Substituted 1,3-Dipropylxanthines as Irreversible Antagonists of A_1 Adenosine Receptors

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This report describes the synthesis of 29 xanthines containing a chemoreactive chloroaryl, β-chloroethylamino, α,β-unsaturated carbonyl, bromoacetyl, 3-(fluorosulfonyl)benzoyl, or 4-(fluorosulfonyl)benzoyl group as part of an exocyclic 1-, 3-, or 8-substituent. The xanthines inhibited the binding of [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]CPX) to the A_1 adenosine receptor (A_1AR) of DDT_1 MF2 cells at IC_{50}s in the low-nanomolar to low-micromolar range. Seven of the 29 analogues irreversibly inhibited the binding of [^{3}H]CPX without changing the K_D of that ligand; five were 1,3-dipropylxanthines having the following reactive groups as 8-substituents: (bromoacetamido)methyl (**24**), (bromoacetamido)ethyl (**25**), (bromoacetamido)propyl (**26**), [4-(fluorosulfonyl)benzamido]methyl (**33**) or 3-[[4-(fluorosulfonyl)benzoyl]oxy]cyclopentyl (**42**). Both 8-cyclopentyl-3-[3-[[4-(fluorosulfonyl)benzoyl]oxy]propyl]-1-propylxanthine (**53**) and 8-cyclopentyl-1,3-bis[3-[[4-(fluorosulfonyl)benzoyl]oxy]propyl]xanthine (**55**) inhibited [^{3}H]CPX binding irreversibly. Five of the ligands, including **26, 33** (IC_{50} = 49 μM), and **53** (IC_{50} = 9 μM), antagonized the binding of [^{3}H]NECA to the A_{2a}AR of PC12 cells, but unlike binding to the A_1AR, binding to the A_{2a}AR was completely reversible. The potency of **33** (IC_{50} = 2 μM, 72% loss of CPX binding at 1 μM) and **53** (IC_{50} = 0.01 μM, 74% loss of CPX binding at 0.05 μM) and their seletivity for the A_1AR suggest that those two ligands may be useful in studies of the structure and function of that receptor.

Introduction

Chemoreactive and photoreactive irreversible antagonists are useful tools for the investigation of receptor biochemistry and physiology. Chemoreactive ligands have the useful characteristics of high efficiency of incorporation, which allows control of receptor concentrations over a wide range, and applicability *in vivo* as well as *in vitro*. These properties have made them useful in a variety of studies including the identification and mapping of ligand-binding subunits,[1,2] the function of a receptor and its subtypes,[3] the relationship between the extent of receptor occupancy and the magnitude of a functional response,[4–6] and the kinetics of the turnover and cellular processing of receptors.[7,8]

The adenosine A_1 receptor (A_1AR) mediates a variety of physiological responses including slowing of heart rate, inhibition of neurotransmitter release and inhibition of lipolysis.[9] At the molecular level, a guanine nucleotide binding protein couples the A_1AR to a number of effector systems. The most extensively investigated effects of cardiac A_1AR activation are the inhibition of adenylyl cyclase activity and the stimulation of potassium currents.[9,10] Photoaffinity ligands have been synthesized and used to probe the structure of the A_1AR.[11,12] In addition, several chemoreactive antagonists have been reported to irreversibly bind to an A_1AR.[13] One of these, the 1,3--phenylenediisothiocyanate derivative of xanthine amine congener (3-DITC-XAC), has been used to identify the ligand-binding subunit of the A_1AR in membranes from rat brain[14] and to provide evidence for spare A_1ARs in the guinea pig atrioventricular node.[15] Those reports illustrate the usefulness of irreversible alkylating ligands to study the A_1AR.

The present work was initiated to (a) further identify alkylating A_1AR antagonists and (b) by means of structure–activity relationships, to determine some of the structural requirements for the irreversible binding of such ligands. A series of chemoreactive xanthine derivatives were synthesized and tested for potency and irreversible binding to the adenosine A_1AR in DDT_1 MF-2 cells. To assess selectivity several of the ligands were also tested for potency and irreversible binding to the A_{2a}AR of PC12 cells. The synthetic strategy involved the attachment of reactive functional groups to the 3- and 8-positions of the well-known A_1AR antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX). Antagonist structure–activity correlations indicate that the A_1AR can accommodate xanthines having rather bulky N-3 substituents[16] and that a wide variety of C-8 substituents can greatly enhance antagonist activity.[17] The synthetic strategy called for initially evaluating substituents at C-8 because of ease of synthesis and the resistance of such xanthines to metabolism. If a ligand containing a reactive C-8 substituent bound to the receptor irreversibly, the reactive group was then introduced into the N-1 or N-3 substituent.

The first type of substituents were chloroaryl groups, chosen because of the high affinity of the A_1AR for 8-(4-chlorophenyl)-1,3-dipropylxanthine.[18] Chloropyridyl and 4-chloro-3-nitrophenyl substituents contain ortho electron-withdrawing groups and, in the case of the pyridyl

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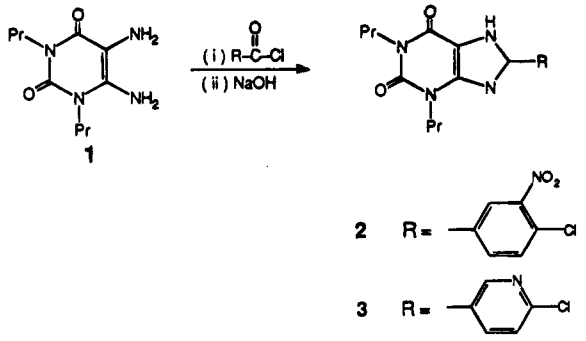
Scheme 1



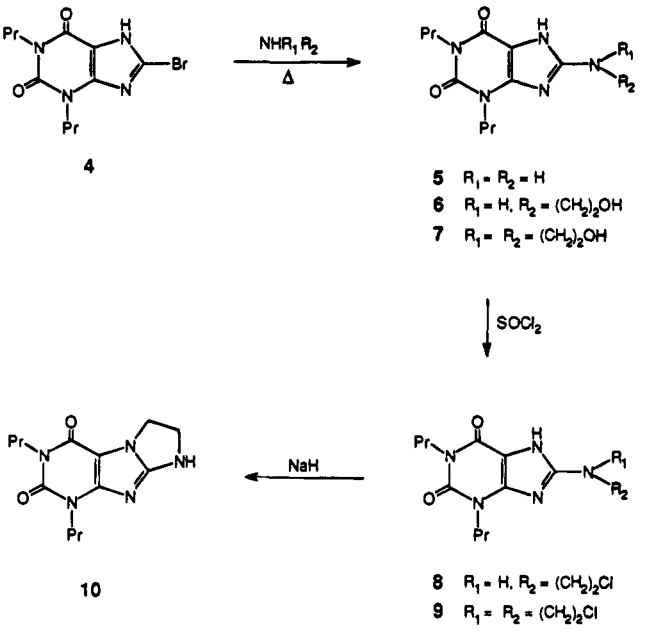
group, a heteroatom that greatly accelerates the rate of aromatic nucleophilic substitutions.[19] The strong alkylating activity of the nitrogen mustards[20] justified their inclusion. Another type of reactive group, the α,β-unsaturated carbonyl, was included because it undergoes addition reactions wherein the nucleophile attacks the carbon β to the carbonyl group, resulting in a covalent bond.[21] Irreversible ligands for several other types of receptor exploit the reactivity of the bromoacetyl group.[22,23] Finally, the usefulness as affinity ligands of adenosines containing the 4-(fluorosulfonyl)benzoyl group[25] called for its introduction into an antagonist molecule. The finding that 8-[[4-(fluorosulfonyl)benzamido]methyl]-1,3-dipropylxanthine (**33**) is an irreversible antagonist prompted the synthesis of additional analogues containing either a 3- or 4-(fluorosulfonyl)-benzoyl group. Varying the length of the carbon chain between the heterocycle and the (fluorosulfonyl)benzamido group may place the reactive moiety in a better orientation to undergo nucleophilic substitution. Since the presence of an 8-cyclopentyl ring is known to greatly enhance the antagonist potency of xanthines, it was of interest to test analogues that contained both the (fluorosulfonyl)benzoyl group and an 8-cyclopentyl ring. This goal was achieved by attaching the (fluorosulfon)-benzoyl group directly to the 8-cyclopentyl ring, or, alternatively, to either the 1- or 3-propyl side chain as well as to both the 1- and the 3-propyl side chain. In order to simplify the synthesis, these groups were connected by an ester, rather than an amide linkage.

