Structure-Activity Relationships of 8-Cycloalkyl-1,3-dipropylxanthines as Antagonists of Adenosine Receptors

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8-Substituted xanthines currently represent the most potent class of adenosine-receptor antagonists. A series of 8-substituted 1,3-dipropylxanthines was prepared and their potency as antagonists of A_1 and A_2 adenosine receptors of human platelets and rat adipocytes, respectively, were determined. No agents studied were as potent as 8-cyclopentyl-1,3-dipropylxanthine as antagonists of the A_1 adenosine receptor, but 8-(2-methylcyclopropyl)-1,3-dipropylxanthine was at least 1000-fold more potent as an antagonist of A_1 than of A_2 adenosine receptors. While most substitutions on the 8-cycloalkyl moiety caused decreased potency to inhibit both A_1 and A_2 adenosine receptors, 8-[*trans*-4-(acetamidomethyl)cyclohexyl]-1,3-dipropylxanthine was nearly equipotent as an antagonist of the two receptors and appeared to be the most potent antagonist of A_2 adenosine receptors reported to date.

Adenosine exerts diverse pharmacological effects and endogenous adenosine is presumed to be an important physiological regulator in mammalian systems. Many of the effects of adenosine appear to result from interaction with at least two cell surface receptors[1,2] termed A_1 and A_2 adenosine receptors. This interaction causes inhibition (A_1) or stimulation (A_2) of intracellular adenylate cyclase activity. Most alkylxanthines inhibit both A_1 and A_2 adenosine receptors in a manner that appears to be the result of competitive antagonism of agonist binding. Adenosine can also exert an inhibitory effect on broken-cell preparations of adenylate cyclase by interaction with a site termed the P site, but it appears unlikely that adenosine exerts significant physiological effects by interaction with this intracellular site.[3] Xanthines do not inhibit the interaction of adenosine with the P site.

Substituted xanthines represent the most potent class of adenosine-receptor antagonists reported to date. Our work[4] and that of others[5] have established that structural modification of the 1- and 3-position of the xanthine nucleus does not greatly alter the ability of the compounds to inhibit either the A_1 or the A_2 adenosine receptor subtypes, although 1,3-dipropylxanthine appears to be the most potent antagonist in this series.[4,5]

The most dramatic alteration in potencies of the xanthines as antagonists of adenosine receptors results from substitution in the 8-position of this heterocyclic system. Substitution of alkyl, cycloalkyl, and aromatic groups on the 8-position of 1,3-dipropylxanthine generates compounds that are, in general, more potent than the parent 1,3-disubstituted compound as antagonists of adenosine receptors.[4-10] Modification of the 8-position has led to compounds that are highly selective A_1 antagonists by virtue of causing a much greater increase in affinity of the xanthine for the A_1 adenosine receptor subtype than for the A_2 adenosine receptor subtype. The most striking example is 1,3-dipropyl-8-cyclopentylxanthine. The parent 1,3-dipropylxanthine inhibits adenosine receptors of rat fat cells (A_1) and human platelets (A_2) with K_i values of 0.94 and 1.9 μM, respectively.[4] 1,3-Dipropyl-8-cyclopentylxanthine has a higher affinity for both receptor subtypes than the parent compound, but is about 150-fold more potent as anantagonist of A_1 adenosine receptors (K_i = 0.47 nM) than of A_2 adenosine receptors (K_i = 69 nM).

We have investigated the structure-activity relationships of 8-cycloalkyl-substituted 1,3-dipropylxanthines in an attempt to determine the characteristics of the domain of the A_1 adenosine receptor that are responsible for the rather remarkable affinity of the 8-cycloalkylxanthines for this receptor subtype. Many of the compounds reported here were also designed with the additional goal of obtaining potent antagonists with reactive functional groups that could be used to construct affinity chromatographic media for purification of adenosine receptors. A limited series of 8-cycloalkenylxanthines and the corresponding hydrogen halide addition products have been previously reported to be less potent than the parent cycloalkylxanthine.[11]

Results and Discussion

Chemistry. All 8-cycloalkylxanthines were prepared by reaction of 1,3-dipropyl-5,6-diaminouracil with an activated form of cycloalkanecarboxylic acid, i.e., an anhydride or acid chloride, or in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, followed by treatment with hot 2.5 M NaOH to effect ring closure. These are standard procedures for preparation of xanthines and are only variations on the method originally reported by Traube.[12] All 8-amino-substituted xanthines were prepared by reaction of 1,3-dipropyl-8-bromoxanthines with the appropriate amine. Generalized methods are described in the Experimental Section with details presented in Table I. In this paper the terms cis and trans refer to the relationship between the xanthine and the indicated substituent on the cycloalkyl ring.

