqueous medium was directly into the incubation tube. We examined the effects of varying concentrations of DMSO (ranging from 0.5 to 6%) on the apparent affinity of compound **53b** (Figure 6A). The apparent affinity of compound **53b** at $A_2$ receptors was constant within the range of 0.5–6% DMSO. In addition, the total specific binding of [$^3$H]CGS 21680 to striatal membranes was maintained, even at 6% DMSO. However, $A_1$-affinity appeared to be somewhat dependent on DMSO concentration (at 0.5 and 1% DMSO), and at 6% DMSO the total specific binding of [$^3$H]PIA (data not shown) diminished to roughly 30% of its value at 1% DMSO. At the lowest concentration (0.5% DMSO), higher concentrations of the drug were required to displace [$^3$H]PIA. This effect of increase in the apparent $K_i$ value at ≤1% DMSO most likely relates to the xanthine precipitating from the solution, since the UV absorption (Figure 6B) does not increase in a linear fashion with the amount of xanthine added to a fixed aqueous volume. The UV absorption decreases beyond 20 $\mu$M, suggesting supersaturation.

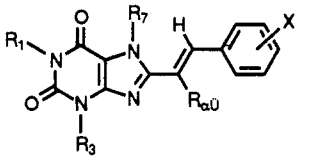
Related, non-styrylxanthines (Table II) were tested in adenosine receptor binding for comparison to the 8-styryl derivatives. Cyclohexylcaffeine, **2**, which was found to be$A_2$-selective in effects on adenylate cyclase[10] was nonselective in binding. The saturated aniline derivative **57** was ~300-fold less potent at $A_2$ receptors than the corresponding styryl derivative **22b**. Ring-constrained styryl analogues, **58**, containing a 8-(2-benzofuran) group were synthesized. Both the 7-H and 7-Me analogues were only weak antagonists of binding at adenosine receptors (Table II).

## Discussion

Potent and $A_2$-selective adenosine antagonists, suitable as pharmacological tools, have long been lacking. $A_2$-selective antagonists may also have application as therapeutic agents, e.g. in the treatment of Parkinson's disease.[24] The nonselective non-xanthine antagonist CGS 15943, **3**, was under development as an antiasthmatic.[25]

It was only recently that 8-styrylxanthines were reported as the first potentially useful compounds by Shimada et

**Table I.** Affinities of 8-Styrylxanthine Derivatives in Radioligand Binding Assays at Rat Brain $A_1$- and $A_2$-Receptors[a]



$R_1 = R_3$ and $R_\alpha$ = H unless noted

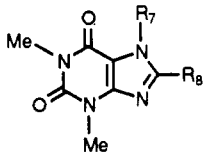
| compd | $R_1$, $R_3$ | $R_7$ | X | $K_i$[a] $A_1$ | $K_i$[a] $A_2$ | $A_1/A_2$ ratio |
|---|---|---|---|---|---|---|
| **15a** | Me | H | H | 654 ± 170 | 291 ± 40 | 2.3 |
| **15b** | Me | Me | H | 3890 ± 1150 | 94 ± 36 | 41 |
| **16**[e] | Me | H | H | 2190 ± 400 | 2110 ± 810 | 1.4 |
| **17a** | Me | H | 2-MeO | 1730 ± 420 | 645 ± 144 | 2.7 |
| **17b** | Me | Me | 2-MeO | 4760 ± 720 | 267 ± 84 | 18 |
| **18** | Me | H | 3-OH | 702 ± 40 | 303 ± 55 | 2.4 |
| **19a** | Me | H | 3-MeO | 1830 ± 821 | 378 ± 155 | 4.8 |
| **19b** | Me | Me | 3-MeO | 5430 ± 1470 | 84.8 ± 24.0 | 64 |
| **20a** | Me | H | 3-$CF_3$ | 881 ± 251 | 343 ± 58 | 2.6 |
| **20b** | Me | Me | 3-$CF_3$ | 3330 ± 410 | 134 ± 44 | 25 |
| **21a** | Me | H | 3-$NO_2$ | 1060 ± 150 | 438 ± 106 | 2.4 |
| **21b** | Me | Me | 3-$NO_2$ | 2140 ± 480 | 195 ± 44 | 11 |
| **22a** | Me | H | 3-$NH_2$ | 288 ± 60 | 202 ± 79 | 1.4 |
| **22b** | Me | Me | 3-$NH_2$ | 1690 ± 360 | 57 ± 3 | 30 |
| **23** | Me | Me | 3-(AcNH) | 9470 ± 2540 | 39 ± 21 | 240 |
| **24** | Me | Me | 3-($HOOC(CH_2)_2CONH$) | 35100 ± 11700 | 143 ± 45 | 250 |
| **25** | Me | Me | 3-(*t*-BOC-NH) | 23600 ± 2500 | 784 ± 100 | 30 |
| **26** | Me | Me | 3-[(*t*-BOC)$_2$-N] | 10800 ± 1300 | 740 ± 77 | 15 |
| **27a** | Me | H | 3-F | 2720 ± 360 | 516 ± 99 | 5.3 |
| **27b** | Me | Me | 3-F | 15780 ± 2860 | 83 ± 18 | 190 |
| **28** | Me | Me | 3-Cl | 28200 ± 7000 | 54 ± 19 | 520 |
| **29a** | Me | H | 4-MeO | 858 ± 320 | 472 ± 132 | 1.8 |
| **29b** | Me | Me | 4-MeO | 14200 ± 3500 | 327 ± 75 | 44 |
| **30a** | Me | H | 4-$Me_2N$ | 3030 ± 300 | 12800 | 0.24 |
| **30b** | Me | Me | 4-$Me_2N$ | 5.6%[b] ($3 \times 10^{-5}$) | 9270 ± 150 | >1 |
| **31a** | Me | H | 2,3-$(MeO)_2$ | 1600 ± 250 | 600 ± 204 | 2.7 |
| **31b** | Me | Me | 2,3-$(MeO)_2$ | 5390 ± 1020 | 716 ± 144 | 7.5 |
| **32a** | Me | H | 3,4-$(MeO)_2$ | 5340 ± 1440 | 1100 ± 250 | 4.8 |
| **32b** | Me | Me | 3,4-$(MeO)_2$ | 13790 ± 2420 | 197 ± 33 | 70 |
| **33a** | Me | H | 3,5-$(MeO)_2$ | 3044 ± 520 | 120 ± 36 | 25 |
| **33b** | Me | Me | 3,5-$(MeO)_2$ | 12.5 ± 6.3%,[b] ($10^{-5}$) | 75.3 ± 29.1 | >200 |
| **34a** | Me | H | 3,5-$F_2$ | 2330 ± 830 | 366 ± 77 | 6.4 |
| **34b** | Me | Me | 3,5-$F_2$ | 14750 ± 3890 | 65 ± 9 | 230 |
| **35** | Me | Me | 3,5-$(MeO)_2$-4-OH | 8700 ± 4100 | 450 ± 66 | 19 |
| **36** | Me | Me | 4-AcO-3,5-$(MeO)_2$ | 6330 ± 1680 | 68 ± 22 | 93 |
| **37** | Me | Me | 4-(4-$PhCH_2O$)-3,5-$(MeO)_2$ | 4120 ± 460 | 139 ± 7 | 30 |
| **38** | Me | Me | 4-(4-$NH_2$-BuO)-3,5$(MeO)_2$ | 6170 ± 1010 | 173 ± 43 | 36 |
| **39** | Me | Me | 4-[4-(*t*-BOC-NH)BuO]-3,5-$(MeO)_2$ | 11031 | 265 ± 105 | 42 |
| **40** | Me | Me | 4-(4-$NH_2$-*trans*-$CH_2CH$=$CHCH_2O$-3,5-$(MeO)_2$ | 6280 ± 1580 | 228 ± 20 | 28 |
| **41** | Me | Me | 4-(4-AcNH-*trans*-$CH_2CH$=$CHCH_2O$)-3,5-$(MeO)_2$ | 17 ± 7%[b] ($10^{-5}$) | 216 ± 40 | >50 |
| **42** | Me | Me | 4-[4-*t*-BOC-NH-*trans*-$CH_2CH$=$CHCH_2O$-3,5-$(MeO)_2$ | 11 ± 5%[b] ($10^{-5}$) | 353 ± 62 | >40 |
| **43a** | Me | H | 2,3,4-$(MeO)_3$ | 26 ± 10%[b] ($10^{-5}$) | 1610 ± 260 | >5 |
| **43b** | Me | Me | 2,3,4-$(MeO)_3$ | 6920 ± 330 | 206 ± 81 | 34 |
| **44a** | Me | H | 3,4,5-$(MeO)_3$ | 2280 ± 530 [>100000][c] | 360 ± 170 [71][c] | 6.3 [>1100] |
| **44b** | Me | Me | 3,4,5-$(MeO)_3$ | 9200 ± 3560 [>100000][c] | 131 ± 54 [18][c] | 70 [>5600] |
| **44c** | Me | Et | 3,4,5-$(MeO)_3$ | 6290 ± 680 | 882 ± 239 | 7.1 |
| **44d** | Me | 2-OHEt | 3,4,5-$(MeO)_3$ | 26 ± 9%[b] ($10^{-5}$) | 22%[b] ($10^{-5}$) | |
| **44e** | Me | propargyl | 3,4,5-$(MeO)_3$ | 4040 ± 370 | 525 ± 220 | 7.7 |
| **44f** | Me | $C_6H_5Et$ | 3,4,5-$(MeO)_3$ | 32 ± 9%[b] ($10^{-5}$) | 14%[b] ($10^{-5}$) | |
| **45a** | Et | H | 3,4,5-$(MeO)_3$ | 852 ± 277 | 269 ± 7 | 3.2 |
| **45b** | Et | Me | 3,4,5-$(MeO)_3$ | 2790 ± 960 | 81 ± 17 | 34 |
| **46** | allyl | Me | 3,4,5-$(MeO)_3$ | 1930 ± 100 [>100000][c] | 131 ± 69 [15][c] | 13 [>6700] |
| **47** | Pr | H | H | 55 ± 28 [1800 or 22[d]][c] | 44 ± 19 [26 or 85[d]][c] | 1.3 [69 or 0.26] |
| **48** | Pr | Me | 3-$NO_2$ | 272 ± 68 | 56.2 ± 6.8 | 4.8 |
| **49** | Pr | Me | 3-$NH_2$ | 113 ± 21 | 18.9 ± 5.3 | 6.0 |
| **50a** | Pr | H | 3-F | 78 ± 17 | 153 ± 31 | 0.51 |
| **50b** | Pr | Me | 3-F | 301 ± 64 | 33 ± 15 | 9.1 |
| **51a** | Pr | H | 3-Cl | 167 ± 39 | 216 ± 66 | 0.77 |
| **51b** | Pr | Me | 3-Cl | 874 ± 222 | 61.3 ± 17.6 | 14 |