Results and Discussion

Chemistry. Scheme 1 depicts the synthetic route[26,27] to 8-substituted 1,3-dipropylxanthines **2** and **3**. 5,6-Diamino-1,3-dipropyluracil (**1**) was acylated with the appropriately substituted acid chloride in pyridine at 0 °C. Alternatively, the acylation could be performed with the corresponding carboxylic acid by refluxing in toluene with the removal of water by means of a Dean–Stark trap. The resulting amide was cyclized by refluxing in aqueous sodium hydroxide. This method, applied to 6-chloronicotinoyl chloride and 4-chloro-3-nitrobenzoyl chloride, yielded compounds **2** and **3**, respectively. Refluxing commercially available 6-hydroxynicotinic acid in thionyl chloride containing a catalytic amount of *N,N'*-dimethylformamide (DMF) afforded 6-chloronicotinyl chloride in one step.

The synthesis of xanthines containing a nitrogen mustard substituent proceded from the 8-bromoxanthine. The reaction of 1,3-dipropylxanthine with bromine in acetic acid[28] gave the 8-bromo derivative, **4**, which on heating at 170 °C with either ammonia, ethanolamine, or diethanolamine yielded compounds **5**, **6**, and **7**, respectively (Scheme 2). Refluxing **6** or **7** in an excess of neat thionyl chloride afforded the corresponding nitrogen mustards, **8** or **9**.

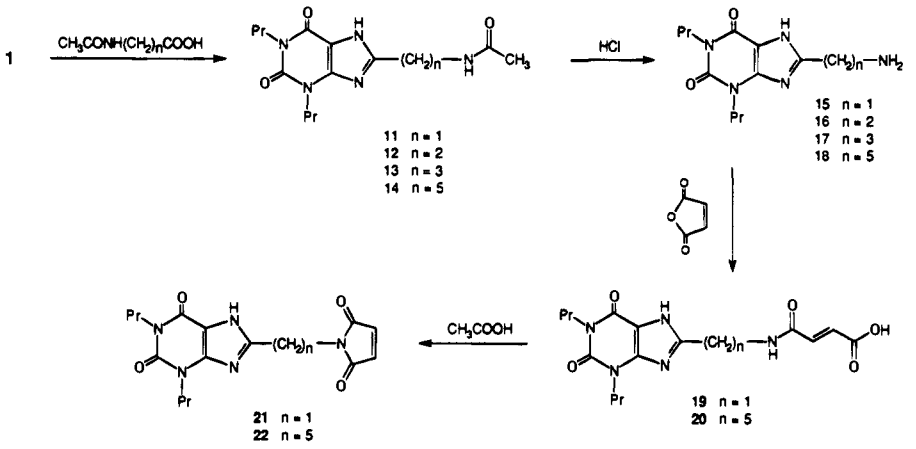
Scheme 2



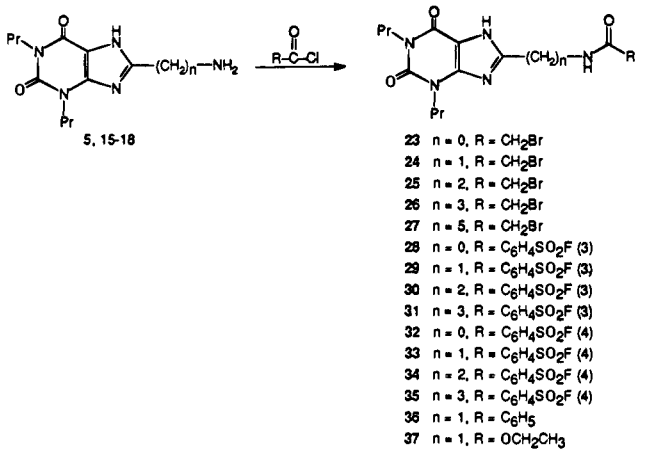
Aziridines are generally prepared from β-amino alcohols by the methods of Gabriel or Wenker.[29] The synthesis of a series of *N*-arylaziridines as possible chemosterilants has been reported.[30] In that synthesis, the conversion of a 2-arylaminoethanol to a displaceable halide or tosylate, followed by a base-catalyzed cyclization, afforded the *N*-arylaziridine. It was hoped that treatment of 8-[(2-chloroethyl)amino]-1,3-dipropylxanthine (**8**) with sodium hydride in dry methyl sulfoxide (DMSO) would allow the isolation of the aziridine. The methylene protons of the expected aziridine are chemically equivalent, and so the ^{1}H NMR spectrum of the desired aziridine should contain a four-proton singlet. Instead, the spectrum of the product contained a pair of two-proton triplets, identified as the methylene protons of the 8-substituent of **8**. Such a result suggested that instead of alkylation of the 8-amino group to form an aziridine, alkylation occurred at N-7 to form the more stable five-membered ring of the imidazo[2,1-*f*]purine, **10**.

The reaction of 8-bromo-1,3-dipropyxanthine, **4**, with maleimide was investigated in order to obtain an α,β-unsaturated carbonyl function in the 8-position. Only starting material was isolated after refluxing **4** and maleimide with *n*-butyllithium in THF. Since amines are known to react with cyclic anhydrides to form cyclic imides, it was thought that 8-amino-1,3-dipropylxanthine and maleic anhydride might react to yield the target molecule. However, **5** failed to react with maleic acid, at reflux in a variety of solvents. Reasoning that an aminomethyl group would be aliphatic in character and thus more reactive, **1** was reacted with *N*-acetylglycine to yield 8-(acetamidomethyl)-1,3-dipropylxanthine, **11**; acid hydrolysis afforded 8-(aminomethyl)-1,3-dipropylxanthine, **15** (Scheme 3). Stirring **15** with maleic anhydride afforded the intermediate α,β-unsaturated acid, **19**, that, on reflux in glacial acetic acid, yielded the desired maleimide **20**. Condensing **1** with higher acetamidoalkanoic acids gave intermediates **16**–**18**; *N*-acetyl-6-aminocaproic acid gave **22**.

Scheme 3



Scheme 4



A variety of xanthines containing an amino group in the 8-substituent had now been prepared. The acylation of these amines with bromoacetyl bromide, 3- or 4-(fluorosulfonyl)benzoyl chloride, benzoyl chloride, or ethyl chloroformate afforded the corresponding amides **23**–**27** (Scheme 4).

Scheme 5 outlines the synthesis of 1,3-dipropylxanthines with reactive substituents attached to an 8-cyclopentyl group through an ester linkage. The reaction of **1** with cyclopent-3-enecarboxylic acid yielded, after cyclization, 8-cyclopent-3-enyl-1,3-dipropylxanthine (**38**). Hydroboration with borane–THF complex, followed by oxidation with hydrogen peroxide, allowed the isolation of the required alcohol, 8-(3-hydroxycyclopentyl)-1,3-dipropylxanthine (**39**) as a mixture of the *cis* and *trans* isomers. The base-catalyzed acylation of **39** with either bromoacetyl bromide or with 3- or 4-(fluorosulfonyl)-benzoyl chloride yielded the potential alkylating agents **40**–**42**.