Pharmacological Studies. 8-Cyclohexyl- and 8-cyclopentyl analogues of 1,3-dipropylxanthine are potent antagonists of A_1 and A_2 adenosine receptors (Tables II and III). Substitution of a carboxylic acid onto the 2- or 4-position of the cyclohexyl or the 2-position of the cy-

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Table I. Preparative Data and Physical Properties of 8-Substituted 1,3-Dipropylxanthines

no.	R′	synthetic method	reactn time (NaOH)	recrystn solvent	mp, °C	yield, %	formula[c]
	R = cyclohexyl						
1	*cis*-4-COOH	A	1 h	$EtOH/H_2O$	227–230	40	$C_{18}H_{26}N_4O_4$
2	*trans*-4-COOH	*a*	6 h	$EtOH/H_2O$	286–290	41	$C_{18}H_{26}N_4O_4$
3	*cis*-2-COOH	D	40 min	$MeOH/H_2O$	179–183	28	$C_{18}H_{26}N_4O_4$
4	*trans*-2-COOH	D	30 min	$MeOH/H_2O$	215–218	12	$C_{18}H_{26}N_4O_4$
5	*trans*-4-CO_2CH_3	F[b]		$MeOH/H_2O$	196–197	30	
6	*trans*-4-NH_2	C	1 h	$EtOH/H_2O$	>300	66	$C_{17}H_{27}N_5O_2\cdot HCl\cdot {}^1/_2H_2O$
7	*cis*-4-NH_2	C[d]	1 h	EtOH/EtOAc	243–244	13	$C_{17}H_{27}N_5O_2\cdot CF_3CO_2H$
8	*cis*-3-NH_2	C[d]	1 h	$EtOAc/Et_2O$	216–220	7	$C_{17}H_{27}N_5O_2\cdot CF_3CO_2H\cdot {}^1/_2H_2O$
9	*trans*-4-NHAc	B	40 min	$EtOH/H_2O$	307–310	52	$C_{19}H_{29}N_5O_3$
10	*cis*-4-NHAc	B	30 min	$EtOH/H_2O$	189–192	54	$C_{19}H_{29}N_5O_3$
11	*cis*-3-NHAc	B	30 min	$EtOH/H_2O$	287–291	46	$C_{19}H_{29}N_5O_3$
12	*trans*-4-CH_2NH_2	C	1 h	EtOH	>300	34	$C_{18}H_{29}N_5O_2\cdot HCl\cdot {}^1/_2EtOH$
13	*trans*-4-CH_2NHAc	B	1 h	$EtOH/H_2O$	250–252	40	$C_{20}H_{31}N_5O_3$
14	*trans*-4-$CON(CH_3)CH_2CH_2N(CH_3)_2$	F		EtOH/EtOAc	211–219	43	$C_{23}H_{38}N_6O_3\cdot HCl\cdot {}^3/_2H_2O$
15	*cis*-4-$CON(CH_3)CH_2CH_2N(CH_3)_2$	F		EtOAc/hexane	141–145	76	$C_{23}H_{38}N_6O_3$
16	*trans*-4-$CONHC_3H_7$	A	1 h	$EtOH/H_2O$	291–295	27	$C_{21}H_{33}N_5O_3$
	R = cyclopentyl						
17	*cis*-2-COOH	*a*		$EtOH/H_2O$	180–184	55	$C_{17}H_{24}N_4O_4$
18	*trans*-2-COOH	A	1 h	$MeOH/H_2O$	160–163	38	$C_{17}H_{24}N_4O_4$
19	*cis*-2-$CON(CH_3)CH_2CH_2N(CH_3)_2$	F		EtOAc/hexane	113–117	52	$C_{22}H_{36}N_6O_3$
20	*trans*-2-$CON(CH_3)CH_2CH_2N(CH_3)_2$	F		EtOH/EtOAc/hexane	117–181	67	$C_{22}H_{36}N_6O_3\cdot C_4H_4O_4$
	R =						
21	2-carboxycyclopent-1-enyl	D	1 h	$EtOH/H_2O$	214–216	60	$C_{17}H_{22}N_4O_2\cdot H_2O$
22	*trans*-2-carboxycyclobutyl	B	20 min	$EtOH/H_2O$	200–204	74	$C_{16}H_{22}N_4O_4$
23	pyrrolidino	*a*		*a*	273–275 dec	71	$C_{15}H_{23}N_5O_2$
24	cyclopentylamino	E		$EtOH/H_2O$	190–192	57	$C_{16}H_{25}N_5O_2$
25	cyclopentylmethyl	B	20 min	$EtOH/H_2O$	158–160[e]	14	$C_{17}H_{26}N_4O_2$
26	cyclobutylamino	E		$EtOH/H_2O$	185–187	84	$C_{15}H_{23}N_5O_2$
27	cyclohexylamino	E		$MeOH/H_2O$	186–189	23	$C_{17}H_{27}N_5O_2$
28	2-methylcyclopropyl	B	20 min	$EtOH/H_2O$	186–189	11	$C_{15}H_{22}N_4O_2$
29	1-adamantyl	*a*	3 h	EtOH	194–196	37	$C_{21}H_{30}N_4O_2$