**Table I.** (Continued)

| compd | $R_1, R_3$ | $R_7$ | X | $K_i$[a] $A_1$ | $K_i$[a] $A_2$ | $A_1/A_2$ ratio |
|---|---|---|---|---|---|---|
| **52a** | Pr | H | 3,4-$(MeO)_2$ | 71 ± 11 [1700][c] | 48.5 ± 8.6 [6700][c] | 1.3 [0.25] |
| **52b**[f] | Pr | Me | 3,4-$(MeO)_2$ | 577 ± 42 [1500][c] | 31.1 ± 11.8 [7.8][c] | 19 [190] |
| **53a** | Pr | H | 3,5-$(MeO)_2$ | 632 ± 152 | 210 ± 140 | 3.0 |
| **53b** | Pr | Me | 3,5-$(MeO)_2$ | 2630 ± 20 | 24.0 ± 6.0 | 110 |
| **54a** | Pr | H | 3,5-$F_2$ | 146 ± 25 | 346 ± 97 | 0.42 |
| **54b** | Pr | Me | 3,5-$F_2$ | 382 ± 40 | 53 ● 15 | 7.2 |
| **55a** | Pr | H | 2,3,4-$(MeO)_3$ | 97 ± 19 | 64.0 ± 15.6 | 1.5 |
| **55b** | Pr | Me | 2,3,4-$(MeO)_3$ | 379 ● 128 | 68.5 ± 12.6 | 5.5 |
| **56a** | Pr | H | 2,4,5-$(MeO)_3$ | 143 ± 19 | 323 ± 74 | 0.44 |
| **56b** | Pr | Me | 2,4,5-$(MeO)_3$ | 689 ± 239 | 327 ± 52 | 2.1 |

[a] Expressed in nM (single determination or mean ± SEM for three or more determinations) vs [$^3$H]PIA (1 nM) at rat $A_1$-receptors and vs [$^3$H]CGS 21680 (5 nM) at rat striatal $A_2$-receptors. [b] Percent displacement of specific binding at the concentration indicated in parentheses. [c] Values in brackets are from ref 11 and represent $K_i$ values vs [$^3$H]NECA in rat striatum and vs [$^3$H]CHA in guinea pig brain, unless noted. [d] Affinites at both $A_1$- and $A_2$-receptors measured in rat brain (Erickson et al.[19]). [e] $R_\alpha$ = F. [f] KF17837.

**Table II.** Affinities of Related Xanthine Derivatives in Radioligand Binding Assays at Rat Brain $A_1$- and $A_2$-Receptors[a]




| compd | $R_7$ | $R_8$ | $K_i$[a] $A_1$ | $K_i$[a] $A_2$ | $A_1/A_2$ ratio |
|---|---|---|---|---|---|
| **2** | Me | cyclohexyl | [28000][b] | 17100 | 1.6 |
| **57** | Me | 2-(3-aminophenyl)ethyl | 15%[c] ($10^{-5}$) | 18000 | |
| **58a** | H | 7-methoxybenzofuran-2-yl | 1770 ± 70 | 3900 ± 940 | 0.5 |
| **58b** | Me | 7-methoxybenzofuran-2-yl | | 4740 | |

[a] Expressed in nM (single determination or mean ± SEM for three or more determinations) vs [$^3$H]PIA (1 nM) at rat $A_1$-receptors and vs [$^3$H]CGS 21680 (5 nM) at rat striatal $A_2$-receptors. [b] Reference 10. [c] Percent displacement of specific binding at the concentratioin indicated in parentheses.

al.[11] These authors found that 8-styryl derivatives of 1,3-dimethylxanthines were the most selective for $A_2$-receptors (selectivities greater than 5000-fold were reported), but the affinities of the corresponding 1,3-propyl analogues at both subtypes were greater (the most potent compound having a $K_i$ value of 7.8 nM at $A_2$ receptors).

The selectivity factors in the present study were generally much less than those reported by Shimada et al.[11] The principal reason may be that $A_1$-affinity in this study was measured in the same species as $A_2$-affinity (rat), whereas Shimada et al. measured $A_1$ affinity in guinea pig brain and $A_2$ affinity in rat brain. The species dependence of affinity of alkylxanthines at both $A_1$ and $A_{2a}$ receptors is well-documented.[21,22] Invariably, the affinity of xanthines at $A_1$-receptors is higher in the rat than in the guinea pig, but the interspecies affinity ratios have been found to vary from only 2-fold for theophylline to as much as 20-fold for 8-phenyltheophylline.[21] Indeed, the reported variation in $A_1$-affinities for 8-styrylxanthines in different species differ even more: up to 33-fold (e.g. compound **47** has an $A_1$-affinity in rat of 55 nM versus 1800 nM in guinea pig;[11] Erickson et al.[19] have determined a $K_i$ value at rat $A_1$-receptors of 22 nM). Thus, comparing guinea pig $A_1$-values to rat $A_2$-affinities results in artificially high selectivity ratios, and we therefore feel strongly that affinities should be compared within one species in order to yield useful figures.

In addition, some unexplained and substantial differences (e.g. compound **50a**) were observed between $K_i$ values versus [$^3$H]CGS 21680 in this study and versus [$^3$H]NECA in the study of Shimada et al.[11] (both having been measured in rat striatal membranes).

Another potential reason for discrepancies with previous results in binding assays was the amount of DMSO present. Shimada et al.[11] utilized approximately 1% DMSO in the assay medium, and we used 2%. We demonstrated (Figure 6) that at 0.5% DMSO a 1,3-dipropyl-7-methylxanthine derivative, **53b**, did not remain dissolved in aqueous solution at concentrations greater than 10 $\mu$M. This would affect in particular $A_1$-displacement curves for many compounds in this study, for which data points beyond xanthine concentrations of 10 $\mu$M are required. Thus, the addition of insufficient DMSO to the medium (or serial aqueous dilutions) might tend to overestimate the selectivity of the $A_2$-selective xanthines, but would not be expected to alter the apparent affinity at $A_2$ receptors (Figure 6A).