1,3-Dipropyl-8-cyclopentylxanthine analogues containing alkylating groups on the propyl side chains were prepared from 1-allyl-8-cyclopentyl-1-propylxanthine, **44** (Scheme 6). Hydroboration of **43** by means of borane–THF complex followed by oxidation with hydrogen peroxide gave a mixture of the 2- and 3-hydroxypropyl isomers, **44** and **45**. As both isomers had similar retention times on TLC, the mixture of isomers was not detected until the intermediate was reacted further with bromoacetyl bromide to yield two products, the 2- and 3-bromoacetoxypropyl esters, **46** and **47**, respectively, which were separable by reverse-phase HPLC in a 5:2 ratio. 9-Borabicyclo[3.3.1]nonane (9-BBN) has been reported to hydroborate olefins with exceptionally high regio- and stereoselectivity.[31,32] It also possesses greater air and thermal stability than borane. Hydroboration with 9-BBN, followed by oxidation with hydrogen peroxide, gave only the desired 3′-hydroxypropyl isomer, **45**, which on acylation with either 3- or 4-(fluorosulfonyl)benzoyl chloride gave the corresponding esters **52** and **53** (Scheme 7).

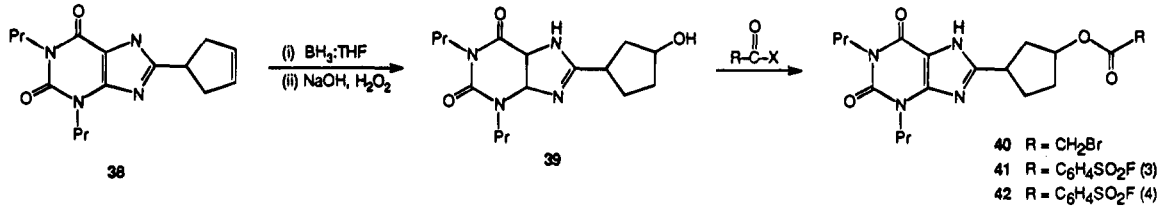
A different strategy gave 8-cyclopentyl-1-[3-[[4-(fluorosulfonyl)benzoyl]oxy]propyl]-3-propylxanthine, **54**, hwich is the 1-isomer of **53**. The synthesis of **55** can proceed from 3-allyl-6-amino-1-propyluracil, obtained by the alkylation of 6-amino-1-propyluracil with allyl bromide.[26] In our hands, however, that alkylation has a low yield and poor reproducibility. Starting the xanthine synthesis with *N*-allyl-*N*′-propylurea will give approximately equal amounts of the 1-allyl-3-propyl- and 3-allyl-1-propylxanthines, **48** and **43**, respectively. Although HPLC could not separate those isomers, preparative reverse-phase HPLC on C-18 silica separated the alcohols generated by 9-BBN/H_2O_2, **50** and **45**, and those alcohols were then acylated to form **53** and **54**. The synthesis starting with *N*,*N*′-diallylurea yielded 8-cyclopentyl-1,3-diallylxanthine, **49**, which, through hydroboration with 9-BBN, oxidation, and acylation, yielded 8-cyclopentyl-1,3-bis[3-[[4-(fluorosulfonyl)benzoyl]oxy]propyl]xanthine, **55**.

Tables 1 and 2 report the physical properties of novel xanthines.

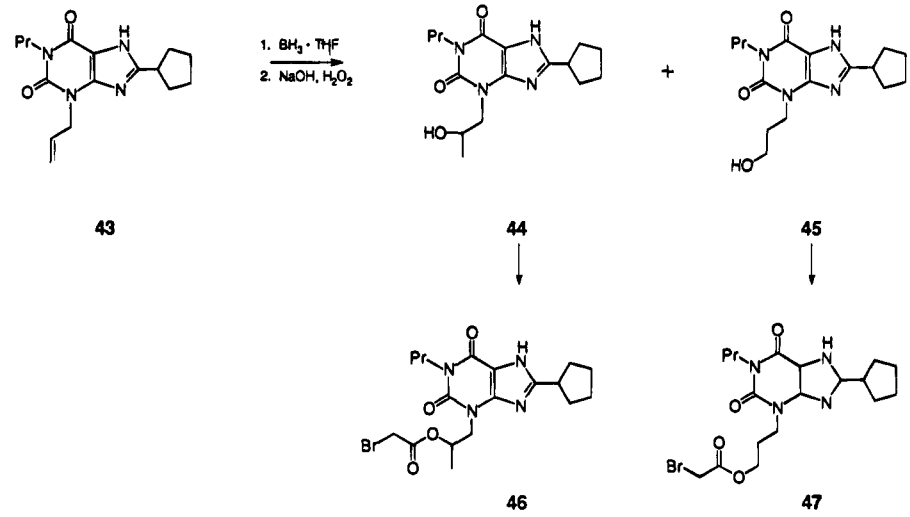
Pharmacology. The chemoreactive xanthine derivatives were tested for potency at and irreversible binding to the A_1AR of DDT_1 MF-2 (DDT) cells and, for selected ligands, to the A_{2a}AR of PC12 cells. The IC_{50} of the inhibition of specific [^{3}H]CPX binding to the A_1AR or of [^{3}H]-*N*-ethyladenosine-5′-uronamide ([^{3}H]NECA) to the A_{2a}AR measured antagonist potency. Preincubating the cells with antagonist for **2** h, followed by repeated washes (5 for DDT_1 MF-2 cells and 7 for PC12 cells) to remove free ligand and then measurement of radioligand binding to cell membranes, assessed the extent of inactivation of receptors. Analysis of binding data by the method of Scatchard[33] yielded estimates of receptor density (B_{max}) and affinity (K_D). The assessments of irreversible inactivation used an initial concentration of the reactive ligand that occupied greater than 50% of the receptor. Table 3 reports the results for assays at the A_1AR and Tables 4 reports the results for the A_{2a}AR.

1,3-Dipropylxanthines having haloaryl groups at-

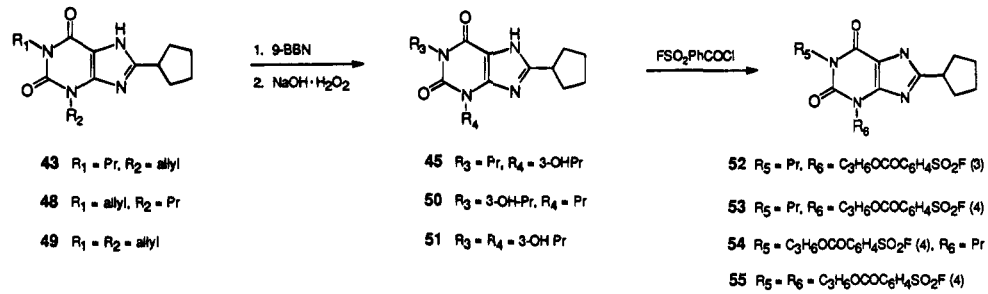
Scheme 5



Scheme 6



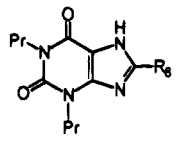
Scheme 7



tached to C-8 (**2**, **3**) antagonized [^{3}H]CPX binding at low-micromolar concentrations and the two 8-substituted nitrogen mustards (**8**, **9**) inhibited [^{3}H]CPX binding in the low-nanomolar range. However, these compounds did not inactivate the receptor; that is, washing antagonist-treated membranes fully restored [^{3}H]CPX binding. The lack of irreversible binding by compound **10** was perhaps not surprising since, as discussed in the chemistry section, a stable five-membered imidazole ring was formed during the synthesis instead of the reactive aziridine. The maleimidoxanthine derivative with a single methylene spacer (**21**) showed weak potency (IC_{50} >100 μM) for interacting with the A_1AR, but some irreversible receptor inhibition was observed. Expanding the spacer to five methylene groups (**22**) increased the potency to the low micromolar range but also reduced the irreversible binding. Bromoacetamides **24**–**27** were active in the low micromolar range. Increasing the number of methylene spacers from 0 to 3 (**23**–**26**) increased the potency by 28-fold; however, all of the compounds in this group showed minimal irreversible inactivation of the receptor (a <30% loss of [^{3}H]CPX binding).

