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NON-XANTHINE HETEROCYCLES: ACTIVITY AS ANTAGONISTS OF A_1- AND A_2-ADENOSINE RECEPTORS

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Abstract—A variety of non-xanthine heterocycles were found to be antagonists of binding of [^{3}H]phenylisopropyladenosine to rat brain A_1-adenosine receptors and of activation of adenylate cyclase via interaction of *N*-ethylcarboxamidoadenosine with A_2-adenosine receptors in human platelet and rat pheochromocytoma cell membranes. The pyrazolopyridines tracazolate, cartazolate and etazolate were several fold more potent than theophylline at both A_1- and A_2-adenosine receptors. The pyrazolopyridines, however, were still many fold less potent than 8-phenyltheophylline and other 8-phenyl-1,3-dialkylxanthines. A structurally related N^6-substituted 9-methyladenine was also a potent adenosine antagonist with selectivity for A_1 receptors. None of several aryl-substituted heterocycles, including a thiazolopyrimidine, imidazopyridines, benzimidazoles, a pyrazoloquinoline, a mesoionic xanthine analog and a triazolopyridazine exhibited the high potency typical of 8-phenyl-1,3-dialkylxanthines. A furyl-substituted triazoloquinazoline was very potent at both A_1 and A_2 receptors. A pteridin-2,4-dione, 1,3-dipropyllumazine, was somewhat less potent than theophylline at A_1- and A_2-adenosine receptors, whereas 1,3-dimethyllumazine was much less potent. A benzopteridin-2,4-dione, alloxazine, was somewhat more potent than theophylline. Other heterocycles with antagonist activity were the dibenzazepine carbamazepine and β-carboline-3-ethyl carboxylate. The phenylimidazoline clonidine had no activity, whereas a related dihydroxyphenylimidazoline was a weak non-competitive adenosine antagonist.

Xanthines, such as theophylline, caffeine and various 8-aryl-1,3-dialkylxanthines, have been widely used as antagonists of A_1- and A_2-adenosine receptors [1]. The 8-aryl-1,3-dialkylxanthines are very potent at both subclasses of adenosine receptors [2–11]. Certain 8-aryl-1,3-dipropylxanthines exhibit selectivity for A_1 receptors [4, 8, 9] and certain caffeine analogs exhibit some selectivity for A_2 receptors [12, 13]. Other classes of heterocyclic compounds exhibit antagonist activity at adenosine receptors. These include: 9-methyladenines [10, 14], various pyrazolopyridines (etazolate, cartazolate, tracazolate) [15–18], various pyrazolopyrimidines [19, 20], imidazopyrazines [21], a phenyl-substituted pyrazoloquinoline (CGS 8216) [22, 23], a furyl-substituted triazoloquinazoline (CGS 15943a) [24], a triazolopyridazine (CL218872) [15], various mesoionic analogs of xanthines [25], pteridin-2,4-diones (lumazine) and benzopteridin-2,4-diones [10, 26], β-carbolines [15, 27], barbiturates [28, 29] and dibenzazepines (carbamazepine) [30–33]. A phenylaminoimidazoline, clonidine, has been reported to antagonize adenosine responses in physiological experiments [34].

The non-xanthine adenosine antagonists have been largely neglected in behavioral and physiological studies, and little is known of structure–activity relationships within such heterocycles or relationships of such structures to those of active xanthines. In an effort to define further potent and/or selective adenosine receptor antagonists for biochemical and pharmacological studies, a range of heterocyclic compounds (Fig. 1) have been compared as inhibitors of binding of [^{3}H]N^6-phenylisopropyladenosine to A_1-adenosine receptors of rat brain membranes and as antagonists of activation of adenylate cyclase via interaction of *N*-ethylcarboxamidoadenosine with A_2-adenosine receptors in membranes from human platelets and/or rat pheochromocytoma (PC12) cells. Several of the heterocycles were as potent or more potent than theophylline at the A_1 and A_2 receptors, but none were highly selective.

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METHODS

Materials. The sources from which we obtained our materials were as follows: tracazolate from ICI Americas, Wilmington, DE; cartazolate and etazolate from the Squibb Institute, Princeton, NJ; 9-methyladenine and N^6-cyclohexyl-9-methyladenine from Dr. R. A. Olsson, University of South Florida, Tampa, FL; ARL-115 from Thomae GmbH, Biberach, West Germany; CGS 8216, CGS 15943a and carbamazepine from the Ciba-Geigy Corp., Summit, NJ; the mesoionic xanthine analogues from Dr. R. A. Glennon, Virginia Commonwealth University, Richmond, VA; etonitazene and *N*-acetyletonitazene from Drs. K. C. Rice and K. A. Jacobson, National Institutes of Health, Bethesda, MD; *N*-ethylcarboxamidoadenosine (NECA) from Research Bichemicals, Wayland, MA; 2-phenyl-1-methylbenzimidazole from the Aldrich Chemical