Functional studies, for example to characterize the antagonism of adenosine agonist-elicited increases in cyclic AMP, will be an important component of future work with these xanthine derivatives.

The most significant findings in this study are as follows: (1) the position of styryl ring substitution (meta favored) is a determinant of potency and selectivity (compare **17b**, **19b**, and **29b**), (2) increasing the size of small alkyl groups at the 1- and 3-xanthine position (e.g. **45b** versus **44b**) increases potency at both receptors and decreases $A_2$-selectivity, and (3) $A_2$-selectivity and moderate affinity are maintained with long chain extension from the para position of the styryl ring (e.g. **41**). It would seem that this position of the 8-styryl group when bound to the receptor is located in a relatively insensitive region. This finding is not unexpected given previous similar observations for para-substituted 8-phenylxanthines.[20] $A_2$-Selectivities of thousands of fold reported previously[11] were not observed in this study, although the selectivities of up to 520-fold (compound **28**), promise to be useful in physiological studies. $A_2$-Antagonists of particular interest are compounds **23**, **24**, **27b**, **28**, **33b**, and **34b** ($A_2$-selectivity of 200-fold or greater); compounds **23**, **28**, **49**, **50b**, **52b**, **53b**, and **54b** ($A_2$-affinity 50 nM or less); and compounds **22b**, **38**, and **40** (amine functionalized congeners). Compound **24** also has enhanced water solubility; the maximal solubility in a 0.1 M potassium phosphate solution at pH 7.4 was 19 mM.

**Table III.** Characterization of Xanthine Derivatives

| compd | % yield | mp (°C) | formula | analysis | compd | % yield | mp (°C) | formula | analysis |
|---|---|---|---|---|---|---|---|---|---|
| **15a** | 51 | >280 | $C_{15}H_{14}N_4O_2 \cdot {}^1/_2H_2O$ | H,N;C[b] | **38** | 90[e] | 207–212 | $C_{22}H_{29}N_5O_5$ | c |
| **15b** | 81 | 220–222 | $C_{16}H_{16}N_4O_2 \cdot {}^1/_4H_2O$ | C,H;N[b] | **39** | 15[d] | 184.5–186.5 | $C_{27}H_{37}N_5O_7 \cdot {}^1/_2H_2O$ | C,H,N[c] |
| **16** | 57 | >280 | $C_{15}H_{13}N_4O_2F$ | C,H,N | **40** | 18 | 200–206 | $C_{22}H_{27}N_5O_5$ | H,N;C[b,c] |
| **17a** | 31 | >300 | $C_{16}H_{16}N_4O_3 \cdot {}^2/_5H_2O$ | C,H,N | **41** | 7 | 229–232 dec | $C_{24}H_{29}N_5O_6$ | C,H,N[c] |
| **17b** | 74 | 238–240 | $C_{17}H_{18}N_4O_3$ | C,H,N | **42** | 75 | 192–195 | $C_{27}H_{35}N_5O_7$ | C,H,N[c] |
| **18** | 3 | >300 | $C_{15}H_{14}N_4O_3$ | C,H,N[c] | **43a** | 72 | 165 | $C_{18}H_{20}N_4O_5$ | C,H;N[b] |
| **19a** | 65 | >280 | $C_{16}H_{16}N_4O_3$ | C,H,N | **43b** | 43 | 189–193 | $C_{19}H_{22}N_4O_5 \cdot {}^1/_2H_2O$ | C,H;N[b] |
| **19b** | 61 | 212–215 | $C_{17}H_{18}N_4O_3 \cdot {}^1/_2H_2O$ | C,H;N[b] | **44a** | 63 | >280 | $C_{18}H_{20}N_4O_5$ | C,H,N |
| **20a** | 55 | >300 | $C_{16}H_{13}N_4O_2F_3$ | C,H,N,F | **44b** | 82 | 245–247 | $C_{19}H_{22}N_4O_5$ | C,H,N |
| **20b** | 84 | 232–236 | $C_{17}H_{15}N_4O_2F_3 \cdot {}^1/_2H_2O$ | C,H,N | **44c** | 84 | 225–229 | $C_{20}H_{24}N_4O_5$ | C,H,N |
| **21a** | 56 | >300 | $C_{15}H_{13}N_5O_4$ | C,H,N | **44d** | 70 | 251–254 | $C_{20}H_{24}N_4O_2$ | C,H,N |
| **21b** | 84 | 306–308 | $C_{16}H_{15}N_5O_4$ | C,H,N | **44e** | 79 | 235–237 | $C_{21}H_{22}N_4O_5$ | C,H,N |
| **22a** | 85 | >300 | $C_{15}H_{15}N_5O_2 \cdot {}^1/_2H_2O$ | C,H,N | **44f** | 71 | 215–218 | $C_{26}H_{28}N_4O_5$ | C,H,N |
| **22b** | 92 | 222–224 | $C_{16}H_{17}N_5O_2 \cdot 0.85H_2O$ | C,H,N[c] | **45a** | 20[a] | 286–289 | $C_{20}H_{24}N_4O_5 \cdot {}^1/_4H_2O$ | C,H,N[c] |
| **23** | 77 | >300 | $C_{18}H_{19}N_5O_3 \cdot {}^3/_5H_2O$ | C,H,N[c] | **45b** | 64 | 207–210 | $C_{21}H_{26}N_4O_5$ | C,H,N[c] |
| **24** | 78 | >300 | $C_{20}H_{21}N_5O_5 \cdot 0.7H_2O$ | C,H,N[c] | **47** | 52[a] | 257–260 | $C_{19}H_{22}N_4O_2$ | C,H,N |
| **25** | 59 | >300 | $C_{21}H_{25}N_5O_4 \cdot {}^1/_2H_2O$ | C,H;N[b,c] | **48** | 91 | 215–217 | $C_{20}H_{23}N_5O_4$ | C,H,N |
| **26** | 27 | 175–177 | $C_{26}H_{33}N_5O_6 \cdot {}^2/_5H_2O$ | C,H,N[c] | **49** | 92 | 145–148 | $C_{20}H_{25}N_5O_2 \cdot {}^3/_4H_2O$ | C,H,N |
| **27a** | 87 | >310 | $C_{15}H_{13}N_4O_2F \cdot {}^1/_2H_2O$ | C,H;N[b,c] | **50a** | 61 | 264–265 | $C_{19}H_{21}N_4O_2F$ | C,H,N |
| **27b** | 75 | 208–209 | $C_{16}H_{15}N_4O_2F$ | C,H,N | **50b** | 83 | 155–157 | $C_{20}H_{23}N_4O_2F \cdot {}^1/_4H_2O$ | C,H,N[c] |
| **28** | 10 | 205 | $C_{16}H_{15}N_4O_2Cl$ | C,H,N | **51a** | 18[a] | 257–259 | $C_{19}H_{21}N_4O_2Cl$ | C,H,N |
| **29a** | 4 | >320 | $C_{16}H_{16}N_4O_3$ | C,H,N | **51b** | 67 | 164–166 | $C_{20}H_{23}N_4O_2Cl$ | C,H,N[c] |
| **29b** | 55 | 220–222 | $C_{17}H_{18}N_4O_3$ | C,H,N[c] | **52a** | 48 | 250–253 | $C_{21}H_{26}N_4O_4 \cdot {}^1/_4H_2O$ | C,H,N |
| **30a** | 43 | >230 | $C_{17}H_{19}N_5O_2$ | c | **52b** | 78 | 164–164 | $C_{22}H_{28}N_4O_4 \cdot {}^3/_4H_2O$ | C,H,N[c] |
| **30b** | 29 | >230 | $C_{18}H_{21}N_5O_2$ | C,H;N[b,c] | **53a** | 100 | 150–152 | $C_{21}H_{26}N_4O_4 \cdot {}^2/_5H_2O$ | C,H,N[c] |
| **31a** | 32[a] | 299–301 | $C_{17}H_{18}N_4O_4$ | C,H,N | **53b** | 59 | 166–167 | $C_{22}H_{28}N_4O_4$ | C,H,N[c] |
| **31b** | 49 | 233.5–235 | $C_{18}H_{20}N_4O_4 \cdot {}^1/_2H_2O$ | C,H,N | **54a** | 78 | 275–278 | $C_{19}H_{20}N_4O_2F_2 \cdot {}^3/_4H_2O$ | C,H,N |
| **32a** | 4 | >295 | $C_{17}H_{18}N_4O_4$ | C,H,N[c] | **54b** | 85 | 161–163 | $C_{20}H_{22}N_4O_2F_2 \cdot 0.9H_2O$ | C,H,N |
| **32b** | 63 | 230–232 | $C_{18}H_{20}N_4O_4 \cdot {}^1/_2H_2O$ | C,H,N | **55a** | 32 | 241–244 | $C_{22}H_{28}N_4O_5$ | C,H,N |
| **33a** | 18 | >320 | $C_{17}H_{18}N_4O_4$ | C,H,N | **55b** | 88 | 107.5–109 | $C_{23}H_{30}N_4O_5$ | C,H,N |
| **33b** | 63 | 228–230 | $C_{18}H_{20}N_4O_4$ | C,H,N | **56a** | 11 | 252–254 | $C_{22}H_{28}N_4O_5$ | C,H,N |
| **34a** | 76 | >310 | $C_{15}H_{12}N_4O_2F_2$ | C,H,N | **56b** | 82 | 193–194 | $C_{23}H_{30}N_4O_5 \cdot {}^1/_2H_2O$ | C,H,N |
| **34b** | 87 | 238–239 | $C_{16}H_{14}N_4O_2F_2 \cdot {}^3/_4H_2O$ | C,H,N | **57** | 67 | 158–160 | $C_{16}H_{19}N_5O_2$ | C,H,N[c] |
| **35** | 54 | 269 dec | $C_{20}H_{22}N_4O_6$ | C,H,N[c] | **58a** | 78 | >280 | $C_{16}H_{14}N_4O_4 \cdot {}^1/_2H_2O$ | C,H,N |
| **36** | 57 | 274–279 | $C_{18}H_{20}N_4O_5 \cdot {}^1/_4H_2O$ | C,H,N[c] | **58b** | 99 | 273–275 | $C_{17}H_{16}N_4O_4$ | C,H,N |
| **37** | 75 | 190–194 | $C_{25}H_{26}N_4O_5 \cdot {}^1/_2H_2O$ | C,H,N | | | | | |