The reactive fluorosulfonyl groups of analogues **28**–**35** attach to either position 3 or 4 of the benzoyl group, and up to five methylene residues separate them from the purine base. All inhibited [^{3}H]CPX binding in the low micromolar range and also irreversibly inactivated the receptor. Although the 3- and 4-substituted derivatives had similar IC_{50}s, at a concentration of 10 μM the 4-fluorosulfonyl derivatives inactivated the receptor to a greater extent than the 3-substituted derivatives. Such a result suggests that a 4-fluorosulfonyl moiety may come into closer proximity than a 3-fluorosulfonyl moiety to a nucleophile in the receptor. In contrast, Jacobson et al.[13] have reported that the 1,3-diisothiocyanatophenylene derivative of xanthine amine congener (3-DITC-XAC) is a more potent and efficacious irreversible antagonist of the A_1AR than 4-DITC-XAC. The reverse order of potency may be due to the different reactive species and the greater separation of the reactive group of 3-DITC-XAC from the xanthine pharmacophore. With the exception of **33**, all of the 8-fluorosulfonyl benzamides increased the K_D of [^{3}H]CPX binding, suggesting that the membranes retained some unbound antagonist that competed with [^{3}H]CPX for binding to the A_1AR. Additional experiments showed that **33** produced a concentration-dependent decrease in [^{3}H]CPX without a change in K_D. 8-(Benzamidomethyl)-1,3-dipropylxanthine, **36**, which lacks the reactive group of **33**, competitively inhibited [^{3}H]CPX binding but did not irreversibly inactivate the A_1AR. Since

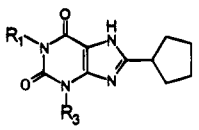
Table 1. Physical Properties of Novel 8-Substituted Xanthines



no.	R_8	synthesis	purification[a]	yield, %	mp, °C	formula	analysis
2	3-C_5H_3N-2-Cl	A	D/W	64	296–7	$C_{16}H_{18}ClN_5O_2 \cdot {}^1/_3H_2O$	C, H, N, Cl
3	C_6H_3-4-Cl-3-NO_2	A	D/W	65	270–2	$C_{17}H_{17}ClN_5O_4$	C, H, N, Cl
8	NHC_2H_4Cl	D	M/W	75	196–8	$C_{13}H_{20}ClN_5O_2$	C, H, N, Cl
9	$N(C_2H_4Cl)_2$	D	M/W	69	155–8	$C_{15}H_{23}Cl_2N_5O_2$	C, H, N, Cl
10	see Scheme 2		E/H	70	177–9	$C_{13}H_{18}N_5O_2$	C, H, N
19	$CH_2NHCOCH{=}CHCOOH$	F	M/W	72	183–5	$C_{16}H_{21}N_5O_5$	C, H, N
20	$C_5H_{10}NHCOCH{=}CHCOOH$	F	M/W	75	87–9	$C_{20}H_{29}N_5O_5$	C, H, N
21	$CH_2C_4H_2NO_2$	G	M/W	88	253–4	$C_{16}H_{19}N_5O_4$	C, H, N
22	$C_5H_{10}C_4H_2NO_2$	G	M/W	88	176–7	$C_{20}H_{27}N_5O_4$	C, H, N
23	$NHCOCH_2Br$	H	M/W	76	199–200	$C_{13}H_{18}BrN_5O_3$	C, H, N, Br
24	$CH_2NHCOCH_2Br$	H	M/W	78	193–4	$C_{14}H_{20}BrN_5O_3$	C, H, N, Br
25	$C_2H_4NHCOCH_2Br$	H	M/W	88	208–9	$C_{15}H_{22}BrN_5O_3$	C, H, N, Br
26	$C_3H_6NHCOCH_2Br$	H	M/W	72	198–9	$C_{16}H_{24}BrN_5O_3$	C, H, N, Br
27	$C_5H_{10}NHCOCH_2Br$	H	M/W	61	194–6	$C_{18}H_{28}BrN_5O_3$	C, H, N, Br
28	$NHCOC_6H_4$-3-SO_2F	I	M/W	49	239–41	$C_{18}H_{20}FN_5O_5S$	C, H, N, F
29	$CH_2NHCOC_6H_4$-3-SO_2F	J	M/W	88	211–2	$C_{19}H_{22}FN_5O_5S$	C, H, N, F
30	$C_2H_4NHCOC_6H_4$-3-SO_2F	J	M/W	65	219–20	$C_{20}H_{24}FN_5O_5S$	C, H, N, F
31	$C_3H_6NHCOC_6H_4$-3-SO_2F	J	M/W	77	199–200	$C_{21}H_{26}FN_5O_5S$	C, H, N, F
32	$NHCOC_6H_4$-4-SO_2F	I	M/W	68	240	$C_{18}H_{20}FN_5O_5S$	C, H, N, F
33	$CH_2NHCOC_6H_4$-4-SO_2F	J	M/W	75	264–5	$C_{19}H_{22}FN_5O_5S$	C, H, N, F
34	$C_2H_4NHCOC_6H_4$-4-SO_2F	J	M/W	68	230–1	$C_{20}H_{24}FN_5O_5S$	C, H, N, F
35	$C_3H_6NHCOC_6H_4$-4-SO_2F	J	M/W	75	239–42	$C_{21}H_{24}FN_5O_5S$	C, H, N, F
36	$CH_2NHCOC_6H_5$	J	M/W	65	201–2	$C_{19}H_{23}N_5O_3$	C, H, N
37	$CH_2NHCOOC_2H_5$	A	M/W	75	176–7	$C_{15}H_{23}N_5O_4$	C, H, N
38	c-C_5H_7	B	D/W	54	203–4[b]	$C_{16}H_{22}N_4O_2$	C, H, N
39	c-C_5H_9O		M/W	75	187–8	$C_{16}H_{24}N_4O_3$	C, H, N
40	c-$C_5H_8OCOCH_2Br$	H	HPLC[c]	74	113–4	$C_{18}H_{25}BrN_4O_4$	C, H, N, Br
41	c-$C_5H_8OCOC_6H_4$-3-SO_2F	K	HPLC[d]	58	175–6	$C_{23}H_{27}FN_4O_6S$	C, H, N, F
42	c-$C_5H_8OCOC_6H_4$-4-SO_2F	K	HPLC[e]	40	205–6	$C_{23}H_{27}FN_4O_6S$	C, H, N, F

[a] Abbreviations for recrystallization solvents are as follows: D/W, DMSO–water; M/W, methanol–water; E/H, ethyl acetate–hexanes. [b] Literature: 198–9 °C.[35] HPLC is reverse-phase HPLC with elution by methanol–water gradients; the initial and final methanol percentages being: [c]70 and 90; [d]80 and 100; and [e]80, isocratic.

Table 2. Physical Properties of Novel 1- and 3-Substituted 8-Cyclopentylxanthines[a]



no.	R_1 and R_3	synthesis	purification	yield, %	mp, °C	formula	analysis
45	$R_1 = C_3H_7$; $R_3 = C_3H_6OH$	L	M/W	75	207–8	$C_{16}H_{24}N_4O_3$	C, H, N, Cl
46	$R_1 = C_3H_7$; $R_3 = C_3H_6OCOCH_2Br$	H	HPLC[b]	50	139–40	$C_{16}H_{25}BrN_4O_4$	C, H, N
47	$R_1 = C_3H_7$; $R_3 = CH(CHOCOCH_2Br)CH_3$	H	HPLC[b]	18	—[c]	$C_{18}H_{25}BrN_4O_4$	C, H, N, Br
51	$R_1 = R_3 = C_3H_6OH$	L	M/W	65	210–1	$C_{16}H_{24}N_4O_4$	C, H, N, Br
52	$R_1 = C_3H_7$; $R_3 = C_3H_6OCOC_6H_4$-3-SO_2F	M	M/W	80	183–4	$C_{23}H_{27}FN_4O_6S$	C, H, N, F
53	$R_1 = C_3H_7$; $R_3 = C_3H_6OCOC_6H_4$-4-SO_2F	M	M/W	77	172–4	$C_{23}H_{27}FN_4O_6S$	C, H, N, F
54	$R_1 = C_3H_6OCOC_6H_4$-4-SO_2F; $R_3 = C_3H_7$	M	M/W	76	187–8	$C_{23}H_{27}FN_4O_6S$	C, H, N, F
55	$R_1 = R_2 = C_3H_6OCOC_6H_4$-4-$SO_2F$	M	M/W	61	154–7	$C_{30}H_{30}F_2N_4O_{10}S_2$	C, H, N, F

[a] Abbreviations as in Table 1. [b] Elution with a gradient of 50–70% methanol in water. [c] Not determined.

the fluorosulfonyl group can react with several amino acids, including serine, tyrosine, and histidine,[34] further work will be necessary to identify the amino acid to which **33** binds.