[a] See Experimental Section. [b] Prepared from the corresponding acid chloride. [c] All compounds were within 0.4% of the calibrated values for C, H, N analysis for the formulas listed except compound 11 (H calcd-found = 0.48%). [d] Procedure C was followed, except the residue was washed with diethyl ether. [e] Previously reported in ref 24 (mp 158–159 °C).

Table II. K_i Values for Antagonism of A_1 and A_2 Adenosine Receptors by Derivatives of 8-Cyclohexyl-1,3-dipropylxanthine

no.	cyclohexyl substituent	K_i, μM A_1	K_i, μM A_2
	H	0.0025[a]	0.12[a]
1	*cis*-4-CO_2H	0.43 ± 0.012	>10
2	*trans*-4-CO_2H	0.059 ± 0.016	1.5 ± 0.13
3	*cis*-2-CO_2H	>10	>10
4	*trans*-2-CO_2H	>3	>3
5	*trans*-4-CO_2CH_3	0.13 ± 0.07	0.56[b]
6	*trans*-4-NH_2	0.090 ± 0.03	>10
7	*cis*-4-NH_2	0.16 ± 0.001	>10
8	*cis*-3-NH_2	0.050 ± 0.01	>10
9	*trans*-4-NHAc	0.012 ± 0.001	>10
10	*cis*-4-NHAc	0.070 ± 0.02	1.0 ± 0.02
11	*cis*-3-NHAc	0.041 ± 0.01	1.0 ± 0.4
12	*trans*-4-CH_2NH_2	0.020 ± 0.002	0.37 ± 0.02
13	*trans*-4-CH_2NHAc	0.008 ± 0.002	0.020 ± 0.01
14	*trans*-4-$CON(CH_3)CH_2CH_2N(CH_3)_2$	0.017 ± 0.002	0.26 ± 0.04
15	*cis*-4-$CON(CH_3)CH_2CH_2N(CH_3)_2$	0.085 ± 0.01	0.45 ± 0.12
16	*trans*-4-$CONHC_3H_7$	0.085 ± 0.005	>3

[a] Data from this laboratory.[4] [b] One assay with duplicate determinations.

Table III. K_i Values for Antagonism of A_1 and A_2 Adenosine Receptors by 8-Cyclopentyl-1,3-dipropylxanthines

no.	cyclopentyl substituent	K_i, μM A_1	K_i, μM A_2
	H	0.000 47[a]	0.069[a]
17	*cis*-2-CO_2H	0.14 ± 0.04	>10
18	*trans*-2-CO_2H	0.10 ± 0.006	>10
19	*cis*-2-$CON(CH_3)CH_2CH_2N(CH_3)_2$	>3	>3
20	*trans*-2-$CON(CH_3)CH_2CH_2N(CH_3)_2$	0.053 ± 0.017	>10

[a] Data from this laboratory.[4]

clopentyl group in either the cis or the trans geometry reduced potency to inhibit both receptor subtypes. However, the position and the geometry of the carboxyl group did influence the extent of the reduction in affinity. Thus, while the *cis*- and *trans*-2-carboxy analogues of 8-cyclohexyl-1,3-dipropylxanthine (**3**, **4**) and 8-(*cis*-4-carboxycyclohexyl)-1,3-dipropylxanthine (**1**) had K_i values greater than 0.4 μM as antagonists of A_1 and A_2 adenosine receptors, 8-(*trans*-4-carboxycyclohexyl)-1,3-dipropylxanthine (**2**) inhibited A_1 adenosine receptors with a K_i of 59 nM. While this compound was relatively potent as an antagonist of the A_1 adenosine receptor, it was nonetheless only about one-twentieth as potent as the parent 8-cyclohexylxanthine. The reduction in potency appeared to be due to steric hindrance, and not repulsion of the negative charge of the carboxylate group, because the corresponding methyl ester (**5**, Table II) and propylamide (**16**, Table II) were even less potent than compound **2** as antagonists of the A_1 adenosine receptor.