[a] Yield calculated from 1,3-dialkyl-6-amino-5-nitrosouracil. [b] Analyses: % N found (calcd) **15b**, 17.60 (18.62); **19b**, 15.86 (16.71); **25**, 16.00 (16.66); **27a**, 17.58 (18.11); **30b**, 18.15 (20.63); **43a**, 14.06 (15.06); **43b**, 13.37 (14.17); %C found (calcd) **15a**, 62.42 (61.85); **40**, 58.66 (59.28). [c] Accurate mass, measured (ppm from calculated), in EI mode, unless noted: **18**, 298.1055 (−3.7); **22b**, 311.1373 (5.6); **23**, 253.1483 (−1.4); **24**, 411.1556 (3.2); **25**, 411.1894 (−3.1); **26**, 511.2450 (3.7); **27a**, 300.1018 (2.3); **29b**, 326.1371 (−2.4); **30a**, 325.1537 (−0.5); **30b**, 339.1688 (−4.1); **32a**, 342.1326 (−0.6); **35**, 372.1436 (0.7); **36**, 414.1543 (0.9); **38** (FAB), 444.2255 (0.8); **39**, 543.2684 (−1.7); **40**, 441.2001 (−2.5); **41**, 483.2131 (2.7); **42**, 541.2544 (1.4); **45a** (FAB), 401.1812 (−1.3); **45b**, 414.1898 (−1.3); **50b**, 370.1795 (−2.7); **51b**, 386.1492 (−4.5); **52b**, 412.2110 (−0.1); **53a**, 398.1937 (−4.3); **53b**, 412.2093 (−4.3); **57**, 313.1521 (−5.7). [d] From compound **44b**. [e] From compound **39**.

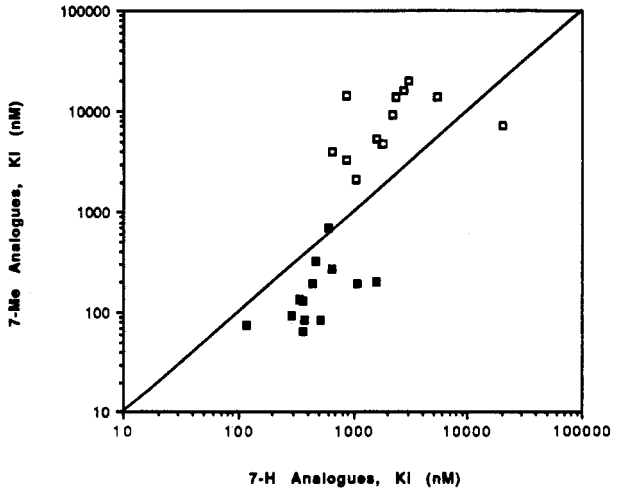



**Figure 4.** Correlation of affinity at adenosine receptors for 7-H versus 7-Me analogues of 1,3-dimethyl-8-styrylxanthine derivatives. Inhibition constants in nM given for $A_1$-receptors (□) and $A_2$-receptors (■).

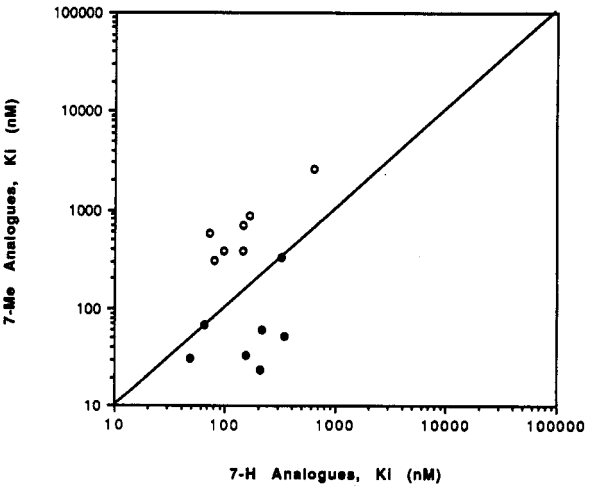



**Figure 5.** Correlation of affinity at adenosine receptors for 7-H versus 7-Me analogues of 1,3-dipropyl-8-styrylxanthine derivatives. Inhibition constants in nM given for $A_1$-receptors (○) and $A_2$ receptors (●).

A goal of this study is to identify adenosine antagonists that may be utilized as radioligands. Compound **53b** is of sufficiently high affinity and $A_2$-selectivity to be useful in binding assays when radiolabeled. An additional objective is to design functionalized congeners for eventual covalent labeling of the receptor, as was carried out for $A_2$ receptors using an agonist ligand[23] and for $A_1$-receptors.[20] The arylamino derivatives **22b** and **49** and the alkylamino derivatives **38** and **40** are chemically functionalized for further reaction leading to $A_2$-selective conjugates.