Since an 8-cyclopentyl group increases the affinity of a xanthine for the A_1AR, that substituent served as the point of attachment for the reactive groups. The bromoacetyl ester of 8-(3-hydroxycyclopentyl)-1,3-dipropylxanthine (**40**) inhibited [^{3}H]CPX binding with an IC_{50} of 20 nM. However, **40** produced only a small loss of receptors and an increase in the K_D of [^{3}H]CPX binding to the remaining receptors. Similarly, a high antagonist potency but a small loss of binding sites characterized the 3- and 4-fluorosulfonyl esters of 1,3-dipropyl-8-(3-hydroxycyclopentyl)xanthine, **41** and **42**.

Reactive groups attached to the 3-propyl group of CPX are also potent A_1AR antagonists. The bromoacetyl esters **46** and **47** inhibited [^{3}H]CPX binding at low nanomolar concentrations, but neither irreversibly inactivated the receptor. Likewise, the 3-(fluorosulfonyl)-benzoyl ester of 8-cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine, **52**, was active in the low-nanomolar range but, at concentrations as high as 1 μM, caused little irreversible inactivation of the receptor. The IC_{50} of the 4-(fluorosulfonyl)benzoyl ester, **53**, was also in the low-nanomolar range, but, in contrast to **52**, it caused 60% and 74% reductions of [^{3}H]CPX binding at concentrations of 10 and 50 nM, respectively. Moreover, **53** did not change the K_D of [^{3}H]CPX binding, evidence that unbound antagonist was completely washed out of the

Table 3. Antagonist Binding to the A_1AR

no.	IC_{50} vs [^{3}H]CPX, μM	% loss of specific [^{3}H]CPX binding (concn)[a]	K_D [^{3}H]CPX binding post-xanthine, nM[b]
2	2.5 ± 0.2	0 (10)	
3	7.0 ± 1.2	0 (10)	
8	0.008 ± 0.003	0 (10)	
9	0.09 ± 0.02	0 (10)	
21	>100	32 (100)	0.73
22	2.2 ± 0.6	16 (10)	
23	17 ± 4	18 (50)	1.3
24	11 ± 3	17 (10)	
25	4 ± 0.8	28 (10)	
26	0.6 ± 0.1	28 (10)	
27	1.2 ± 0.3	7 (10)	
28	3.5 ± 0.5	54 (10)	0.79
29	14 ± 2.1	38 (10)	0.69
		58 (50)	0.94
30	0.7 ± 0.2	65 (10)	1.1
31	2.6 ± 0.1	30 (10)	0.86
32	4.2 ± 1.5	88 (10)	1.1
33	2 ± 0.2	25 (0.1)	
		72 (1)	
		85 (10)	
34	0.7 ± 0.08	72 (10)	0.74
35	0.8 ± 0.13	75 (10)	1.8
36	6.3 ± 0.9	0 (10)	
40	0.02 ± 0.009	18 (1)	0.71
41	0.08 ± 0.004	7 (1)	1.3
42	0.06 ± 0.005	16 (1)	
46	0.011 ± 0.003	0 (1)	1.1
47	0.009 ± 0.002	0 (1)	1.5
52	0.02 ± 0.006	11 (1)	
53	0.01 ± 0.003	60 (0.01)	
		74 (0.05)	
54	0.087 ± 0.008	0 (1)	
55	3.5 ± 0.6	50 (10)	0.82

[a] Cells were pretreated with a xanthine derivative at the concentration (μM) in parentheses, and membranes were assayed for B_{max} and K_D as described in the Experimental Section. Control B_{max} was 247 ± 17 fmol/mg of protein. [b] Control K_D was 0.53 ± 0.08 nM. Only values greater than the control are reported.

Table 4. Antagonist Binding to the A_2AR

no.	IC_{50} vs [^{3}H]NECA (μM)	% loss of specific [^{3}H]NECA binding (concn)[a]	K_D [^{3}H]NECA binding post-xanthine, nM[b]
26	28 ± 8	0 (50)	28
29	45 ± 12	0 (50)	40
33	49 ± 9	0 (50)	51
35	26 ± 3	0 (50)	43
53	9 ± 2	0 (50)	53
54	>100	ND[c]	ND

[a] Cells were pretreated with a xanthine derivative at the concentration (μM) in parentheses, and membranes were assayed for B_{max} and K_D as described in the Experimental Section. Control B_{max} was 214 ± 9 fmol/mg of protein. [b] Control K_D was 10 ± 0.6 nM. [c] ND, not determined.

membranes. Such results support the conclusion that **53** is a potent irreversible antagonist of the A_1AR. The marked difference in capacity for irreversible binding between **52** and **53** indicates that small differences in the position of a reactive group can cause major changes in irreversible binding. The 4-(fluorosulfonyl)benzoyl ester of 8-cyclopentyl-1-(3-hydroxypropyl)-3-propylxanthine, **54**, was active in the sub-micromolar range, but it did not cause irreversible inactivation of the receptor. Finally, analogue **55**, which contains 4-(fluorosulfonyl)-benzoyl esters in both the 1- and 3-substituents, had an IC_{50} in the low-micromolar range and irreversibly inactivated the receptor. However, affinity for [^{3}H]CPX was reduced, indicating that washout of antagonist was incomplete.

Several of the xanthines were tested for potency and irreversibility of the antagonism of binding of [$_3$H]NECA to the $A_{2a}AR$ of PC12 cell membranes. Table 4 summarizes those observations. Xanthines containing as a C-8 substituent a bromoacetamide (**26**) or a fluorosulfonyl (**29**, **33**, and **35**) group antagonized [3H]NECA binding at micromolar concentrations. However, those analogues did not bind irreversibly, and each increased the K_D of [^{3}H]NECA binding, an indication that unbound agonist was still present after cell and membrane washing. Analogue **53** antagonized [^{3}H]NECA binding with an IC_{50} in the low micromolar range, but unlike its action at the A_1AR, it did not irreversibly inactivate the $A_{2a}AR$. Whereas analogue **54** interacted with the A_1AR at nanomolar concentrations, it only weakly interacted with the $A_{2a}AR$ (IC_{50} > 100 μM).

In summary, a variety of 1-, 3-, and 8-substituted xanthines have been synthesized and assayed for irreversible antagonism of the A_1AR. The assays showed that a 4-(fluorosulfonyl)benzoyl group, attached to either a 3- or 8-substituent via an amide or ester linkage, were the most efficacious of the irreversible A_1AR antagonists. Two analogues, 1,3-dipropyl-8-[[4-(fluorosulfonyl)-benzamido]methyl]xanthine, **33**, and 8-cyclopentyl-3-[3-[[4-(fluorosulfonyl)benzoyl]oxy]propyl]-1-propylxanthine, **53**, had several desirable characteristics: High affinity for the A_1AR, concentration-dependent inactivation of the receptor and ease of washing out of unbound ligand. Those properties indicate that the two antagonists will be useful for studies of the structure and function of the A_1AR.

Experimental Section

The 1,3-substituted 5,6-diaminouracils were synthesized by literature methods.[26,27] Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained using a Varian EM360L spectrometer at 60 MHz. Unless otherwise stated, DMSO-d_6 was used as a solvent, and resonances are reported as the chemical shift, δ, from a TMS internal standard. M-H-W Laboratories, Phoenix, AZ, performed the elemental analyses, which were acceptable if within 0.4% of the theoretical composition.