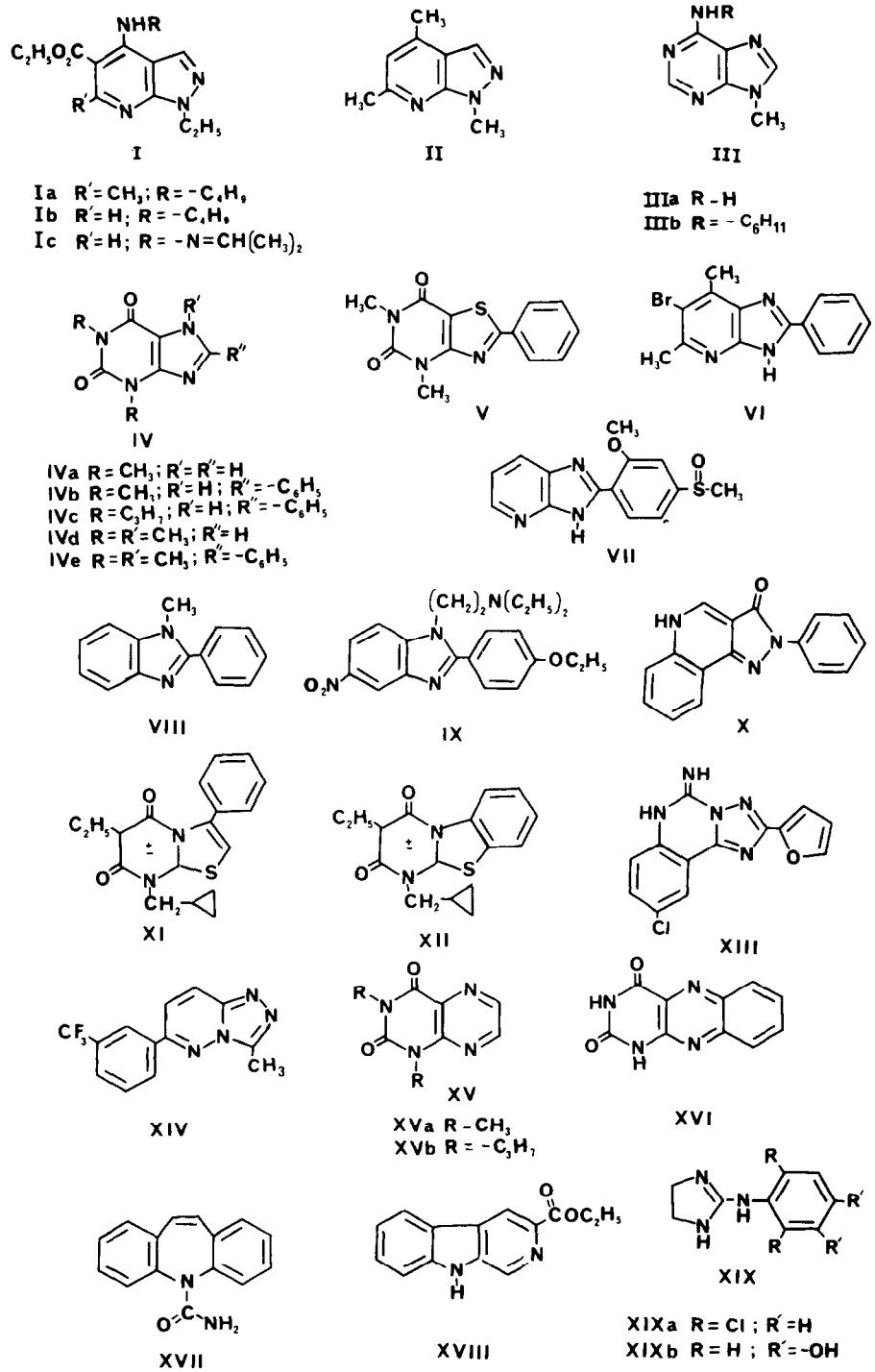


Fig. 1. Structures of xanthine and non-xanthine heterocycles.

Co., Milwaukee, WI; rolipram from Schering AG, Berlin, West Germany; alloxazine from the Sigma Chemical Co., St. Louis, MO; clonidine, oxymetazoline, ST 600, and 2-(3,4-dihydroxyphenylamino)imidazoline (DPI) from Smith Kline & French Laboratories, Philadelphia, PA; [^{3}H]N^6-phenylisopropyladenosine (49.9 Ci/mmol) and [^{3}H]xanthine amine congener (157 Ci/mmol, see Ref. 35) from New England Nuclear, Boston, MA; and [α-^{32}P]ATP (30 Ci/mmol) from Amersham, Arlington Heights, IL. Other compounds were from standard sources or were synthesized (see Schemes I–III) as described in the following sections.

1,4,6-*Trimethyl*-1H-*pyrazolo*[3,4-*b*]*pyridine (II)*. [Scheme I]. A solution of 7.37 g (0.16 mol) of methylhydrazine in 15 ml ethanol was added dropwise to a stirred solution of 27.15 g (0.16 mol) of ethylethoxy-

methylene cyanoacetic acid in 35 ml ethanol. After the exothermic reaction was over, the reaction mixture was refluxed for 2 hr. The solvent could be removed to yield a precipitate of 5-amino-1-methyl-4-ethylcarboxypyrazole or the reaction mixture could be used in the next step.

To the ethanol solution of 5-amino-1-methyl-4-ethylcarboxypyrazole was added 14 g of NaOH in 100 ml H_2O and 100 ml EtOH, and the reaction mixture was refluxed for 4 hr. The solvent volume was reduced *in vacuo*, and the reaction mixture was neutralized with conc. HCl. The resulting precipitate was filtered, washed with H_2O and $CHCl_3$ and dried to give 13.85 g (61.4%) of 5-amino-1-methyl-4-carboxypyrazole. This was decarboxylated by heating at 170–175°. The solid melted on heating and recrystallized upon cooling to give 9.51 g (100%) of 5-amino-1-methylpyrazole.