The addition of an amino group to the 3- or 4-position of the cyclohexyl moiety of 1,3-dipropyl-8-cycloalkylxanthine, like addition of a carboxylic acid group, reduced the potency of the parent compound as an antagonist of adenosine receptors (Table II). Acetylation of the amino function had little effect on the potency of these compounds as antagonists of A_1 adenosine receptors, but acetylation of the *cis*-4-amino- and *cis*-3-amino-substituted compounds (**10** and **11**) appeared to increase the potency as antagonists of the A_2 adenosine receptor. Interestingly,

Table IV. K_i Values for Antagonism of A_1 and A_2 Adenosine Receptors by 8-Substituted 1,3-Dipropylxanthines

no.	R substituent	K_i, μM	
		A_1	A_2
	cyclopentyl	0.000 47[a]	0.069[a]
	piperidino	0.022[a]	0.49[a]
21	2-carboxycyclopent-1-enyl	>10	>10
22	*trans*-2-carboxycyclobutyl	0.66 ± 0.07	>10
23	pyrrolidino	0.076 ± 0.013	>3
24	cyclopentylamino	0.0080 ± 0.004	0.13 ± 0.006
25	cyclopentylmethyl	0.069 ± 0.016	>10
26	cyclobutylamino	0.0026 ± 0.0006	0.12 ± 0.06
27	cyclohexylamino	0.014 ± 0.006	0.68 ± 0.15
28	2-methylcyclopropyl	0.011 ± 0.003	>10
29	1-adamantyl	0.082 ± 0.0015	>5
	cyclopropyl	0.042[a]	0.24[a]

[a] Data from this laboratory.[4]

the *trans*-4-aminomethyl compound **12** and, especially, the corresponding acetamide (**13**) were potent antagonists of both A_1 and A_2 adenosine receptors. Indeed, 1,3-dipropyl-8-[*trans*-4-(acetamidomethyl)cyclohexyl]xanthine (**13**) was, in our hands, the most potent antagonist of A_2 adenosine receptors reported to date. The potency of this compound is remarkable considering the other acetamido derivatives shown in Table II had K_i values of 1 μM or above as antagonists of the A_2 adenosine receptor.

PD115,199, the *N,N′,N′*-trimethylethylenediamine amide of 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine, has been reported to be a potent antagonist of both A_1 and A_2 adenosine receptors.[13] We prepared a series of *N,N′,N′*-trimethylethylenediamine amides of the carboxylic acid derivatives reported in Table II. The effects of this substitution are complex. The *cis*- and *trans*-4-amide derivatives were considerably more potent than the corresponding carboxylic acid derivatives as antagonists of the A_2 adenosine receptor (compare compounds **1** and **2** with **15** and **14**). On the other hand, the *cis*-2-amidocyclopentyl derivative (**19**, Table III) appeared to be less potent than the corresponding carboxylic acid (**17**) as an antagonist of the A_1 adenosine receptor while the *trans*-2-amide derivative (**20**) was more potent than the parent carboxylic acid (**18**). The relative effect on affinity for the A_2 adenosine receptor could not be determined because the K_i values were greater than the solubility of the compounds.

Martinson et al.[4] reported that 8-piperidino-1,3-dipropylxanthine was a relatively potent antagonist of A_1 and A_2 adenosine receptors with K_i values of 22 and 490 nM, respectively. We have expanded this series in light of the high potency of the 8-cyclopentyl and 8-cyclohexyl analogues to determine if increased potency would derive from other cycloalkylamino substituents (Table IV). In contrast to the increased potency derived from substitution of cyclopentyl for cyclohexyl, the pyrrolidino analogue (**23**, Table IV) was less potent than the piperidino analogue as an antagonist of either A_1 or A_2 adenosine receptors. However, 1,3-dipropyl-8-(cyclobutylamino)xanthine (compound **26**) was equipotent with 1,3-dipropyl-8-cyclohexylxanthine despite being isomeric with the pyrrolidino analogue (**23**). Likewise, the cyclopentylamino analogue (**24**) was more potent than the isomeric piperidino compound. The amino function appeared to contribute to potency since isosteric substitution of a methylene (**25**) for an amino group (**24**) gave a compound with reduced potency as an antagonist of A_1 and A_2 adenosine receptors. Thus, the electronic effects of the 8-amino substituent appear to help increase the affinity of the xanthines for the adenosine receptors if the amino group is not constrained in a ring such as pyrrolidine. It was noted earlier[13] that the amino function of 1,3-dialkyl-8-aminoxanthines is only slightly basic and, thus, undoubtedly alters the charge distribution of the xanthine nucleus at the pH of the adenylate cyclase assays. Indeed, the ultraviolet absorption maximum (λ max) of 1,3-dipropylxanthine at neutral pH is at 271 nm while the λ max of 1,3-dipropyl-8-piperidinoxanthine is 304 nm at pH 2–13. These data could be interpreted to suggest that the 8-aminoxanthines bind to the receptors in a different orientation than the 8-cycloalkylxanthines or, more likely, that the 8-amino groups exists predominately in the imino form and, thus, constrains the amino substituents to a different orientation than that presented by the tetrahedral 8-carbon analogues.