1,3-Dipropyl-8-(6-chloro-3-pyridyl)xanthine (2). Method A. 6-Chloronicotinoyl chloride (4.6 g, 26.3 mmol) in dry methylene chloride (50 mL) was added dropwise to a solution of 1 (4.4 g, 19.4 mmol) in dry pyridine (20 mL) under an atmosphere of nitrogen. The solution was stirred for 2 h at room temperature, water (50 mL) was added to quench the reaction, and the solvent was evaporated under reduced pressure to afford an orange solid. This crude solid was refluxed for 2 h in 2 N sodium hydroxide (50 mL). After cooling on ice, the pH was adjusted to 7 using concentrated HCl. The solid that precipitated was recrystallized from DMSO and water. ^{1}H NMR (DMSO-d_6) δ 0.90 (2 t, 6H, $CH_2CH_2CH_3$), 1.41–1.92 (m, 4H, $CH_2CH_2CH_3$), 3.89 (t, 4H, $CH_2CH_2CH_3$), 7.69 (d, 1H, pyridyl), 8.50 (2 d, 1H, pyridyl), 9.32 (d, 1H, pyridyl).

1,3-Dipropyl-8-[(2-hydroxyethyl)amino]xanthine. (6). Method B. A suspension of **4** (2.0 g, 6.3 mmol) in ethanolamine (5 mL, 82.8 mmol) and methanol (40 mL) was heated at 160 °C in a stainless steel bomb. After 48 h the methanol was evaporated *in vacuo* and the residue poured onto water. The solid that precipitated was recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.86 (2 t, 6H, $CH_2CH_2CH_3$), 1.39–1.88 (m, 4H, $CH_2CH_2CH_3$), 3.29–3.61 (m, 4H, CH_2CH_2-OH), 3.90 (2 t, 4H, $CH_2CH_2CH_3$), 4.73 (br s, 1H, NH), 7.02 (br t, 1H, OH).

8-[(2-Chloroethyl)amino]-1,3-dipropylxanthine (8). Method C. 8-[(2-Hydroxyethyl)amino]-1,3-dipropylxanthine (1.0 g, 3.4 mmol) in thionyl chloride (5 mL, 3.1 g, 25.8 mmol) was refluxed for 15 min and then poured onto ice. Extraction

with ethyl acetate and evaporation gave an oily residue that was triturated with ethyl acetate/hexane and crystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.85 (2 t, 6H, $CH_2CH_2CH_3$), 1.30-1.85 (m, 4H, $CH_2CH_2CH_3$), 3.60–4.05 (m, 8H, $CH_2CH_2CH_3$ and CH_2CH_2Cl), 6.95 (br s, 1H, NH).

1,3-Dipropyl-6,7-dihydro-2,4-dioxo-1*H*-imidazo[2,1-*f*]purine (10). A solution of **8** (0.6 g, 1.9 mmol) in 10 mL of dry DMSO containing NaH (0.077 g, 1.9 mmol) was stirred at 60 °C for 2 h. A second aliquot of NaH (0.077 g, 1.9 mmol) was added, and stirring at 60 °C continued for an additional 2 h. The mixture was poured into cold ethyl acetate and water (1:1, 50 mL). Extraction of the water layer with additional ethyl acetate (50 mL), drying ($MgSO_4$), and evaporation of the combined organic fractions yielded a brown oil. Recrystallization from ethyl acetate and hexane gave pure **10**: ^{1}H NMR (DMSO-d_6) δ 0.86 (2 t, 6H, $CH_2CH_2CH_3$), 1.48–1.72 (m, 4H, $CH_2CH_2CH_3$), 3.79–4.12 (m, 8H, $CH_2CH_2CH_3$ and CH_2CH_2), 7.39 (br s, 1H, NH).

8-(Acetamidomethyl)-1,3-dipropylxanthine (11). Method D. *N*-Acetylglycine (4.7 g, 40 mmol) and **1** (7.5 g, 33 mmol) were refluxed for 48 h in dry toluene, and water was removed by means of a Dean–Stark trap. Workup consisted of evaporating the solvent and recrystallizing from ethyl acetate and hexane: ^{1}H NMR (DMSO-d_6) δ 0.87 (2 t, 6H, $CH_2CH_2CH_3$), 1.36–2.01 (m, 4H, $CH_2CH_2CH_3$), 1.91 (s, 3H, CH_3), 3.85 (t, 2H, $CH_2CH_2CH_3$), 3.98 (t, 2H, $CH_2CH_2CH_3$), 4.35 (d, 2H, CH_2), 8.42 (br t, 1H, NH).

8-(Aminomethyl)-1,3-dipropylxanthane (15). Method E. Compound **11** (1.0 g, 3.3 mmol) was refluxed in 40 mL of aqueous 2 N HCl for 2 h, cooled, and adjusted to pH 8 with 1 N NaOH. The solid that precipitated was collected and washed with cold water: ^{H} NMR (DMSO-d_6) δ 0.89 (2 t, 6H, $CH_2CH_2CH_3$), 1.30–1.92 (m, 4H, $CH_2CH_2CH_3$), 3.85 (t, 2H, $CH_2CH_2CH_3$), 3.97 (t, 2H, $CH_2CH_2CH_3$), 4.20 (d, 2H, CH_2). 9.00 (br t, 1H, NH).

1,3-Dipropyl-8-(maleamidomethyl)xanthine (19). Method F. A solution of **15** (0.5 g, 1.9 mmol) and maleic anhydride (0.28 g, 2.9 mmol) in dry dioxane (20 mL) was stirred at room temperature. The solid that precipitated was filtered off and recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.87 (2 t, 6H, $CH_2CH_2CH_3$), 1.30–1.89 (m, 4H, $CH_2CH_2CH_3$), 3.85 (t, 2H, $CH_2CH_2CH_3$), 3.96 (t, 2H, $CH_2CH_2CH_3$), 4.26 (d, 2H, CH_2), 6.40 (2 d, 2H, CH=CH), 9.49 (br t, 1H, NH), 13.83 (br s, 1H, COOH).

1,3-Dipropyl-8-(maleimidomethyl)xanthine (21). Method G. Compound **19** (0.43 g, 1.2 mmol) was refluxed in glacial acetic acid for 4 h. Evaporation of the solvent yielded a white solid that was recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.88 (2 t, 6H, $CH_2CH_2CH_3$), 1.29–1.91 (m, 4H, $CH_2CH_2CH_3$), 3.86 (t, 2H, $CH_2CH_2CH_3$), 3.95 (t, 2H, $CH_2CH_2CH_3$), 4.77 (s, 2H, CH_2), 7.21 (S, 2H, CH=CH).

8-(Bromoacetamido)-1,3-dipropylxanthine (23). Method H. A solution of **5** (0.46 g, 1.7 mmol) and bromoacetyl bromide (0.23 mL, 2.6 mmol) in 1,4-dioxane was stirred at room temperature for 12 h. Evaporation of the solvent yielded a white solid that was recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.90 (2 t, 6H, $CH_2CH_2CH_3$), 1.31–1.90 (m, 4H, $CH_2CH_2CH_3$), 3.40 (br s, 1H, NH), 3.93 (m, 4H, $CH_2CH_2CH_3$), 4.15 (s, 2H, CH_2BR).

1,3-Dipropyl-8-[3-(fluorosulfonyl)benzamido]xanthine (28). Method I. A solution of **5** (1.0 g, 4.0 mmol) and 3-(fluorosulfonyl)benzoyl chloride (0.77 mL, 4.8 mmol) in dry dioxane (40 mL) was refluxed for 4 h, and the solvent was evaporated, leaving a brown oil. Trituration with water resulted in the precipitation of a white solid that was recrystallized from methanol and water.