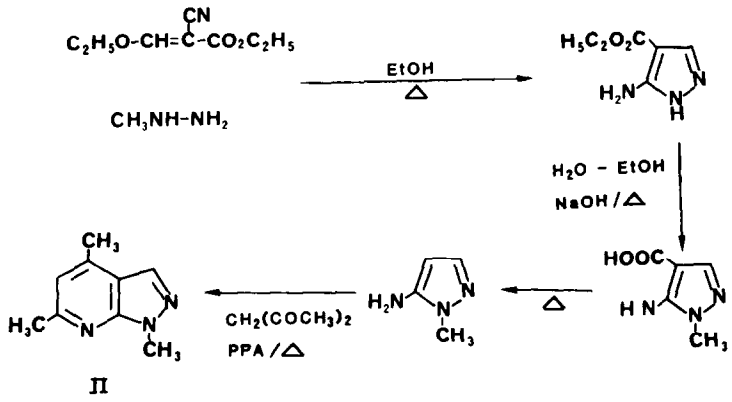
A mixture of 4.85 g (0.05 mol) of 1-methyl-5-aminopyrazole, 10 ml of acetylacetone, and 10 ml of polyphosphoric acid was heated for 30 min at 145–150°. Upon cooling, 100 ml of H_2O was added, and the reaction mixture was neutralized with aqueous ammonia. The resulting white precipitate was filtered and dried to give 4.02 g (50%) of 1,4,6-trimethyl-1*H*-pyrazolo[3,4-b]pyridine (II).

4,6-*Dimethyl*-2-*phenyl*-4,5,6,7-*tetrahydro*-5,7-*dioxothiazolo*[4,5-*d*]*pyrimidine (V)* [Scheme II]. A mixture of 7.75 g (0.05 mol) of 1,3-dimethyl-6-aminouracil, 7.18 g (0.05 mol) of benzylamine hydrochloride, and 7.5 ml of benzylamine was heated at 145° for 3 hr. The product solidified on cooling and was washed with H_2O and EtOAC, filtered, and dried to give 9.15 g (75%) of 1,3-dimethyl-6-benzylaminouracil.

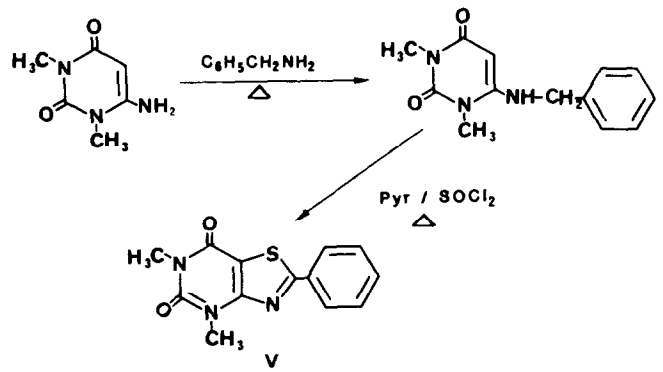
To a solution of 0.66 g (0.0027 mol) of 1,3-dimethyl-6-benzylaminouracil in 2.5 ml pyridine was added dropwise 50 ml of thionyl chloride while cooling. After the addition was complete, the reaction mixture was refluxed for 15 min and the solvent was removed *in vacuo*. Ethanol was added to hydrolyze the excess thionyl chloride and then removed *in vacuo*. Recrystallization with EtOH/Et_2O gave 0.50 g (68%) of 4,6-dimethyl-2-phenyl-4,5,6,7-tetrahydro-5,7-dioxothiazolo[4,5-d]pyrimidine (V).

5,7-*Dimethyl*-2-*phenyl*-6-*bromo*-1H-*imidazo*[4,5-*b*]*pyridine (VI)* [Scheme III]. A mixture of 5 g (0.045 mol) of 4,6-dimethyl-2-aminopyridine and 0.05 g of sodium acetate (anhydrous) in 25 ml acetic anhydride was refluxed for 3 hr. The unreacted anhydride was decomposed by dropwise addition of water. The aqueous solution was neutralized with $NaHCO_3$ and allowed to stand overnight. The resulting precipitate was filtered, washed with water, and dried to give 4.86 g (66%) of 2-acetamido-4,6-dimethylpyridine.

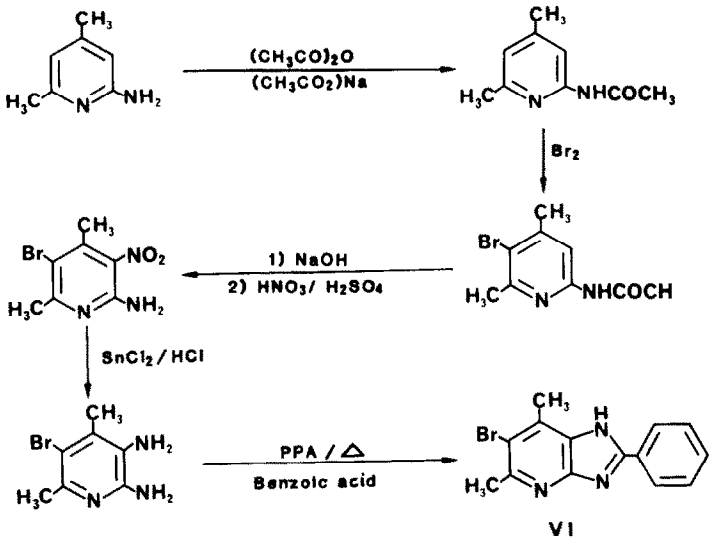
To 2.35 g (0.0143 mol) of 2-acetamido-4,6-dimethylpyridine in 40 ml of MeOH was added 3% Br_2 water (approx. 73 ml) until the yellow color persisted. The resulting white precipitate was filtered and dried to give 2.0 g (58%) of 2-acetamido-5-bromo-4,6-dimethylpyridine.



Scheme I.



Scheme II.



Scheme III.

A solution of 1.9 g (0.0078 mol) of 2-acetamido-5-bromo-4,6-dimethylpyridine in 60 ml of 15% NaOH was refluxed for 2 hr. The volume was reduced under pressure and the precipitate was filtered and dried to give 1.4 g (89%) of 2-amino-5-bromo-4,6-dimethylpyridine.