Finally, Martinson et al.[4] reported that 1,3-dipropyl-8-cyclopropylxanthine was a more potent antagonist of A_1 than of A_2 adenosine receptors (K_i = 42 and 240 nM, respectively). We found that 1,3-dipropyl-8-(2-methylcyclopropyl)xanthine (**28**, Table IV) was a highly selective and potent antagonist of the A_1 compared to the A_2 adenosine receptor with K_i values of 11 nM and >10 μM, respectively. Thus, 1,3-dipropyl-8-(2-methylcyclopropyl)xanthine was at least 1000-fold more potent as an antagonist of A_1 than of A_2 adenosine receptors and appeared to be the most specific antagonist of A_1 adenosine receptors that has been reported, to date. This compound is a mixture of the enantiomers of the cis and trans diastereomers. We are actively pursuing the separation of these isomers in order to determine if one of the them exhibits even greater specificity for the A_1 adenosine receptor.

Experimental Section

Melting points were determined on a Laboratory Device Mel-Temp and are uncorrected. IR spectra were obtained on a Perkin-Elmer Model 257 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker NR-300 instrument with tetramethylsilane as an internal standard. Mass spectra were recorded on a Nermag 10-10C Quadrupole mass spectrometer or a VG 70-70E double-focusing mass spectrometer with direct inlet. Analytical and spectral data (IR, NMR, and MS) were all consistent with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

All solid products were dried under vacuum.

The following compounds were synthesized by published procedures: 1,3-dipropyl-5,6-diaminouracil;[14] *cis*- and *trans*-1,4-cyclohexanedicarboxylic acid monomethyl esters and *trans*-1,4-cyclohexanedicarboxylic acid monopropylamide;[15,16] *trans*-1,2-cyclopentanedicarboxylic acid monomethyl ester and *trans*-1,2-cyclobutanedicarboxylic acid monomethyl ester were prepared by adaptations of the methods reported by Smith;[14] *cis*- and *trans*-4-acetamidocyclohexanecarboxylic acids were synthesized following the method of Ferber[17] but with CH_2Cl_2 in place of AcOH and *cis*-3-acetamidocyclohexanecarboxylic acid and *trans*-4-(acetamidomethyl)cyclohexanecarboxylic acid were prepared by similar procedures. 1,3-Dipropyl-8-bromoxanthine (mp 191–192 °C) was prepared by a procedure reported for the preparation of 1-methyl-3-isobutyl-8-bromoxanthine.[18]

All xanthines were formed by ring closure of the 1,3-dipropyl-5-(acylamino)-6-aminouracil in hot (80 °C) aqueous NaOH (50 mL of 2.5 M NaOH for each 10 mmol of starting 1,3-di-

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propyl-5,6-diaminouracil). Reaction times are given in Table I. The reaction mixture was cooled to room temperature and insoluble material was removed by filtration. The filtrate was acidified by addition of concentrated hydrochloric acid and the resulting precipitate was collected by filtration or, in appropriate cases, the acidified solution was evaporated in order to isolate the hydrochloride salt when the final product was an amine.

General procedures listed in Table I are illustrated by specific examples in the following section.

Method A. 1,3-Dipropyl-8-(*cis*-4-carboxycyclohexyl)xanthine (1). A solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.92 g, 10.0 mmol) in 30 mL of water was added to a suspension of *cis*-1,4-cyclohexanedicarboxylic acid monomethyl ester (1.86 g, 10.0 mmol) and 1,3-dipropyl-5,6-diaminouracil (2.26 g, 10.0 mmol) in 30 mL of water, and the mixture was stirred at room temperature overnight under nitrogen. The resulting precipitate was collected, washed with water, and dried. The solid was treated with 2.5 M NaOH and the product was isolated by filtration after acidification of the solution. Recrystallization from $EtOH/H_2O$ gave 1.45 g of 1.