1,3-Dipropyl-8-[[3-(fluorosulfonyl)benzamido]methyl]xanthine (29). Method J. Compound **15** (0.5 g, 1.9 mmol) and 3-(fluorosulfonyl)benzoyl chloride (0.5 g, 2.3 mmol) were stirred at room temperature for 16 h. Water (10 mL) was added, and the solvent was evaporated under reduced pressure. The residual solid was washed with water and recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.89 (2 t, 6H, $CH_2CH_2CH_3$), 1.30–1.92 (m, 4H, $CH_2CH_2CH_3$), 3.88 (t, 2H, $CH_2CH_2CH_3$), 4.00 (t, 2H, $CH_2CH_2CH_3$), 4.66 (d, 2H, CH_2), 7.79–8.72 (m, 4H, phenyl), 9.62 (br t, 1H, NH).

1,3-Dipropyl-8-[(ethoxycarboxamido)methyl]xanthine (37). A mixture of **15** (1.0 g, 3.8 mmol) and ethyl chloroformate (0.43 mL, 4.5 mmol) in 40 mL of dry dioxane was stirred for 12 h at room temperature. Evaporation of the solvent yielded a white solid. The product was recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) 0.88 (2 t, 6H, $CH_2CH_2CH_3$), 1.18 (t, 3H, CH_3), 1.31–1.92 (m, 4H, $CH_2CH_2CH_3$), 3.92 (t, 2H, $CH_2CH_2CH_3$), 4.00 (t, 2H, CH_2), 4.30 (d, 2H, CH_2), 7.612 (br t, 1H, NH).

8-(3-Cyclopenten-1-yl)-1,3-dipropylxanthine[35] (38). A suspension of **1** (16.7 g, 74 mmol) and 3-cyclopentene-1-carboxylic acid[36] (10 g, 89 mmol) in 100 mL of toluene was refluxed for 12 h in a flask fitted with a Dean–Stark trap. The toluene was evaporated, and the residual acid was removed by co-evaporation with additional toluene. The oily residue was refluxed in 100 mL of 2 N NaOH for 1 h, cooled on ice, and neutralized with 36% HCl. The orange solid that separated was filtered off and recrystallized from DMSO and water; ^{1}H NMR ($CDCl_3$) δ 0.99 (2 t, 6H, $CH_2CH_2CH_3$), 1.66–1.91 (m, 2H, $CH_2CH_2CH_3$), 3.87 (d, 4H, cyclopentenyl 2- and 5-CH_2), 3.68–3.92 (m, 1H, cyclopentene 1-H), 4.04 (t, 2H, $CH_2CH_2CH_3$), 4.13 (t, 2H, $CH_2CH_2CH_3$) 5.82 (s, 2H, cyclopentene CH=CH).

8-(3-Hydroxycyclopentyl)-1,3-dipropylxanthine (39). In an atmosphere of dry nitrogen, **38** (4.77 g, 15.8 mmol) and borane–THF complex (31.5 mmol) in dry THF (100 mL) were stirred for 4 h at room temperature. Water (10 mL) was added cautiously to destroy the excess borane. Sodium hydroxide (3 N, 1.75 mL, 5.3 mmol) and the hydrogen peroxide (30%, 1.6 mL, 15.8 mmol) were added, and the solution was stirred for 1 h at 50 °C. Evaporation of the solvent yielded a white solid that was recrystallized from methanol and water: ^{1}H NMR (DMSO-d_6) δ 0.88 (2 t, 6H, $CH_2CH_2CH_3$), 1.33–2.15 (m, 10H, $CH_2CH_2CH_3$ and cyclopentyl CH_2), 3.46 (br s, 1H, cyclopentyl 1-CH), 3.87 (t, 2H, $CH_2CH_2CH_3$), 3.97 (t, 2H, $CH_2CH_2CH_3$), 4.32 (m, 1H, cyclopentyl 3-CH), 4.62 (br d, 1H, OH).

8-[3-(Bromoacetoxy)cyclopentyl]-1,3-dipropylxanthine (40). Under dry nitrogen a solution of **39** (0.5 g, 1.56 mmol) in 50 mL of 1,4-dioxane was treated with bromoacetyl bromide (0.2 mL, 2.3 mmol), and the mixture was stirred for 1 h at room temperature. The oily residue remaining after evaporation was purified by reverse-phase HPLC as described in Table 1: ^{1}H NMR (DMSO-d_6) δ 0.88 (2 t, 6H, $CH_2CH_2CH_3$), 1.34–2.27 (m, 10H, $CH_2CH_2CH_3$ and cyclopentyl CH_2), 3.42 (br s, 1H, cyclopentyl 1-CH), 3.89 (t, 2H, $CH_2CH_2CH_3$), 3.98 (t, 2H, $CH_2CH_2CH_3$), 4.16 (s, 2H, CH_2Br), 5.35 (br t, 1H, cyclopentyl 3-CH).

1,3-Dipropyl-8-[3-[[3-(fluorosulfonyl)benzoyl]oxy]cyclopentyl]xanthine (41). Method K. To a solution of **39** (0.5 g, 1.6 mmol) and *N*,*N*-dimethylethylamine in 40 mL of dry dioxane was added 3-(fluorosulfonyl)benzoyl chloride (0.29 mL, 1.9 mmol), and the solution was stirred at room temperature for 1 h. After the solvent was evaporated, the residual oil was taken up in methanol and purified using reverse-phase HPLC: ^{1}H NMR (DMSO-d_6) δ 0.90 (2 t, 6H, $CH_2CH_2CH_3$), 1.36–2.62 (m, 10 H, $CH_2CH_2CH_3$ and cyclopentyl CH_2), 3.50 (br s, 1H, cyclopentyl 1-CH), 3.89 (t, 2H, $CH_2CH_2CH_3$), 4.00 (t, 2H, $CH_2CH_2CH_3$), 5.63 (m, 1H, cyclopentyl 3-CH), 7.93–8.75 (m, 4H, phenyl *H*).

8-Cyclopentyl-3-(2-hydroxypropyl)-1-propylxanthine (44) and 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (45). 3-Allyl-8-cyclopentyl-1-propylxanthine, **43** (2.0 g, 6.6 mmol), and 1 M borane–THF complex (13.2 mmol) were stirred at room temperature in dry THF (50 mL) for 4 h. Water (5 mL) was added cautiously to destroy the excess borane. Sodium hydroxide (3 N, 0.8 mL, 2.4 mmol) and then 30% hydrogen peroxide (0.74 mL, 7.3 mmol) were added, and the solution was stirred at 50 °C for 1 h. Evaporation of the solvent yielded a white solid that was recrystallized from methanol and water: yield 1.4 g, 66%.

8-Cyclopentyl-3-[2-(bromoacetoxy)propyl]-1-propylxanthine (46) and 8-Cyclopentyl-3-[3-(bromoacetoxy)propyl]-1-propylxanthine (47). A solution of the mixture of **44** and **45** (0.5 g, 1.6 mmol) and bromoacetyl bromide (0.17 mL, 2.0 mmol) in dioxane (20 mL) was stirred for 2 h at room

temperature. Evaporation of the solvent yielded a white solid. Preparative HPLC on C-18 silica separated two products, **46** and **47**.

46: $R_1 = (CH_2)_2CH_3$, $R_3 = (CH_2)_3OCOCH_2Br$; yield 50%; mp 139–140 °C; ^1H NMR (DMSO-d_6) δ 0.89 (t, 3H, $CH_2CH_2CH_3$), 1.49–2.05 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2O$ and cyclopentyl CH_2), 3.14 (t, 1H, cyclopentyl CH), 3.83 (t, 2H, $CH_2CH_2CH_2O$), 3.92 (s, 2H, CH_2Br), 4.07 (2t, 4H, $CH_2CH_2CH_3$ and $CH_2CH_2CH_2O$).