A solution of 1.4 g (0.007 mol) of 2-amino-5-bromo-4,6-dimethylpyridine in 4 ml H_2SO_4 was warmed to 55° and to this was cautiously added 0.8 ml of conc. HNO_3, so that the reaction temperature did not rise above 55°. The reaction mixture was stirred for 2 hr, while maintaining the temperature at 55°. After cooling, the reaction mixture was poured on 10 g of ice and partially neutralized with 40% NaOH to give a yellow precipitate. This was collected by filtration, washed several times with water, and dried to give 1.65 g (96%) of 2-amino-3-nitro-5-bromo-4,6-dimethylpyridine.

To a cooled solution of 5.09 g (0.0268 mol) of anhydrous stannous chloride in 30 ml conc. HCl was slowly added 1.65 g (0.0067 mol) of 2-amino-3-nitro-5-bromo-4,6-dimethylpyridine. The reaction mixture was heated at 90–95 for 30 min. It was then allowed to cool and made basic with 40% NaOH to give a precipitate which was collected by filtration and dried to give 0.98 g (68%) of 2,3-diamino-5-bromo-4,6-dimethylpyridine.

A mixture of 0.5495 g (0.0045 mol) of benzoic acid and 0.95 g (0.0044 mol) of 2,3-diamino-5-bromo-4,6-dimethylpyridine in 5 ml of polyphosphoric acid was heated at 175° for 4 hr. The reaction mixture was cooled, diluted with 50 ml of water, and filtered. Neutralization of the filtrate gave 0.4 g (30%) of 2-phenyl-6-bromo-5,7-dimethylimidazo[4,5-b]pyridine (VI).

Binding assay. Rat cerebral cortical membranes were prepared, and binding of [^{3}H]N^6-*R*-phenylisopropyladenosine and [^{3}H]xanthine amine congener ([^{3}H]XAC, 8[4-[[[[(2-aminoethyl)amino]-carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine) was assayed as described [35]. [^{3}H]XAC represents a high affinity antagonist ligand for adenosine receptors [35] which unlike the lower affinity antagonist ligand [^{3}H]1,3-diethyl-8-phenylxanthine [7] can be used at temperatures above 0°. Briefly, binding of 1 nM [^{3}H]N^6-*R*-phenylisopropyladenosine or 0.5 nM [^{3}H]XAC was measured in a total volume of 1 ml of 50 mM Tris buffer (pH 7.4) with 0.2 units adenosine deaminase and brain membranes (100 μg protein) after incubation for 90 min at 37° (or in some cases 0°) in the presence of various concentrations of antagonists. Nonspecific binding was determined in the presence of 5 mM theophylline. All assays were done in triplicate. Separation was by addition of 4 ml of ice-cold buffer followed by rapid filtration through Whatman GF/B glass fiber filters in a Brandel M-24R manifold (Brandel Instruments, Gaithersburg, MD), followed by washing twice with 5 ml of ice-cold buffer.

Adenylate cyclase assay. Human platelet and rat pheochromocytoma PC12 cell membranes were prepared, and adenylate cyclase activity was assayed as described [8]. Briefly, assays were in a total volume of 100 μl of 50 mM Tris–HCl, pH 7.4, containing 0.1 mM [α-^{32}P]ATP (0.3 μCi/tube), 0.1 mM cyclic AMP, 1 μg/ml adenosine deaminase, 0.1 mM rolipram, 5 mM creatine phosphate, 0.4 mg/ml creatinine kinase and 2 mg/ml bovine serum albumin. Concentrations of GTP and $MgCl_2$ were, respectively, 1 μM and 1 mM for platelet membranes (10–15 μg protein/tube) and 10 μM and 0.5 mM for PC12 cell membranes (5–10 μg protein/tube). Incubations were for 5 min at 37°. [^{32}P]Cyclic AMP formation was determined as described [8]. Dose–response curves for activation of platelet or PC12 adenylate cyclase by *N*-ethylcarboxyamidoadenosine (NECA) were carried out in the absence or presence of the antagonist. Each experiment was repeated three times.

Data analysis. The EC_{50} or IC_{50} values were obtained from concentration–response curves. K_i values for binding were obtained from IC_{50} values by the Cheng–Prusoff equation using a K_D for [^{3}H]N^6-*R*-phenylisopropyladenosine of 1.0 nM and a K_D for [^{3}H]xanthine amine congener of 1.2 nM. K_B values for adenylate cyclase were calculated using the Schild

equation and the ratio of EC_{50} values for NECA activation in the presence and absence of antagonist.

RESULTS AND DISCUSSION

Structure–activity relationships for xanthines as antagonists for adenosine receptors have been studied extensively in efforts to develop both potent and selective agents. At both A_1- and A_2-adenosine receptors, *n*-propyl moieties at the 1 and 3 positions confer higher potency than ethyl or methyl [3, 10]. The addition of an 8-phenyl moiety to theophylline (IVa, IVb) was discovered to confer a remarkable increase in potency for both A_1 and A_2 receptors [5, 7, 10]. Since those discoveries a variety of 8-phenylxanthines have been synthesized: Certain of these with 1,3-dipropyl moieties (IVc) are potent and selective antagonists for A_1 receptors [2, 4, 8, 9]. The effects of aryl substituents on potency and selectivity are influenced markedly by the nature of the alkyl substituents at the 1 and 3 positions [2]. Thus, the manner of interaction of the planar heterocyclic ring and the planar 8-aryl ring of an 8-aryl-1,3-dialkylxanthine with the receptor appears subject to change due to accommodation for alterations in either the nature of 1,3-alkyl substituents and/or the nature and position of 8-aryl substituents. Furthermore, although an 8-phenyl substituent markedly enhances the affinity of theophylline for adenosine receptors, this effect does not extend to caffeine (IVd, IVe), where an 8-phenyl substituent has only a modest effect [2]. Thus, the nature of the xanthine can influence whether or not the presence of an 8-aryl ring will enhance markedly affinity of the xanthine for adenosine receptors. Although a variety of heterocyclic compounds other than xanthines have been reported as antagonists of adenosine receptors (see Introduction), questions as to whether or not the planar heterocyclic rings of these compounds and of xanthines interact at the same site on the receptor; whether alterations in hydrophobic residues can be utilized as in the 1,3-dialkyl substituents of xanthines to alter activity; and whether or not aryl (or heteroaryl) substituents can augment activity have not been systematically explored. The present study on a variety of nonxanthine antagonists is relevant to these questions and to the possible development of selective A_1- or A_2-adenosine receptor antagonists.