Method B. 1,3-Dipropyl-8-(*trans*-2-carboxycyclobutyl)xanthine (22). A solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.92 g, 10.0 mmol) in 30 mL of water was added to a suspension of *trans*-1,2-cyclobutanedicarboxylic acid monomethyl ester (1.58 g, 10.0 mmol) and 1,3-dipropyl-5,6-diaminouracil (2.26 g, 10.0 mmol) in 30 mL of water. The mixture was stirred for 2 h at room temperature under nitrogen atmosphere. The insoluble material was removed by filtration and the filtrate was evaporated. The residue was heated with 2.5 M NaOH as described before.

The resulting precipitate was collected by filtration after acidification of the solution and washed with water. Recrystallization from $EtOH/H_2O$ gave 2.47 g of 22.

Method C. 1,3-Dipropyl-8-[*trans*-4-(aminomethyl)cyclohexyl]xanthine (12). A solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.92 g, 10.0 mmol) in 70 mL of water was added to a suspension of *trans*-4-[(trifluoroacetamido)methyl]cyclohexanecarboxylic acid (2.53 g, 10.0 mmol) and 1,3-dipropyl-5,6-diaminouracil (2.26 g, 10.0 mmol) in 30 mL of water. The reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. The resulting precipitate was collected and washed with water. The solid was heated at 90 °C for 1 h in 50 mL of 2.5 M NaOH solution. The cooled reaction mixture was brought to pH 11 by addition of concentrated HCl and treated with charcoal. After the charcoal was removed by filtration, the solution was evaporated to dryness. The residue was washed with diethyl ether repeatedly. The solid was dissolved in water and acidified with 2 N HCl. The suspension was evaporated to dryness and the residue was washed with a small amount of EtOH and then extracted with hot EtOH. The extract was concentrated by evaporation of the EtOH. The resulting crystals were collected, washed with EtOH, and dried under reduced pressure to afford the hydrochloride salt of 12 (1.38 g).

Method D. 1,3-Dipropyl-8-(*cis*-2-carboxycyclopent-1-enyl)xanthine (21). To a solution of 1,3-dipropyl-5,6-diaminouracil (3.27 g, 14.5 mmol) in 100 mL of CH_2Cl_2 was added 1-cyclopentene-1,2-dicarboxylic anhydride (2.00 g, 14.5 mmol), and the mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was heated in 50 mL of 2.5 M NaOH at 80 °C for 1 h. The reaction mixture was acidified with concentrated HCl. The resulting precipitate was collected and washed with water. Recrystallization from EtOH/water gave 3.00 g of 21.

Method E. 1,3-Dipropyl-8-(cyclopentylamino)xanthine (24). A mixture of 1,3-dipropyl-8-bromoxanthine (2.0 g, 6.35 mmol) and 10 mL of cyclopentylamine was heated at 180 °C in a pressure tube for 2 days. After removal of the volatiles by evaporation, the residue was washed with water. The solid was dissolved in a mixture of EtOH and water and treated with charcoal. Recrystallization from EtOH/water gave 1.16 g of 24.

Method F. 1,3-Dipropyl-8-[*cis*-4-[[[2-(dimethylamino)ethyl]methylamino]carbonyl]cyclohexyl]xanthine (15). To a cooled solution of 1,3-dipropyl-8-(*cis*-4-carboxycyclohexyl)xanthine (0.36 g, 1.0 mmol) in 20 mL of DMF in an ice bath was added 0.16 mL of thionyl chloride. The mixture was stirred at room temperature for 2.5 h. To the cooled reaction mixture (ice bath) was added a solution of *N,N,N'*-trimethylethyldenediamine (1.5 mL) in 1.5 mL of DMF. After the mixture was stirred at room temperature for 2.5 h, it was evaporated to dryness on a vacuum pump. The residue was partitioned between $CHCl_3$ and water. The dried $CHCl_3$ was evaporated. Recrystallization from EtOAc/hexane gave 0.34 g of 15.

1,3-Dipropyl-8-(1-adamantyl)xanthine (19). To a cooled solution of 1,3-dipropyl-5,6-diaminouracil (2.17 g, 9.6 mmol) in 35 mL of pyridine in an ice bath was added 1-adamantanecarboxylic acid chloride (2.00 g, 10.0 mmol). The mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was heated in 50 mL of 2.5 M NaOH at 80 °C for 3 h. The reaction mixture was allowed to cool and water was added to dissolve the white needles.