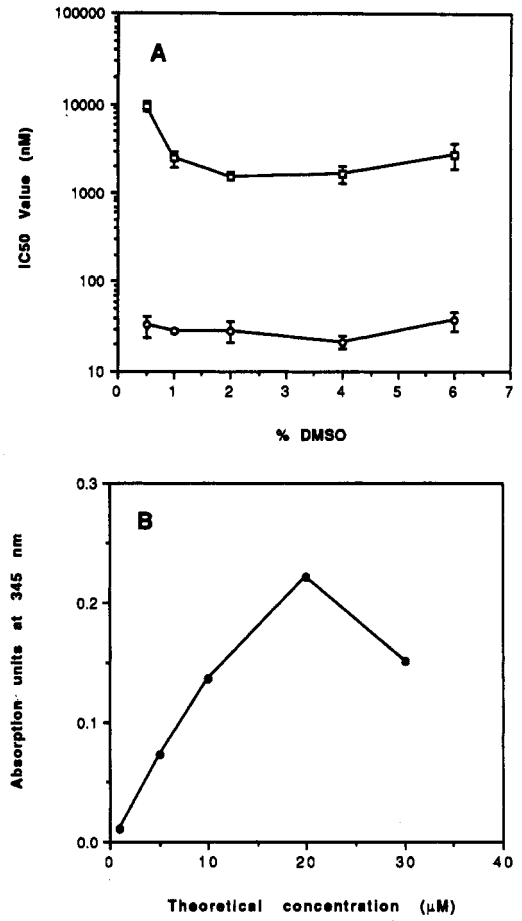



**Figure 6.** (A) Dependence of observed $IC_{50}$ values on DMSO concentration in competitive radioligand binding of 1,3-dipropyl-8-(3,5-dimethoxystyryl)xanthine, **53b**, at $A_1$- and $A_2$-receptors. $IC_{50}$ values are expressed in nM (mean ± SEM for three or more determinations) vs [$^3$H]PIA (1 nM) at rat $A_1$-receptors (□) and vs [$^3$H]CGS21680 (5 nM) at rat striatal $A_2$-receptors (○). (B) UV absorption of water solutions following addition of compound **53b** dissolved in DMSO (theoretical final concentration assuming complete dissolution given on abscissa). The final concentration of DMSO was 0.5% in each case. The peak absorption occured at 345 nm, with a molar extinction coefficient (ε) of 13 200.

## 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 ($NH_3$) and xanthine derivatives by fast atom bombardment mass spectrometry (positive ions in a glycerol matrix) 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 ±0.4% was acceptable. All xanthine derivatives were judged to be homogeneous using thin-layer chromatography following final purification. 7-Methoxy-2-benzofurancarboxylic acid, *trans*-cinnamic acid, and the following derivatives thereof were purchased from Aldrich (St. Louis, MO): α-fluoro, 2-methoxy, 3,4-dimethoxy, 3,5-difluoro, and 3,5-dimethoxy. 3- and 4-methoxy derivatives of *trans*-cinnamic acid were purchased from Fluka (Ronkonoma, NY). The following derivatives of *trans*-cinnamic acid were purchased from Lancaster (Windham, NH): 2,3-dimethoxy, 3,4,5-trimethoxy, 2,3,4-trimethoxy, 2,4,5-trimethoxy, and 3-fluoro. The following derivatives of *trans*-cinnamic acid were from Janssen Chimica (Geel, Belgium): 3-trifluoromethyl, 3-chloro, and 3-nitro. 2-Chloroadenosine was purchased from Research Biochemicals, Inc. (Natick, MA). Compound **46** was the gift of Dr. Ray Olsson (University of Southern Florida, Tampa, FL). 8-Cyclohexylcaffeine, **2**, was the gift of Dr. John W. Daly (NIH). Analytical TLC plates and silica gel (230–400 mesh) were purchased from VWR (Bridgeport, NJ).

**Synthesis of Xanthine Derivatives. General Procedure A: Reaction of Substituted Cinnamic Acid Derivatives with 5,6-Diamino-1,3-dimethyluracil To Yield Amide 9.** The substitued cinnamic acid (1 equiv) was dissolved in a minimum volume of DMF containing 1,3-dialkyl-5,6-diaminouracil (1.5 equiv). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1 equiv) was added, followed by a catalytic amount (0.05 equiv) of 4-(*N*,*N*-dimethylamino)pyridine and 0.05 equiv of imidazole. The mixture was stirred at room temperature for 3 h, and saturated sodium chloride solution was added (for 1,3-dipropyl derivatives water was used here), to form a precipitate or amorphous insoluble fraction. The insoluble residue was filtered and dissolved in 4 N aqueous sodium hydroxide containing sufficient methanol to obtain a clear solution.

The mixture was heated at 60 °C for 2 h or until the complete disappearance of starting material, as judged using TLC (silica plate, $CHCl_3/CH_3OH$/HOAc, 85:10:5, v/v). The mixture was cooled and acidified to pH = 1 with 6 N aqueous hydrochloric acid solution.

The precipitate was washed with water, dried, and further purified using a preparative silica plate (85–95% $CHCl_3$/5–15% methanol/1–5% HOAc). This procedure was used to make compounds **17a**, **29a**, **32a**, **33a**, etc.

**General Procedure B: Methylation of N-7 8-Styrylxanthines.** An 8-styrylxanthine derivative (1 equiv) was dissolved in a minimum of DMF. Excess finely powdered anhydrous potassium carbonate was added and the solution was left in an ultrasonic bath for 10 min. Methyl iodide (5 equiv) was added. The mixture was stirred at 60 °C for 30 min or until the complete disappearance of starting material, as judged using TLC (silica, chloroform/methanol/acetic acid, 95:4:1, v/v). The reaction mixture was cooled, and excess concentrated aqueous ammonia solution was added. The precipitate was washed with water, dried in vacuo, and further purified either by crystallization or by chromatography on a preparative thin-layer plate (85–95% chloroform/5–15% methanol/1–5% acetic acid). This procedure was used to make compounds **17b**, **29b**, **32b**, **33b**, etc.

**1,3-Dimethyl-8-(2-methoxystyryl)xanthine (17a).** Compound **17a** was made from 2-methoxycinnamic acid according to general procedure A and triturated with hot methanol: Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 3.27 (s, 3 H, $N_3$-$CH_3$), 3.35 (s, 3 H, $N_1$-$CH_3$), 3.5 (s, 3 H, $OCH_3$), 3.9 (s, 3 H, $N_7$-$CH_3$), 7.1 (d, 1 H, $J$ = 18 Hz), 7.0–7.2 (m, 2 H), 7.4 (m, 1 H), 7.7 (d, 1 H, $J$ = 8 Hz), 7.8 (d, 1 H, $J$ = 18 Hz). MS (CI/$NH_3$): $m/e$ 313 ($MH^+$, base) 281, 117.

**1,3,7-Trimethyl-8-(2-methoxystyryl)xanthine (17b).** Compound **17b** was made from **17a** according to general procedure B. Mp: 238–240 °C. $^1$H NMR DMSO-$d_6$: δ 3.24 (s, 3 H, $N_3$-$CH_3$), 3.48 (s, 3 H, $N_1$-$CH_3$), 3.90 (s, 3 H, $OCH_3$), 4.06 (s, 3 H, $N_7$-$CH_3$), 7.0–7.14 (m, 2 H), 7.34 (d, 1 H, $J$ = 16 Hz), 7.4 (m, 1 H), 7.9 (d, 1 H, $J$ = 8 Hz), 8.0 (d, 1 H, $J$ = 16 Hz). MS (CI/$NH_3$): $m/e$ 327 ($MH^+$) base peak.

**1,3-Dimethyl-8-[3-(trifluoromethyl)styryl]xanthine (20a).** Compound **20a** was made from 3-(trifluoromethyl)cinnamic acid according to general procedure A. Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 3.26 (s, 3 H, $NCH_3$), 3.48 (s, 3 H, $NCH_3$), 7.19 (d, 1 H, $J$ = 16 Hz), 7.64 (t, 1 H, $J$ = 8 Hz), 7.70 (d, 1 H, $J$ = 7 Hz), 7.72 (d, 1 H, $J$ = 16 Hz), 7.94 (d, 1 H, $J$ = 8Hz), 7.96 (s, 1 H). MS (CI): $m/e$ 350 (base), 329, 292.

**1,3,7-Trimethyl-8-[3-(trifluoromethyl)styryl]xanthine (20b).** Compound **20b** was made from **20a** according to general procedure B. Mp: 232–236 °C. $^1$H NMR DMSO-$d_6$: δ 3.25 (s, 3 H, $NCH_3$), 3.49 (s, 3 H, $NCH_3$), 4.09 (s, 3 H, $N_7$-$CH_3$), 7.58 (d, 1 H, $J$ = 16 Hz), 7.67 (t, 1 H, $J$ = 8 Hz), 7.72 (d, 1 H, $J$ = 8 Hz), 7.78 (d, 1 H, $J$ = 16 Hz), 8.09 (d, 1 H, J = 7 Hz), 8.26 (s, 1 H). MS (EI): $m/e$ 364.