Pyrazolopyridines. The pyrazolopyridines tracazolate, cartazolate, and etazolate (Ia, Ib, Ic) have been reported to inhibit 2-chloroadenosine-stimulated generation of cyclic AMP in guinea pig brain vesicular preparations at an A_2 receptor with estimated K_i values of 13, 2.0 and 3.9 μM respectively [18]. Tracazolate was reported to have K_i values versus binding of N^6-[^{3}H]cyclohexyladenosine to brain membranes of various species ranging from 0.22 μM in calf, to 0.31 μM in rat, to 5.2 μM in human [17]. Surprisingly, in view of the present studies and others [23, 25], it was stated that etazolate and cartazolate were inactive in such binding assays at 50 μM [17]. Tracazolate was reported in another publication to have K_i values at A_1-receptor binding sites in rat brain and rat testes membranes of 0.5 and 0.4 μM respectively [16]. Another study indicated a K_i for tracazolate at A_1-receptor binding sites in rat brain of 1.4 μM [15]. The present studies confirm the earlier reports and indicate that such pyrazolopyridines are potent adenosine receptor antagonists with some selectivity for the A_1 receptors (Table 1). Recently, tracazolate and cartazolate were reported as somewhat selective A_1-receptor antagonists based on binding data for brain A_1- and A_2-receptor sites [25]. The structural features responsible for potent interactions of these pyrazolopyridines with adenosine receptors are unknown. However, an effort to prepare a trimethylpyrazolopyridine (II) analogous to caffeine, but of course lacking oxygen moieties, yielded a compound virtually devoid of activity at adenosine receptors (Table 1). An analogy between the three pyrazolopyridines (Ia, Ib, Ic), which contain a substituted amine moiety on the 6-membered pyridine ring, and 9-methyladenines (IIIb) that contain a similar amine moiety on the 6-membered pyrimidine ring, is an attractive one. N^6-Substituted 9-methyladenines have been proposed to bind to adenosine receptors in the same orientation as N^6-substituted adenosines [14].

The pyrazolopyridines etazolate, cartazolate, and tracazolate have anxiolytic activities, proposed as due to interaction with the GABA–receptor-channel complex, where such compounds augment benzodiazepine binding in the same concentration range as for interactions with adenosine receptors [36, 37]. Analogies between compounds that affect the GABA–receptor-channel complex and also affect adenosine receptors have been made previously [27]. Etazolate also is a potent phosphodiesterase inhibitor [38]. It should be noted that barbiturates represent another class of compounds that enhance diazepam binding to the GABA–receptor-channel complex [39] and have similar potencies at such complexes and as antagonists at adenosine receptors [28, 29], suggesting again structural analogies between certain regulatory sites on the GABA–receptor-channel complex and antagonist sites on adenosine receptors. A wide variety of purines and xanthines inhibit binding of diazepam to the GABA–receptor-channel complex [40] and one N^6-substituted adenosine (EMD 28422) increases the apparent number of diazepam binding sites in brain membranes [41]. Further studies on other N^6-substituted analogs of these anxiolytic pyrazolopyridines (Ia, Ib, Ic) will be necessary to define the structural parameters that contribute to potent antagonist activity at adenosine receptors.

A variety of pyrazolopyrimidines containing various thio substituents (—SH, —SCH_2CONH_2, —$SCHCH_3CONH_2$) in the 6-membered pyrimidine ring are relatively potent adenosine antagonists with selectivity for brain A_1 receptors [19, 20]. Most contain a phenyl substituent in the pyrazole ring instead of the *N*-ethyl substituent of the pyrazolopyridines, tracazolate, cartazolate and etazolate. The relationships between these two structural classes of heterocyclic adenosine receptor antagonists are unclear, nor have such thio-substituted pyrazolopyrimidines been investigated with respect to the GABA–receptor-channel complex.

Table 1. Potencies of xanthine and non-xanthine heterocycles as antagonists at A_1 and A_2 adenosine receptors*