The insoluble oil was collected and dissolved in a small amount of MeOH. To the solution was added 2 N HCl. The resulting crystals were collected by filtration. Recrystallization from EtOH gave 1.33 g of 19.

1,3-Dipropyl-8-(*cis*-2-carboxycyclopentyl)xanthine (17). 1,3-Dipropyl-8-(2-carboxycyclopent-1-enyl)xanthine (21) (0.40 g, 1.1 mmol) was hydrogenated in 50 mL of MeOH over 20 mg of PtO_2 at an initial pressure of 50 psi on a Parr hydrogenation apparatus until no more H_2 was absorbed. After removal of the catalyst by filtration, the solvent was evaporated. The residue was recrystallized from EtOH/water. The resulting crystals (starting compound 21) were removed by filtration. The filtrate was diluted with water and the resulting crystalline material collected to give 0.19 g of 17.

1,3-Dipropyl-8-pyrrolidinoxanthine (23). A mixture of 1,3-dipropyl-8-bromoxanthine (1.85 g, 5.87 mmol) and 10 mL of pyrrolidine was heated at 130 °C in a pressure tube for 21 h. After removal of the volatiles by evaporation, the residue was washed with water, 2.5 M NaOH, water, and 1 N HCl, successively, and dried to give 1.28 g of 23.

1,3-Dipropyl-8-(*trans*-4-carboxycyclohexyl)xanthine (2). A mixture of *trans*-1,4-cyclohexanedicarboxylic acid monomethyl ester (1.86 g, 10.0 mmol), 1,3-dipropyl-5,6-diaminouracil (2.40 g, 10.0 mmol) and *N,N'*-dicyclohexylcarbodiimide (2.14 g, 10.0 mmol) in 100 mL of CH_2Cl_2 was stirred at room temperature overnight. The resulting precipitate was removed by filtration and discarded. The filtrate was evaporated and the residue was heated at 80 °C for 6 h in 2.5 M NaOH solution (50 mL). The reaction mixture was allowed to cool and the insoluble material was removed by filtration. The cooled filtrate was acidified with concentrated HCl. The resulting precipitate was collected, washed with water, and recrystallized from EtOH/water to afford 2 (1.50 g).

***trans*-4-[(Trifluoroacetamido)methyl]cyclohexanecarboxylic Acid.** Trifluoroacetic anhydride (14 mL) was added to an ice-cooled solution of *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (11.60 g, 73.8 mmol) in 100 mL of CH_2Cl_2. The mixture was stirred at room temperature for 24 h. After evaporation of the solvent, ice–water was added to the residue. The resulting solid was collected. Recrystallization from EtOH/water gave 12.25 g of crystalline solid (65.6%): mp 166–170 °C.

The other trifluoroacetamido compounds were synthesized by similar methods: *cis*-4-(trifluoroacetamido)cyclohexanecarboxylic acid, 65%, mp 150–168 °C; *trans*-4-(trifluoroacetamido)cyclohexanecarboxylic acid, 50%, mp 257–261 °C; *cis*-3-(trifluoroacetamido)cyclohexanecarboxylic acid, 42%, mp 132–142 °C.

Membrane Preparations. A partially purified membrane fraction (P-2) was prepared by the method described by Kono et al.[19] from fat cells that were prepared by collagenase digestion of rat epididymal fat pads.[20] Fat cell membranes were stored at −73 °C in 250 mM sucrose, 50 mM glycylglycine, pH 7.4.

Membranes from outdated human platelets (American Red Cross) were prepared as described earlier.[4] Briefly, platelets were suspended in buffer composed of (mM) Tris·HCl (50), pH 7.5, NaCl (150), EDTA (20), and centrifuged at 1500*g* for 20 min. Platelets removed from the top of the cell pellet were resuspended in buffer and centrifuged again. The pellet was resuspended in a solution composed of 16 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$,

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5 mM EDTA, and 0.1 mM phenylmethanesulfonyl fluoride and frozen in liquid nitrogen. The suspension was thawed, and the resulting crude membranes were washed once in 10 mM triethanolamine, pH 7.5, containing 2 mM EDTA and stored at −73 °C in the same buffer.