**1,3-Dimethyl-8-(3-nitrostyryl)xanthine (21a).** Compound **21a** was made from 3-nitrocinnamic acid according to general procedure A (temperature raised to 80 °C for 3 h, recrystallized from methanol). Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 3.25 (s, 3 H, $NCH_3$), 3.48 (s, 3H, $NCH_3$), 7.22 (d, 1 H, $J$ = 16 Hz), 7.70 (t, 1 H, $J$ = 8 Hz), 7.76 (d, 1 H, $J$ = 16 Hz), 8.10 (d, 1 H, $J$ = 8

Hz), 8.18 (d, 1 H, $J$ = 8 Hz), 8.41 (s, 1 H). MS (EI): $m/e$ 327 (base), 310, 280.

**1,3,7-Trimethyl-8-(3-nitrostyryl)xanthine (21b).** Compound **21b** was made from **21a** according to general procedure B. Mp: 306–308 °C. $^1$H NMR DMSO-$d_6$: δ 3.23 (s, 3 H, $NCH_3$), 3.47 (s, 3 H, $NCH_3$), 4.08 (s, 3 H, $N_7$-$CH_3$), 7.63 (d, 1 H, J = 16 Hz), 7.71 (t, 1 H, $J$ = 8 Hz), 7.80 (d, 1 H, $J$ = 16 Hz), 8.18 (d, 1 H, $J$ = 8 Hz), 8.23 (d, 1 H, $J$ = 8 Hz), 8.70 (s, 1 H). MS (EI): $m/e$ 341 (base), 294.

**1,3-Dimethyl-8-(3-aminostyryl)xanthine (22a).** Compound **22a** was made from **21a** by reducing with Zn/acetic acid for 3 h. Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 3.24 (s, 3 H, $NCH_3$), 3.46 (s, 3 H, $NCH_3$), 5.19 (s, 2 H, $NH_2$), 6.56 (d, 1 H, $J$ = 8 Hz), 6.74 (d, 1 H, $J$ = 8 Hz), 6.76 (s, 1 H), 6.84 (d, 1 H, $J$ = 16 Hz), 7.05 (t, 1 H, $J$ = 8 Hz), 7.49 (d, 1 H, $J$ = 16 Hz). MS (CI/$NH_3$): $m/e$ 315 (M + $NH_4^+$), 298 ($MH^+$, base).

**1,3,7-Trimethyl-8-(3-aminostyryl)xanthine (22b).** Compound **22b** was made from **21b** using Zn/acetic acid as reducing agent for 3 h. Mp: 222–224 °C. $^1$H NMR DMSO-$d_6$: δ 3.22 (s, 3 H, N-$CH_3$), 3.46 (s, 3 H, $NCH_3$), 4.00 (s, 3 H $N_7$-$CH_3$), 5.14 (s, 2 H, $NH_2$), 6.58 (d, 1 H, $J$ = 8 Hz, H-4), 6.87 (s, 1 H, H-2), 6.92 (d, 1 H, $J$ = 8 Hz, H-6), 7.07 (t, 1 H, $J$ = 8 Hz, H-5), 7.14 (d, 1 H, $J$ = 16 Hz), 7.51 (d, 1 H, $J$ = 16 Hz). MS (CI/$NH_3$): $m/e$ 312 ($MH^+$).

**1,3,7-Trimethyl-8-[3-(acetylamino)styryl]xanthine (23).** Compound **23** was made from **22b** with acetic anhydride in DMF and DMAP for 1 h. Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 2.06 (s, 3 H, $COCH_3$), 3.23 (s, 3 H, $NCH_3$), 3.47 (s, 3 H, $NCH_3$), 4.03 (s, 3 H, $N_7$-$CH_3$), 7.24 (d, 1 H, $J$ = 16 Hz), 7.34 (t, 1 H, $J$ = 8 Hz), 7.50 (t, 1 H, $J$ = 8 Hz), 7.54 (d, 1 H, $J$ = 8 Hz), 7.61 (d, 1 H, $J$ = 16 Hz), 7.86 (s, 1 H). MS (CI/$NH_3$): $m/e$ 354 ($MH^+$).

**1,3,7-Trimethyl-8-[3-[(3-carboxy-1-oxopropyl)amino]styryl]xanthine (24).** Compound **24** was made from **22b** with succinic anhydride in DMF and DMAP. Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 2.28 (t, 2 H, $J$ = 7 Hz), 2.43 (t, 2 H, $J$ = 7 Hz), 3.23 (s, 3 H, $NCH_3$), 3.47 (s, 3 H, $NCH_3$), 4.03 (s, 3 H, $N_7CH_3$), 7.24 (d, 1 H, $J$ = 16 Hz), 7.32 (t, 1 H, $J$ = 8 Hz), 7.45 (d, 1 H, $J$ = 8 Hz), 7.54 (d, 1 H, $J$ = 8 Hz), 7.61 (d, 1 H, $J$ = 16 Hz), 7.82 (s, 1 H). MS (CI/$NH_3$): $m/e$ 394 (M − OH), 312, 209 (base). UV: $\lambda_{max}$ (methanol) 349 nm, log ε = 4.48. The maximal aqueous solubility following dissolution in $K_2HPO_4$ (0.1 M) was determined to be 19 mM.

**1,3,7-Trimethyl-8-[3-[(*tert*-butyloxy)carbonyl]amino]styryl]xanthine (25).** Compound **25** was made from **22b** with di-*tert*-butyl dicarbonate and DMAP in DMF. Mp: >300 °C. $^1$H NMR DMSO-$d_6$: δ 1.40 (s, 9 H, $CH_3COO$), 3.17 (s, 3 H, $NCH_3$), 3.41 (s, 3 H, $NCH_3$), 3.89 (s, 3 H, $N_7$-$CH_3$), 7.23 (d, 1 H, $J$ = 16 Hz), 7.33 (d, 1 H, $J$ = 8 Hz), 7.51 (t, 1 H, $J$ = 8 Hz), 7.57 (s, 1 H), 7.67 (d, 1 H, $J$ = 16 Hz), 7.75 (d, 1 H, $J$ = 8 Hz). MS (CI/$NH_3$): $m/e$ 414 (M − $CH_3$ + $NH_4^+$, base), 338, 314, 312.

**1,3,7-Trimethyl-8-[3-[bis[(*tert*-butyloxy)carbonyl]amino]styryl]xanthine (26).** Compound **26** was made from **22b** with di-*tert*-butyl dicarbonate and DMAP in DMF. Mp: 175–177 °C. $^1$H NMR DMSO-$d_6$: δ 1.39 (s, 18 H, $CH_3COO$), 3.23 (s, 3 H, $NCH_3$), 3.46 (s, 3 H, $NCH_3$), 4.03 (s, 3 H, $N_7$-$CH_3$), 7.17 (d, 1 H, $J$ = 8 Hz), 7.42 (t, 1 H, $J$ = 8 Hz), 7.43 (d, 1 H, $J$ = 16 Hz), 7.67 (d, 1 H, $J$ = 16 Hz), 7.69 (d, 1 H, $J$ = 8 Hz), 7.74 (s, 1 H). MS (CI/$NH_3$): $m/e$ 514 (M − $CH_3$ + $NH_4^+$), 414 (base).

**1,3-Dimethyl-8-(4-methoxystyryl)xanthine (29a).** Compound **29a** was made from 4-methoxycinnamic acid according to general procedure A. Mp: >320 °C. $^1$H NMR DMSO-$d_6$: δ 3.24 (s, 3 H, $N_3$-$CH_3$), 3.46 (s, 3 H, $N_7$-$CH_3$), 3.78 (s, 3 H, $OCH_3$), 6.85 (d, 1 H, $J$ = 16 Hz), 7.0 (d, 2 H, $J$ = 8 Hz), 7.55 (d, 2 H, $J$ = 8 Hz), 7.6 (d, 1 H, $J$ = 16 Hz). MS (CI/$NH_3$): $m/e$ 313 ($MH^+$, base) 172.

**1,3,7-Trimethyl-8-(4-methoxystyryl)xanthine (29b).** Compound **29b** was made from **29a** according to general procedure B. Mp: >320 °C. $^1$H NMR DMSO-$d_6$: δ 3.22 (s, 3 H, $N_3CH_3$), 3.45 (s, 3 H, $N_1$-$CH_3$), 3.8 (s, 3H, $OCH_3$), 4.0 (s, 3 H, $N_7$-$CH_3$), 7.0 (d, 1 H, $J$ = 8 Hz), 7.2 (d, 1 H, $J$ = 16 Hz), 7.66 (d, 1 H, $J$ = 16 Hz), 7.72 (d, 1 H, $J$ = 8 Hz). MS (CI/$NH_3$): $m/e$ 327 ($MH^+$, base) 205.