Heterocycle	A_1 K_i (μM) versus [^{3}H]PIA binding	A_2 K_B (μM) versus NECA-stimulation of adenylate cyclase	
	Rat brain	Rat PC12 cells	Human platelets
Pyrazolopyridines (PP)			
Ia Tracazolate	0.79 (0.70–0.89)	2.4 (0.7–3.2)	4.0 (3.1–5.3)
Ib Cartazolate	0.52 (0.42–0.66)	4.9 (0.6–4.0)	3.3 (1.2–6.5)
Ic Etazolate	2.7 (1.7–4.3)	3.6 (0.8–1.7)	4.6 (3.1–6.7)
II 1,4,6-Trimethyl-PP	>100 (10%)	—	⩾100
9-Methyladenines†			
IIIa N^6 = H	106 (87–129)	24 (19–30)	24 (21–27)
IIIb N^6 = Cyclohexyl	0.94 (0.40–2.2)	21 (12–38)	7.4 (2.2–25)
Xanthines‡			
IVa Theophylline	13 (11–15)	17 (16–19)	14 (4–18)
IVb 8-Phenyltheophylline	0.076 (0.058–0.098)	1.6 (0.4–6.1)	1.9 (0.5–6.8)
IVc 1,3-Dipropyl-8-phenylxanthine	0.01 (0.0055–0.018)	2.3 (0.6–8.7)	2.1 (1.3–3.6)
IVd Caffeine	44 (31–63)	37 (26–53)	30 (16–54)
IVe 8-Phenylcaffeine	15 (12–18)	15 (7.9–27)	14 (10–20)
Thiazolopyrimidine (TP)			
V 4,6-Dimethyl-2-phenyl-5,7-dioxo-TP	79 (69–91)	—	130 (114–148)
Imidazopyridine (IP)			
VI 6-Bromo-5,7-dimethyl-2-phenyl-IP	3.3 (2.3–4.6)	9.8 (4.0–14)	2.6 (2.0–3.3)
VII ARL 115	52 (47–58)	22 (11–44)	12.5 (10–16)
Benzimidazole (B)			
VIII 1-Methyl-2-phenyl-B	23 (10–50)	—	99 (80–124)
IX Etonitazene§	76 (52–110)	65 (19–220)	—

Pyrazoloquinolinone			
X CGS 8216	3.0 (2.2–4.2)	14 (3.2–61)	8.4 (7.0–10.2)
Mesoionic analogs			
XI Thiazolopyrimidine	33 (24–46)	—	34 (17–67)
XII Benzothiazolo-pyrimidine	15 (13–17)	12 (1.4–63)	6.3 (5.5–7.1)
Triazoloquinazoline			
XIII CGS 15943a	0.006 (0.0035–0.013)	0.0019 (0.0004–0.0087)	0.0021–(0.0011–0.0032)
Triazolopyridazine			
XIV CL 218872	22, 34	90 (50–160)	—
Pteridin-2,4-diones			
XVa 1,3-Dimethyl lumazine	>100 (20%)	—	95 (77–116)
XVb 1,3-Dipropyl-lumazine	20 (12–36)	—	45 (32–63)
XVI Alloxazine	9.1 (7.5–11.1)	20 (7.4–56)	6.0 (2.6–14)
Dibenzazepine			
XVII Carbamazepine	31 (16–62)	200‖	≫100
β-Carboline			
XVIII 3-Ethyl carboxylate	20 (16–25)	26 (6.3–108)	—
Phenylaminoimidazolines			
XIXa Clonidine	≫300 (0%)¶	≫300	—
XIXb DPI	18 (10–34)	60‖	—

* K_i and K_B values were calculated as described in Methods and are geometric means with 95% confidence limits from three experiments. In some cases, the percent inhibition of [^{3}H]PIA binding at the highest concentration tested is given following that concentration in parentheses.

† Data are from Ref. 15.

‡ Data are from Refs. 9, 12 and 14.

§ An analog of IX in which the nitro group was replaced with an *N*-acetamido group was less active at A_1 receptors (K_i 220 μM).

‖ A noncompetitive component was present (see Fig. 2).

¶ Oxymetazoline and the 2-methyl-5-fluoro analog of clonidine ST 600 were also inactive.

9-Methyladenines. Discovered during screening of a wide range of compounds as adenosine antagonists [10], the activity of 9-methyladenine (IIIa) has now been found to be enhanced markedly at A_1 receptors by the presence of N^6-substituents [14]. The effects of different N^6-substituents on activity of 9-methyladenines as adenosine receptor antagonists and the effects of the same N^6-substituents on activity of adenosines as agonists are similar, suggesting that the binding domain for N^6-substituents of adenosine and the N^6-substituents of 9-methyladenines are probably the same for both classes of compounds [14]. Certain N^6-substituted 9-methyladenines (IIIb) are more potent at A_1 than at A_2 receptors, whereas 9-methyladenine (IIIa) is more potent at A_2 receptors (Table 1).

Thiazolopyrimidines, imidazopyridines, benzimidazoles and other heterocycles containing aryl or heteroaryl substituents. Several heterocycles (V–X) containing aryl substituents that might be considered analogous to the 8-phenyl substituent of theophylline are adenosine antagonists, but none were particularly potent (Table 1). Whether this reflects an incorrect orientation for binding of the aryl ring to that subdomain of the receptor or merely a low affinity for the interaction of the heterocyclic portion with another subdomain is unknown. A more extensive series of such heterocycles including the parent compound without an aryl substituent would be required before interpretations can be advanced. The 2-phenyl-pyrazoloquinolinone CGS 8216 (X) has been reported to have a K_i value of 1.3 μM versus binding of [^{3}H]cyclohexyladenosine to A_1 receptors in rat brain membranes and to have a K_i value of 3.1 μM versus A_2-receptor stimulation of cyclic AMP accumulation in guinea pig brain vesicular preparations [15, 22]. This heterocycle has much more potent activity as a benzodiazepine antagonist at the GABA–receptor channel complex [22]. The furyl-substituted triazoloquinazoline CGS 15943a (XIII) has been proposed as a very potent A_2-selective adenosine receptor antagonist [24]. In the present study, CGS 15943a was found to be very potent at both A_1 and A_2 receptors (Table 1). It was about 3-fold more potent at the platelet and PC12 A_2 receptors than at the brain A_1 receptor. However, the potency (K_B 1.9 nM) at the A_2 receptor of PC12 cells (Table 1) was very similar to the potency [K_B 2.1 nM (1.4 to 3.2)] versus N^6-phenylisopropyladenosine inhibition of adenylate cyclase at the A_1 receptor of rat fat cell membranes. The latter unpublished data were obtained as described [8]. The triazolopyridazine CL 218872 (XIV) was relatively selective for the A_1 receptor (Table 1). CL 218872 was reported previously [15] to have a K_i at rat brain A_1 receptors of about 16 μM. It represents yet another heterocycle that interacts at sites on adenosine receptors and, in addition, at sites on the GABA–receptor-ion channel complex, the latter with higher affinity (see Ref. 15). Among the drugs containing an aryl substituent, corresponding to the 8-phenyl of xanthines, is etonitazene (IX), a very potent agonist for μ-opioid receptors [42]. Etonitazene was only a weak adenosine receptor antagonist with a K_i of about 70 μM at both A_1 and A_2 receptors. An 8-phenyl-9-oxo analog of theophylline has been reported to have low activity ($K_i > 100$ μM) at A_2 receptors in human fibroblasts [10].