47: $R_1 = (CH_2)_2CH_3$, $R_3 = CH_2CH(OCOCH_2Br)CH_3$; yield 18%; ^1H NMR (DMSO-d_6) δ 0.87 (t, 3H, 1-$CH_2CH_2CH_3$), 1.25 (d, 3H, 3-CH_2CHCH_3), 1.53–2.07 (m, 10H, $CH_2CH_2CH_3$ and cyclopentyl CH_2), 3.17 (t, 1 H, cyclopentyl CH), 3.84 (t, 2H, $CH_2CH_2CH_3$), 3.86 (s, 2H, CH_2Br), 4.02–4.24 (m, 3H, 3-CH_2CHCH_3).

8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (45). Method L. A solution of **43** (4.0 g, 13.2 mmol) and 9-BBN (0.5 M, 79.4 mL, 39.7 mmol) in dry THF (40 mL) was refluxed for 4 h. After cooling, water (20 mL) was added carefully. Sodium hydroxide (3 N, 1.6 mL, 4.9 mmol) and 30% hydrogen peroxide (1.49 mL, 14.6 mmol) were added, and the solution was stirred for 2 h at 50 °C. Evaporation of the solvent afforded a white solid that was purified by recrystallization from methanol and water: Yield 3.3 g, 78%; ^1H NMR ($CHCl_3$) δ 0.96 (t, 3H, $CH_2CH_2CH_3$), 1.43–2.37 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2OH$ and cyclopentyl CH_2), 3.19 (br t, 1H, cyclopentyl CH), 3.52 (m, 2H, $CH_2CH_2CH_2OH$), 4.05 (t, 2H, $CH_2CH_2CH_3$), 4.33 (t, 2H, $CH_2CH_2CH_2OH$), 4.70 (t, 1H, OH).

8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (45) and 8-Cyclopentyl-1-(3-hydroxypropyl)-3-propylxanthine (50). Method D, applied to the mixture of 1(3)-allyl-5,6-diamino-3(1)-propyluracil, obtained by the condensation of *N*-allyl-*N'*-propylurea with cyclopentanecarboxylic acid, gave a mixture of **43** and **48**. A solution of that mixture of xanthines (1.2 g, 4 mmol) and 0.5 M 9-BBN (8 mL, 4 mmol) in 30 mL of dry THF was refluxed overnight. HPLC showed that the reaction had consumed less than half the starting material, and so an additional 8 mL of 9-BBN was added and reflux continued for 5 h. HPLC revealed the reaction had not progressed, so it was quenched with water (5 mL). Oxidation according to method L by means of 3 N NaOH (0.5 mL, 1.5 mmol) and 30% H_2O_2 (0.45 mL, 4.4 mmol) and then evaporation gave a white solid. Preparative HPLC on C-18 silica developed with a linear gradient of 50% to 80% methanol in water eluted, in order, **45** (87 mg, 0.27 mmol), **50** (105 mg, 0.33 mmol), and starting material.

8-Cyclopentyl-1,3-bis(3-hydroxypropyl)xanthine (51). A solution of **49** (4.0 g, 13.3 mmol) in 40 mL of THF under a dry nitrogen atmosphere was treated with 0.5 M 9-BBN (106.5 mL, 53 mmol) for 4 h at reflux. Cooling, quenching with 20 mL of water, oxidation with 3 N NaOH (2 mL, 6 mmol), followed by 30% H_2O_2 (10 mL, 88 mmol) and concentration *in vacuo* precipitated a white solid that was filtered off and recrystallized from methanol and water: ^1H NMR ($CDCl_3$) δ 1.43–2.20 (m, 12 h, $CH_2CH_2CH_2OH$ and cyclopentyl CH_2), 3.23 (br t, 1H, cyclopentyl CH), 3.20–4.63 (m, 8H, CH_2CH_2, CH_2OH), 4.21 (t, 1H, OH), 4.30 (t, 1H, OH).

8-Cyclopentyl-3-[3-[[3-(fluorosulfonyl)benzoyl]oxy]propyl]-1-propylxanthine (52). Method M. A solution of **45** (0.5 g, 1.6 mmol) and 3-(fluorosulfonyl)benzoyl chloride (0.29 mL, 1.9 mmol) in dry dioxane (40 mL) was refluxed for 12 h. Evaporation gave a brown oil that was triturated with and then recrystallized from methanol and water: ^1H NMR ($CHCl_3$) δ 0.95 (t, 3H, $CH_2CH_2CH_3$), 1.50–2.54 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2O$, and cyclopentyl CH_2), 3.15 (t, 1H, cyclopentyl CH), 4.00 (m, 2H, $CH_2CH_2CH_2O$), 4.38 (t, 2H, $CH_2CH_2CH_3$), 4.48 (t, 2H, $CH_2CH_2CH_2O$), 7.60–8.66 (m, 4H, phenyl).

Biochemical Methods. Solutions. Stock solutions of the xanthine derivatives were prepared fresh on the day of the experiment by dissolving in DMSO to give a concentration of 10 mM. At the time of use, the compounds were diluted to the desired concentration and Hank's balanced saline solution (HBSS). Control assays contained the same final concentration of DMSO.

Cell Culture and Membrane Isolation. DDT_1 MF-2 cells grew as monolayers in Dulbecco's modification of Eagle's medium containing (5% fetal bovine serum, penicillin G (100 units/mL), streptomycin (0.1 mg/mL), and amphotericin B (2.5 μg/mL). Monolayers of PC12 cells were grown in dishes treated for 60 min at room temperature with a solution of poly-L-lysine (10 μg/mL). Incubation was at 37 °C in water-saturated air containing 5% CO_2. Initial cell density was (0.5–1) $\times 10^4$ cells/cm^2 and subculture was once (PC12) or twice (DDT_1 MF-2) weekly, using divalent cation-free phosphate-buffered saline (HBSS) containing 1 mM EDTA to detach the cells. Experiments used cultures that were 1 day preconfluent. Assays for the irreversible inactivation of the A_1AR or A2aAR consisted of removing the culture medium, washing the cells twice with 10 mL of HBSS and incubating them for 2 h at 37 °C in 20 mL of HBSS containing the antagonist. At the end of the exposure, the medium containing the antagonist was removed and the cells were washed (5 times for DDT_1 MF-2 cells and 7 times for PC12 cells) by incubating for 3 min with fresh, ligand-free HBSS.

The preparation of cell membranes began with the replacement of medium with 5 mL of 50 mM Tris·HCl, pH 7.4, containing 5 mM $MgCl_2$ (Tris-Mg), scraping off the cells with a rubber policeman and centrifugation for 10 min at 48000*g*. The pellet was resuspended in ice-cold Tris-Mg, dispersed with a Tekmar homogenizer for 10 s at setting 3, followed by centrifugation for 10 min at 48000*g*. Resuspension in Tris-Mg complete the preparation. Assays of membrane protein content[37] employed bovine serum albumin as a standard.

Radioligand Binding Assay. The specific binding of [^{3}H]-CPX to DDT_1 MF-2 and [^{3}H]NECA to PC12 cell membranes measured the A_1AR and A_{2a}AR density, respectively. Briefly, an assay consisted of incubating membranes (0.1–0.2 mg of protein) for 2 h at room temperature in 0.2 mL of Tris-Mg containing 0.06–4 nM [^{3}H]CPX of 1–10 nM [^{3}H]NECA. Mixtures containing [^{3}H]NECA also contained adenosine deaminase, 2 units/mL. Similar mixtures containing 10 μM N^6-cyclopentyladenosine (for [3H]CPX binding) or 10 μM NECA (for [^{3}H]NECA binding) defined unspecific binding. Filtration through GF/B glass fiber filters and washing three times with Tris-Mg separated free from bound ligand, which was then quantitated by liquid scintillation spectrometry. Assays of the binding of the xanthine antagonists to the A_1-AR or A2aAR employed Tris-Mg containing 1 nM [^{3}H]CPX or [^{3}H]NECA, respectively, and 7–10 concentrations of the xanthine. All assays were performed in triplicate; replicates differed by <6%.

Scatchard[33] plots determined the K_D and B_{max} of [^{3}H]CPX and [^{3}H]NECA binding and Hill plots served for the measurement of the IC_{50} of the inhibition of radioligand binding by the xanthines.

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