**1,3-Dimethyl-8-[4-(dimethylamino)styryl]xanthine (30a).** A solution of 4-(dimethylamino)cinnamic acid (0.1 g, 0.52 mmol), 1-hydroxybenzotriazole (0.14 g, 1.04 mmol), and EDAC (0.19 g, 1.04 mmol) in DMF (1 mL) was sonicated for 1 h. 1,3-Dimethyl-5,6-diaminouracil (0.088 g, 0.52 mmol) was added, and the mixture was heated for 3 h at 80 °C. The dark red solution was cooled to room temperature, and the product was obtained as a deep yellow precipitate (0.045 g). An additional crop was obtained by cooling the mother liquor in an ice bath and adding 10 volumes of brine (combined yield 38%). $^1$H NMR $CD_3OD$: δ 7.54 (d, 1 H, $J$ = 15.5 Hz), 7.45 (d, 2 H, 8.8 Hz), 6.74 (d, 2 H, $J$ = 8.8 Hz), 6.56 (d, 1 H, $J$ = 15.5 Hz), 3.42, 3.27 (s, 3 H, $CH_3$), 3.00 (s, 6 H, $N(CH_3)_2$). MS (CI): $m/e$ 344 ($MH^+$).

The above amide (0.045 g, 0.13 mmol) was suspended in methanol (1 mL), and 4 N NaOH (1 mL) was added. The resulting solution was stirred at 80–90 °C for 1.5 h. HCl (18%) was added carefully to the ice-cooled reaction solution to pH = 7–8. A yellow precipitate was obtained (0.018 g, 43%). $^1$H NMR DMSO-$d_6$: δ 7.54 (d, 1 H, $J$ = 16 Hz), 7.44 (d, 2 H, $J$ = 8.5 Hz), 6.74 (d, 2 H, $J$ = 16 Hz), 6.74 (d, 2 H, $J$ = 16 Hz), 3.47, 3.25 (s, 3 H, $CH_3$), 2.97 (s, 6 H, $N(CH_3)_2$). MS (CI): $m/e$ 326 ($MH^+$).

**1,3-Dimethyl-8-(2,3-dimethoxystyryl)xanthine (31a).** Compound **31a** was made from 2,3-dimethoxycinnamic acid according to general procedure A (recrystallized from methanol). Mp: 299–301 °C. $^1$H NMR DMSO-$d_6$: δ 3.25 (s, 3 H, $N_3$-$CH_3$), 3.47 (s, 3 H, $NCH_3$), 3.78 (s, 3 H, $OCH_3$), 3.82 (s, 3 H, $OCH_3$), 7.05 (d, 1 H, $J$ = 17 Hz), 7.05 (dd, 1 H, $J$ = 2, 8 Hz), 7.11 (t, 1 H, $J$ = 8 Hz) 7.26 (dd, 1 H, $J$ = 2, 8 Hz), 7.84 (d, 1 H, $J$ = 17 Hz) MS (CI/$NH_3$): $m/e$ 360 (M + $NH_4^+$), 343 (base peak).

**1,3,7-Trimethyl-8-(2,3-dimethoxystyryl)xanthine (31b).** Compound **31b** was made from **31a** according to the general procedure B. Mp: 233–235 °C. $^1$H NMR DMSO-$d_6$: δ 323 (s, 3 H, $NCH_3$), 3.47 (s, 3 H, $NCH_3$), 3.78 (s, 3 H, $OCH_3$), 3.83 (s, 3 H, $OCH_3$), 4.02 (s, 3 H, $N_7$-$CH_3$), 7.06 (d, 1 H, $J$ = 8 Hz), 7.10 (t, 1 H, $J$ = 8 Hz), 7.32 (d, 1 H, $J$ = 16 Hz), 7.51 (d, 1 H, $J$ = 8 Hz), 7.90 (d, 1 H, $J$ = 16 Hz). MS (EI): $m/e$ 356 (base), 325.

**1,3-Dimethyl-8-(3,4-dimethoxystyryl)xanthine (32a).** Compound **32a** was made from 3,4-dimethoxycinnamic acid according to general procedure A. Mp: >320 °C. $^1$H NMR DMSO-$d_6$: δ 3.25 (s, 3 H, $N_3$-$CH_3$), 3.46 (s, 3 H, $N_1$-$CH_3$), 3.78 (s, 3 H, $OCH_3$), 3.82 (s, 3 H, $OCH_3$), 6.96 (d, 1 H, $J$ = 16 Hz), 6.98 (d, 1 H, $J$ = 8 Hz), 7.14 (d, 1 H, $J$ = 8 Hz), 7.25 (s, 1 H). MS (CI/$NH_3$): $m/e$ 343 ($MH^+$), 172 (base peak).

**1,3,7-Trimethyl-8-(3,4-dimethoxystyryl)xanthine (32b).** Compound **32b** was made from **32a** according to general procedure B. Mp: 230–232 °C. $^1$H NMR DMSO-$d_6$: δ 3.29 (s, 3 H, $N_3$-$CH_3$), 3.52 (s, 3 H, $N_7$-$CH_3$), 3.85 (s, 3 H, $OCH_3$), 3.9 (s, 3 H, $OCH_3$), 4.09 (s, 3 H, $N_7$-$CH_3$), 7.05 (d, 1 H, $J$ = 8 Hz), 7.25 (d, 1 H, $J$ = 16 Hz), 7.30 (d, 1 H, $J$ = 8 Hz), 7.48 (s, 1 H), 7.66 (d, 1 H, $J$ = 16 Hz). MS (CI): $m/e$ 357 ($MH^+$ base), 209.

**1,3-Dimethyl-8-(3,5-dimethoxystyryl)xanthine (33a).** Compound **33a** was made from 3,5-dimethoxycinnamic acid according to general procedure A. Mp: >320 °C. $^1$H NMR DMSO-$d_6$: δ 3.24 (s, 3 H, $N_3$-$CH_3$), 3.46 (s, 3 H, $N_1$-$CH_3$), 3.78 (s, 6 H, $OCH_3$), 6.5 (s, 1 H), 6.78 (s, 2 H), 7.02 (d, 1 H, $J$ = 16 Hz), 7.54 (d, 1 H, $J$ = 16 Hz). MS (CI): $m/e$ 343 ($MH^+$ base), 166, 136.

**1,3,7-Trimethyl-8-(3,5-dimethoxystyryl)xanthine (33b).** Compound **33b** was made from **33a** according to general procedure B. Mp: 228–230 °C. $^1$H NMR DMSO-$d_6$: δ 3.22 (s, 3 H, $N_3$-$CH_3$), 3.45 (s, 3 H, $N_1$-$CH_3$), 3.79 (s, 6 H, $OCH_3$), 4.04 (s, 3 H, $N_7$-$CH_3$), 6.5 (s, 1 H), 6.97 (s, 2 H), 7.32 (d, 1 H, $J$ = 16 Hz), 7.58 (d, 1 H, $J$ = 16 Hz).

**General Procedure C: Demethylation Followed by O-Alkylation of 8-(3,5-Dimethoxy-4-hydroxystyryl)xanthines.** 1,3,7-Trialkyl-8-(3,4,5-trimethoxystyryl)xanthine (1 equiv) was dissolved in a minimum of DMF, and 1.5 equiv of sodium thiophenoxide was added. The solution was heated to 150–160 °C for 20 min or until judged complete using TLC. Ether was added, and the resulting orange precipitate was isolated by filtration. This solid was dissolved in DMF, and water and 6 N HCl were added until pH 2 to give **35** as a yellow precipitate. The appropriate halide (2 equiv for monohalide and 8 equiv for dihalide) was added to a solution of **35** in DMF, followed by finely powdered, anhydrous $K_2CO_3$. The solution was left in an ultrasonic bath for 15 min and further heated at 50–80 °C for 2 h or until judged complete using TLC. The reaction mixture was cooled and extracted with petroleum ether. The crude product was precipitated by water (for product of reaction with monohalides) or reacted further (for dihalides) with concentrated aqueous ammonia and chromatographed on preparative TLC

using 90–95% chloroform/5–10% methanol/1% aqueous ammonia. This procedure was used to make compounds **37–40**.