The imidazopyridine ARL 115 (VII) and various analogs have cardiotonic activity [43]. However, it appears unlikely that the relatively weak antagonist activity of ARL 115 at adenosine receptors (Table 1) contributes to *in vivo* cardiotonic action of such compounds.

Mesoionic xanthine analogs. A number of mesoionic analogs of xanthines have been screened as antagonists of binding of [^{3}H]cyclohexyladenosine to rat brain A_1 receptors and as antagonists of A_2-receptor-mediated stimulation by 2-chloroadenosine of cyclic AMP accumulation in guinea pig brain slices [25]. None appeared markedly potent or selective. Two of these mesoionic compounds (XI, XII) proved in the present study to be relatively weak and non-selective antagonists for the rat brain and human platelet adenosine receptors (Table 1).

Pteridin-2,4-diones. 1,3-Dialkylpteridine-2,4-diones are structurally analogous to 1,3-dialkylxanthines. Such compounds and related benzopteridine-2,4-diones, such as alloxazine (XVI), were reported to be antagonists of A_2-adenosine receptors stimulatory to adenylate cyclase in human fibroblast cells [10]. Alloxazine, a benzopteridin-2,4-dione lacking alkyl substituents, was later reported to be a potent antagonist of brain A_1 receptors [26]. In the present study, 1,3-dimethyl- and 1,3-dipropyllumazine (XVa and XVb), analogous to theophylline and 1,3-dipropylxanthine, proved to be much less potent than the xanthines at both A_1 and A_2 receptors (Table 1). Surprisingly, alloxazine (XVI), which does not contain the alkyl substituents requisite for potency in the xanthine series, was quite potent at both A_1 and A_2 receptors (Table 1). The present potencies of alloxazine as an antagonist of A_2 receptors are in concordance with the K_i value of 1.1 μM for alloxazine at A_2 receptors in human fibroblasts [10] and the K_i value of 2.7 μM versus binding of [^{3}H]*N*-ethylcarboxamidoadenosine to rat striatal A_2 receptors [26].

β-Carbolines. One other class of heterocycles with activity at the GABA–receptor-channel complex has been reported to have activity at adenosine receptors: Thus, β-carboline-3-ethyl carboxylate, a benzodiazepine antagonist, was stated to have a K_i value of 1 μM at A_2-adenosine receptors in human fibroblasts [27]. β-Carboline-3-ethyl carboxylate proved to be much weaker as an antagonist of A_2 receptors in PC12 membranes (Table 1). It was also very weak at brain A_1 receptors. In binding studies at A_1 receptors of rat brain membranes, β-carboline-3-carboxylate was reported to have a K_i value of 20 μM [15]. In the present study, β-carboline-3-ethyl carboxylate had comparable potency at A_1 and A_2 receptors. Other agents, such as diazepam, RO 15-1788 and zopliclone that are active at the GABA–receptor-channel complex, were reported to be inactive in binding studies at A_1-adenosine receptors of rat brain membranes [15, 23]. RO 15-1788 was very weak ($K_B \gg 100$ μM) as an antagonist of A_2-adenosine receptors in PC12 membranes (unpublished data).

Dibenzazepines. The anticonvulsant carbamazepine and structural analogs have been reported as relatively potent and somewhat selective A_1-adeno-

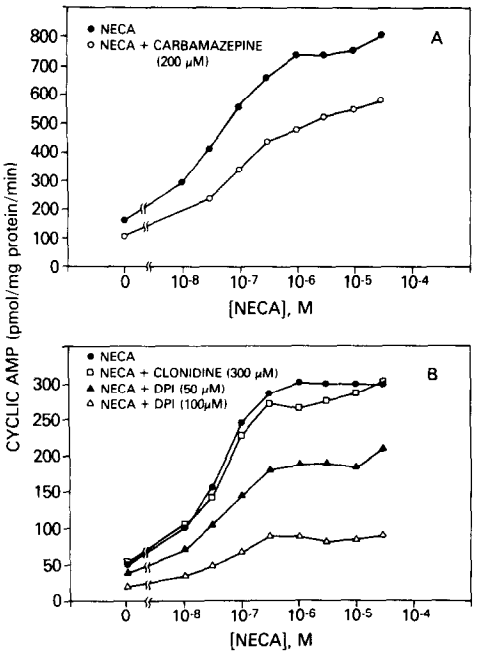


Fig. 2. Inhibition of NECA-stimulated adenylate cyclase activity by (A) carbamazepine and (B) DPI in membranes from rat PC12 cells. Mean values of three determinations are shown.