Adenylate Cyclase Assays. The assay of adipocyte adenylate cyclase was based on that reported by Londos et al.[21] as modified by Martinson et al.[4] Incubations (30 min, 24 °C) were initiated by addition of [α-^{32}P]ATP (1 μCi) to each assay tube. Each tube (100 μL) contained 100 μM [α-^{32}P]ATP, 100 mM NaCl, 50 mM triethanolamine hydrochloride, pH 7.5, 4 mM $MgCl_2$, 1 mg/mL bovine serum albumin, 100 μM papaverine hydrochloride, 1 μM forskolin, 10 μM GTP, 2 mM creatine phosphate, 40 units/mL creatine kinase, 5 units/mL adenosine deaminase, and 2–6 μg of membrane protein. Reactions were terminated by sequential addition of zinc acetate (containing [^{3}H] cyclic adenosine monophosphate) and Na_2CO_3 followed by centrifugation. The cyclic adenosine monophosphate in the supernatant fraction was purified by sequential chromatography over Dowex-50 resin and neutral alumina.[22] ^{32}P and ^{3}H content were determined by liquid scintillation spectrometry. Recovery of ^{32}P was corrected on the basis of recovery of ^{3}H. Adenylate cyclase activity of platelet membranes was assayed in a similar manner except the mixture contained 2.1 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EGTA, 40 units/mL myokinase, and 20–40 μg of membrane protein and no forskolin or NaCl. Incubations were conducted for 20 min at 30 °C. Product accumulation was linear with time and membrane protein concentration in both systems. Protein was determined by the Bio-Rad protein assay with bovine gamma globulin as standards.

K_i Determinations. Inhibition constants were derived by transformation of the data according to Arunlakshana and Schild.[23] A plot of log (CR-1) on log [antagonist], where CR represents agonist EC_{50} in presence of divided by agonist EC_{50} in absence of antagonist, was derived by linear least-squares analysis of data from each experiment. Slopes of the plots were not significantly different from 1. K_i values for a single antagonist were derived from experiments with at least two membrane preparations except in cases where apparent K_i values exceeded the solubility of the compound. Agonists employed were N-ethyladenosin-5′-uronamide (NECA) (platelet) and (−)-N^6-((R)-phenylisopropyl)adenosine (R-PIA) (adipocyte). Agonists and antagonists were added to the assays from stock solutions (usually DMSO) so that the solvent was present at 1–2%. In every experiment appropriate solvents were included in the control tubes (agonist but no antagonist).

Agonists of adenosine receptors inhibit adipocyte and stimulate platelet adenylate cyclases by interaction with A_1 and A_2 adenosine receptors, respectively. In this study, as in the previous study,[4] R-PIA (10^{-5} M) caused a 50–60% reduction in forskolin (1 μM) stimulated adenylate cyclase of fat cell membranes (EC_{50} = 16 nM) and NECA (10^{-4} M) caused an approximately 200% increase in adenylate cyclase activity of the platelet membrane preparations (EC_{50} = 0.23 μM). In addition, the ability of at least one concentration of 1-methyl-3-isobutylxanthine to inhibit each membrane preparation was monitored to assure that the antagonism was similar to that previously reported.[4]

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Benzodiazepine Receptor Binding Activity of 9-(1-Phenylethyl)purines

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Several α-methyl analogues of the 9-benzylpurines that bind to the benzodiazepine receptor (BZR) were synthesized and tested for BZR-binding activity. Although introduction of a *m*-amino group and an 8-bromo substituent gave an additive increase in BZR affinity with 9-(3-aminobenzyl)-8-bromo-6-(dimethylamino)-9*H*-purine (4), addition of an α-methyl group to 4 resulted in a loss in BZR affinity. This loss in affinity is apparently due to repulsive, steric interactions between the 8-bromo and 9-(1-phenylethyl) substituents, which results in a conformation that is not optimal for interaction with the BZR. Several compounds were tested on a modified Geller–Seifter conflict schedule, but none exhibited significant anxiolytic activity.

The anxiolytic activity of the benzodiazepines (BZs) is mediated through high-affinity receptors (BZRs) in the central nervous system.[1-3] A variety of compounds have been proposed as possible endogenous ligands of the BZR,[4] including the purines, inosine, and hypoxanthine.[5,6] We recently reported the potent BZR-binding activity of a series of 9-benzylpurines;[7,8] the most active compound was 8-bromo-9-(3-formamidobenzyl)purine **5** (Table I), which was over 1000-fold more active than the unsubstituted parent **1** and had an IC_{50} of only half that of diazepam. Although **5** had potent affinity for the BZR, neither **5** nor any of its weaker binding congeners exhibited significant diazepam-like activity in the Geller–Seifter conflict paradigm.[9,10] To further explore the effect of structural changes on BZR-binding activity, we prepared several α-methyl analogues of the 9-benzylpurines in search of an

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