The 4-hydroxy intermediate **35** was also recrystallized (MeOH/$H_2O$). $^1H$ NMR DMSO-$d_6$: $\delta$ 3.23 and 3.46 (each s, 3 H, $N_1$- and $N_3$-$CH_3$); 3.83 (s, 6 H, 3,5-($OCH_3$)), 4.03 (s, 3 H, $N_7$-$CH_3$), 7.08 (s, 2 H, Ar), 7.18 and 7.61 (each d, 1 H, C=C, $J$ = 15.7 Hz), 8.82 (br s, 1 H, ArOH).

**1,3,7-Trimethyl-8-[3,5-dimethoxy-4-(benzyloxy)styryl]xanthine (37).** Compound **37** was made from benzyl bromide according to general procedure C. Mp: 190–195 °C. $^1H$ NMR $CDCl_3$: $\delta$ 3.42 (s, 3 H, $N_3CH_3$), 3.63 (s, 3 H $N_5CH_3$), 3.89 (s, 6 H, $OCH_3$), 5.06 (s, 2 H, $OCH_2$), 6.8 (s, 2 H), 6.78 (d, 1 H, $J$ = 16 Hz), 7.3–7.5 (m, 5 H), 7.7 (d, 1 H, $J$ = 16 Hz). MS (CI): $m/e$ 463 ($MH^+$ base), 375, 357.

**1,3,7-Trimethyl-8-[3,5-dimethoxy-4-[(4-aminobutyl)oxy]styryl]xanthine (38).** Compound **38** was made from 1,4-dibromobutane according to general procedure C. MS (CI): $m/e$ 444 ($MH^+$ base), 373, 359.

**1,3,7-Trimethyl-8-[3,5-dimethoxy-4-[[4-[[(*tert*-butyloxy)carbonyl]amino]butyl]oxy]styryl]xanthine (39).** Compound **39** was made from **38** using di-*tert*-butyl dicarbonate in $CHCl_3$ (30 min). The chloroform was removed under a stream of $N_2$, and the crude product was purified using a preparative plate (silica, ethyl acetate/petroleum ether, 70:30). $^1H$ NMR $CDCl_3$: $\delta$ 1.41 (s, 9 H, $CH_3$), 1.6–1.8 (m, 4 H, $CH_2$), 3.2 (m, 2 H, $CH_2NH$), 4.0 (m, 2 H, $OCH_2$), 3.39 (s, 3 H, $N_3$-$CH_3$), 3.6 (s, 2 H, $N_7$-$CH_3$), 3.88 (s, 6 H, $OCH_3$), 4.05 (s, 3 H, $N_7$-$CH_3$), 6.74 (s, 2 H), 6.75 (d, 1 H, $J$ = 16 Hz), 7.7 (d, 1 H, $J$ = 16 Hz). MS (CI): $m/e$ 544 ($MH^+$ base) 44, 359.

**1,3,7-Trimethyl-8-[3,5-dimethoxy-4-[(4-amino-*trans*-butenyl)oxy]styryl]xanthine (40).** Compound **40** was made from 1,4-dibromo-*trans*-2-butene according to general procedure C. $^1H$ NMR $CDCl_3$: $\delta$ 3.41 (s, 3 H, $N_3$-$CH_3$), 3.63 (s, 3 H, $N_1$-$CH_3$), 3.91 (s, 6 H, $OCH_3$), 4.06 (s, 3 H, $N_7$-$CH_3$), 4.43 (s, 2 H, $CH_2NH_2$), 5.94 (s, 2 H, $OCH_6$), 6.78 (s, 2 H), 6.79 (d, 1 H, $J$ = 16 Hz). MS (CI): $m/e$ 442 ($MH^+$ base) 373, 357, 124.

**7-Ethyl-1,3-trimethyl-8-(3,4,5-trimethoxystyryl)xanthine (44c).** Compound **44c** was made from compound **44a** according to general procedure B, except that ethyl iodide was used instead of methyl iodide. $^1H$ NMR DMSO-$d_6$: $\delta$ 1.34 (t, 3 H, $CH_3Et$, $J$ = 7 Hz), 3.25 and 3.47 (each s, 3 H, $NCH_3$), 3.70 (s, 4 H, 4-$OCH_3$), 3.86 (s, 6 H, 3,5=($OCH_3$)), 4.54 (q, 2 H, $N_7$-$CH_2$), 7.13 (s, 2 H, Ar), 7.30 and 7.68 (each d, 1 H, C=C, $J$ = 16 Hz).

**1,3-Dipropyl-7-methyl-8-styrylxanthine (47).** 5-Amino-6-nitroso-1,3-dipropyluracil[10,20] was suspended in DMF (10 mmol/100 mL) and hydrogenated over 5% Pd/C at 40 psi overnight. The clear solution was filtered through Celite and could be stored at −20 °C.

*trans*-Cinnamic acid (0.47 g) and EDAC (0.65 g) were added to 2.1 mmol of the above solution and stirred for 4 h. An additional 0.3 g of EDAC was added. After an additional 2 h, half-saturated NaCl solution was added and the mixture was extracted with ethyl acetate (6×). The organic layer was dried over $Na_2SO_4$ and evaporated to an oil, which was used without further purification.

The above oil was dissolved in methanol (30 mL) and treated with 4 N NaOH (20 mL). After refluxing for 15 min, the mixture was cooled, ice was added, and it was acidified using 6 N HCl. A precipitate formed and was recovered by filtration. The NMR and MS were consistent with the assigned structure of **47**. Recrystallized from DMF/water.

$\epsilon_{342}$ for **47** in methanol ($\lambda_{max}$) was 35 100. A smaller absorption peak was at 265 nm.

**1,3,7-Trimethyl-8-[2-(3-aminophenyl)ethyl]xanthine (57).** Compound **54** was made from **21b** with $H_2$/Pd at 50 psi in DMF for 3 h. Mp: 158–160 °C. $^1H$ NMR DMSO-$d_6$: $\delta$ 2.82 (t, 2 H, $J$ = 8 Hz), 2.96 (t, 2 H, $J$ = 8 Hz), 3.20 (s, 3 H, $NCH_3$), 3.42 (s, 3 H, $NCH_3$), 3.69 (s, 3 H, $N_7$-$CH_3$), 4.95 (s, 2 H, $NH_2$), 6.34–6.39 (3 H, H-2, H-4, H-6), 6.90 (t, 1 H, $J$ = 8 Hz, H-5). MS (CI/$NH_3$): $m/e$ 314 (MH+).

**Biological Methods. Receptor Binding.** Rat cerebral cortical membranes and striatal membranes were prepared[12,13] 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%.

Inhibition of binding of 1 nM [$^3H$]-$N^6$-(2-phenylisopropyl)-adenosine (Du Pont NEN, Boston, MA) to $A_1$-receptors in rat cerebral cortex membranes was measured as described.[17] Membranes (~100 μg of protein/tube) were incubated for 1.5 h at 37 °C in a total volume of 0.5 mL of 50 mM Tris HCl, at pH 7.4. Test drugs were dissolved in DMSO and added in 10-μL aliquots, resulting in a final DMSO concentration of 2%. Bound and free radioligand were separated by addition of 3 mL of a buffer containing 50 mM Tris HCl at pH 7.4 at 5 °C followed by vacuum filtration using a Brandel cell harvester (Brandel, Gaithersburg, MD) and a Whatman GF/B glass-fiber filter with additional washes totaling 9 mL of buffer. Nonspecific binding was determined with 10 μM 2-chloroadenosine.

Inhibition of binding of 5 nM [$^3H$]CGS 21680 was carried out as follows. Membranes (~80 μg of protein/tube, prepared according to ref 8) were incubated for 1 h at 25 °C in a total volume of 0.5 mL of 50 mM Tris HCl containing 10 mM $MgCl_2$ at pH 7.4. Test drugs were dissolved in DMSO and added in 10-μL aliquots, resulting in a final DMSO concentration of 2%. Nonspecific binding was defined using 20 μM 2-chloroadenosine. Filtration was carried out using a Brandel cell harvester, as above, using Tris HCl/$MgCl_2$ as the washing buffer.

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 GraphPAD program (Institute for Scientific Information), were converted to apparent $K_i$ values using $K_D$ values[12,13] of 1.0 and 14 nM for [$^3H$]PIA and [$^3H$]CGS 21680 binding, respectively, and the Cheng–Prusoff equation.[18]

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