sine receptor antagonists [30–33]. The relationship to *in vivo* anticonvulsant activity of such dibenzazepines is unclear. The present results on antagonism of brain A_1-receptor binding and A_2-receptor activation of human platelet adenylate cyclase are consonant with the previous reports on brain A_1 and A_2 receptors. The antagonism at the A_2 receptor appeared to involve a noncompetitive component (Fig. 2A). The several-fold more potent effects of carbamazepine and analogs on binding of the antagonist [^{3}H]1,3-diethyl-8-phenylxanthine to brain membranes than on binding of the agonist [^{3}H]N^6-cyclohexyladenosine [32] appeared worthy of further study. For that reason the effects of carbamazepine and various other heterocycles on binding of the antagonist [^{3}H]XAC to A_1 receptors in rat brain membranes were compared to the effects on binding of the agonist [^{3}H]R-PIA (Table 2). There was no significant difference for carbamazepine in this comparison, nor were any of the other heteroaryl antagonists in any case significantly more potent versus [^{3}H]XAC binding than versus the agonist [^{3}H]PIA binding when assays were at 37°. Remarkably, the clonidine analog DPI (see below), while effective versus agonist binding, had no effect on antagonist binding. At 0°, the same temperature at which previous studies on binding of the antagonist [^{3}H]1,3-diethyl-8-phenylxanthine had been conducted [32]. carbamazepine and N^6-cyclohexyl-9-methyladenine were found to be somewhat more potent than at 37° versus [^{3}H]XAC binding (Table 2), whereas theophylline and 8-phenyltheophylline were not.

Phenylimidazolidines. Clonidine, a phenylaminoimidazoline, reported to antagonize adenosine actions in various physiological paradigms [34], could be considered as an arylamino heterocycle in analogy to 8-aryl xanthines. Clonidine (XIXa) and various analogs had no effect on the A_1 receptor (Table 1, see footnote). Remarkably, a 3,4-dihydroxyphenylimidazoline (XIXb, DPI) did inhibit binding of [^{3}H]phenylisopropyladenosine to A_1 receptors. Clonidine and DPI could not be tested in the platelet system, since the α_2-adrenergic agonist activity of such compounds is known to result in inhibition of adenylate cyclase. Indeed, clonidine and DPI did inhibit with IC_{50} values of 0.7 and 1 μM, respectively, adenylate cyclase in human platelet membranes (data not shown). Unlike clonidine, DPI appeared to be a full inhibitory agonist at α_2-adrenergic receptors and caused the same maximal inhibition in platelet membranes as epinephrine (for prior literature on DPI see Ref. 44). In rat pheochromocytoma PC12 membranes, which do not have α_2-adrenergic receptors, DPI did antagonize A_2-adenosine receptor-mediated activation of adenylate cyclase, whereas

Table 2. Comparison of potencies of xanthines and selected non-xanthine heterocycles as antagonistis of binding of an agonist N^6-[^{3}H]*R*-phenylisopropyladenosine (PIA) and an antagonist [^{3}H]xanthine amine congener (XAC) to A_1-adenosine receptors in rat brain membranes*

Heterocycle	K_i (μM) versus [^{3}H]PIA binding 37°	[^{3}H]XAC binding 37°	[^{3}H]XAC binding 0°
Ib Cartazolate	0.52 (0.42–0.66)	0.43 (0.19–0.99)	—
IIIb N^6-Cyclohexyl-9-methyladenine	0.94 (0.40–2.2)	1.2 (0.84–1.7)	0.31 (0.23–0.40)
IVa Theophylline	13 (11–15)	7.6 (5.1–12)†	7.5 (4.6–12)
IVb 8-Phenyltheophylline	0.076 (0.058–0.098)	0.074 (0.054–0.102)†	0.063 (0.004–0.09)
X CGS 8216	3.0 (2.2–4.2)	3.1 (1.3–7.1)	—
XIII CGS 15943a	0.0066 (0.0035–0.013)	0.0094 (0.0034–0.026)	—
XVI Alloxazine	9.1 (7.5–11)	61 (39–96)	—
XVII Carbamazepine	31 (16–62)	53 (29–97)	20 (9.8–42)
XIXa Clonidine	⩾300 (0%)	⩾300 (0%)	
XIXb DPI	18 (10–34)	⩾100 (0%)	

* K_i and K_B values were calculated as described in Methods and are geometric means with 95% confidence limits from three experiments. In some cases, the percent inhibition of binding at the highest concentration is given in parentheses following that concentration. K_i values versus [^{3}H]PIA are from Table 1.

† Data are from Ref. 36.

clonidine did not. The inhibition by DPI appeared to be noncompetitive (Fig. 2B).

In summary, clearly, further heterocycles need to be prepared and evaluated as adenosine antagonists before the relationship of xanthine binding to that of other heterocycles can be properly assessed. Indeed, little is as yet known as to the nature of structure–activity relationships for heterocycle interactions with that subdomain of adenosine receptors. At present, the 1,3-dialkylxanthines appear to represent a near optimal interaction, although recently the heteroaryl substituted tricyclic quinazoline (XIII) has been reported as a very potent A_2-selective antagonist [24]. It should be noted that several of the present heterocycles, in particular N^6-cyclohexyl-9-methyladenine (IIIb), an imidazopyridine (VI) and alloxazine (XVI) are more potent at A_2 receptors of human platelets than at A_2 receptors of rat pheochromocytoma cells, suggesting as has data on, xanthines [8] and adenosine analogs [45] that these two A_2-adenosine receptors are not identical. Since extensive structural studies on xanthines provide indications of directions toward development of only the A_1-selective antagonist, further studies on other heterocycles also need to be pursued in attempts to define whether or not certain structural alterations provide directions for development of A_2-selective antagonists. The present results do not provide any clear indications for new directions, which may mean that the heterocyclic domain of A_1 receptors has inherently higher binding affinity for all such entities than does the domain of A_2 receptors.

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