Benzo[1,2-*c*:5,4-*c*']dipyrazoles: Non-Xanthine Adenosine Antagonists

Norton P. Peet,* George A. Dickerson,† Abdulminium H. Abdallah,† John W. Daly,‡ and Dieter Ukena‡,§

Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215, and Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Building 8, Room 1A-15, Bethesda, Maryland 20892. *Received January 25, 1988*

3,5-Dimethylbenzo[1,2-*c*:5,4-*c*']dipyrazoles, optionally substituted in the 1-, 7-, and 8-positions, were synthesized from resorcinols. These compounds display affinity for adenosine A_1 (rat brain) and A_2 (human platelet) receptors. In addition, these compounds reverse contractions of guinea pig tracheal cylindrical segments induced by potassium chloride, histamine, acetylcholine, and 5-hydroxytryptamine, as well as reverse bronchospasm induced by aerosolized histamine in the conscious guinea pig.

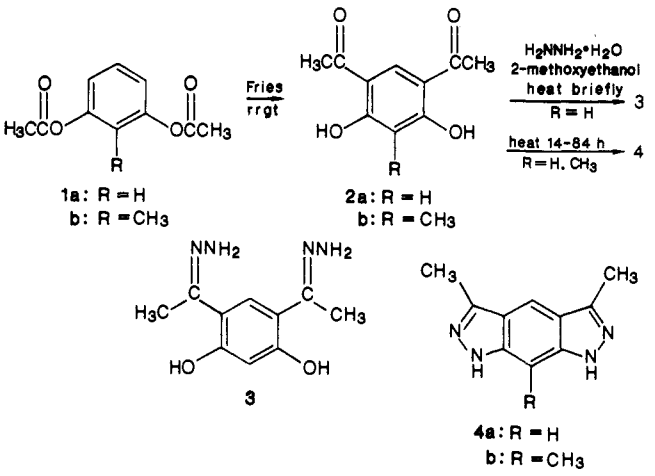
Two classes of membrane receptors for adenosine have been identified. A_1 receptors, inhibitory to adenylate cyclase, mediate cardiac depression, bronchoconstriction, renal vasoconstriction, and decreased lipolysis; A_2 receptors, stimulatory to adenylate cyclase, mediate vasodilation, increased steroid synthesis, and decreased platelet aggregation. Several other physiological effects, including sedation and analgesia, have also been attributed to agonism at one or the other of these receptor types.[1-4]

Because the actions of adenosine are so numerous, the potential for using adenosine receptor agonists and antagonists as therapeutic agents is great.[2,5] Antagonists of the xanthine type are currently in use. Caffeine is given to cause CNS stimulation,[6] diuresis,[7] and cerebral vasoconstriction (treatment of migraines),[8] while theophylline is used to cause bronchodilation (treatment of asthma).[2] Although such pharmacological effects were previously ascribed solely to phosphodiesterase inhibition, recent evidence points to adenosine antagonism as another often primary mechanism of action.[6,9,10,11] For instance, concentrations of caffeine and theophylline known to produce CNS stimulation are much lower than those required to inhibit phosphodiesterase.[6,7,12] In tissues where A_2 receptors predominate, xanthines oppose adenosine-elicited rises in cAMP; a phosphodiesterase inhibitor would be expected to potentiate these rises.[13] Antiasthmatic effects of xanthines, however, may involve primarily phosphodiesterase inhibition.[14]

The objective of many studies in the past few years has been the development of potent and selective antagonists for adenosine receptors.[15-24] Such agents are expected to be more useful therapeutically than caffeine or theophylline, due to a decreased likelihood of side effects.[17] The xanthine derivatives have been studied the most extensively.[3] However, other classes of adenosine antagonists are being researched: pteridines,[7] benzopteridines,[7] benzothiazolopyrimidines,[25] etazolate and similar compounds,[5,26] pyrazolopyrimidines,[5,27] pyrazolopyrimidin-7-ones,[28] pyrazoloquinolines,[29] and triazoloquinazolines[29] (Figure 1). Of these, alloxazine (a benzopteridine),[7] etazolate,[5,26] certain pyrazolopyrimidines,[5,27] certain pyrazolopyrimidin-7-ones,[28] and a triazoloquinazoline (CGS 15943A)[29] are reported to be at least twice as potent as theophylline at either A_1 or A_2 receptors. Recently, several additional non-xanthine heterocycles were compared as antagonists at A_1 and A_2 adenosine receptors.[30]

In the present study, a new class of non-xanthine adenosine antagonists is described. A series of benzo[1,2-*c*:5,4-*c*']dipyrazoles were synthesized and tested in two different systems for adenosine receptor antagonism and

Scheme I



* Address correspondence to this author at Merrell Dow Research Institute, P.O. Box 156300, Cincinnati, Ohio 45215-6300.

† Merrell Dow Research Institute, Indianapolis Center, Indianapolis, IN 46268.

‡ National Institute of Diabetes, Digestive and Kidney Diseases.

§ On leave from the Pharmakologisches Institut der Universitat Heidelberg, F.R.G., with support of the Deutsche Forschungsgemeinschaft (Uk 4.1-1).

(1) Daly, J. W. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **1985**, *19*, 29.
(2) Williams, M. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 315.
(3) Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.
(4) Taylor, M. D.; Moos, W. H.; Hamilton, H. W.; Szotek, D. S.; Patt, W. C.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Heffner, T. G.; Mertz, T. E. *J. Med. Chem.* **1986**, *29*, 346.
(5) Davies, L. P.; Brown, D. J.; Chow, S. C.; Johnston, G. A. R. *Neurosci. Lett.* **1983**, *41*, 189.
(6) Daly, J. W.; Butts-Lamb, P.; Padgett, W. *Cell Mol. Neurobiol.* **1983**, *3*, 69.
(7) Bruns, R. F. *Biochem. Pharmacol.* **1981**, *30*, 325.
(8) Neiforth, K. A.; Cohen, M. L. In *Principles of Medicinal Chemistry*, 2nd ed.; Foye, W. O., Ed.; Lea and Febiger: Philadelphia, 1981; Chapter 13.
(9) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Haleen, S. J.; Steffen, R. P. *J. Med. Chem.* **1985**, *28*, 1071.
(10) Mann, J. S.; Holgate, S. T. *Br. J. Clin. Pharmacol.* **1985**, *19*, 685.
(11) Gleason, J. G.; Perchonok, C. D.; Torphy, T. J. *Annu. Rep. Med. Chem.* **1986**, *21*, 73.
(12) Snyder, S. H.; Katims, J. J.; Annau, Z.; Bruns, R. F.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3260.
(13) Sattin, A.; Rall, T. W. *Mol. Pharmacol.* **1970**, *6*, 13.
(14) Perrsen, C. G. A. *Trends Pharmacol.* **1982**, *3*, 312.
(15) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2077.
(16) Fredholm, B. B.; Jacobson, K. A.; Jonzon, B.; Kirk, K. L.; Li, Y. O.; Daly, J. W. *J. Cardiovasc. Pharmacol.* **1987**, *9*, 396.
(17) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem.* **1985**, *28*, 487.
(18) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1985**, *28*, 1334.
(19) Daly, J. W.; Padgett, W. L.; Shamim, M. T. *J. Med. Chem.* **1986**, *29*, 1305.
(20) Daly, J. W.; Padgett, W. L.; Shamim, M. T. *J. Med. Chem.* **1986**, *29*, 1520.

0022-2623/88/1831-2034$01.50/0

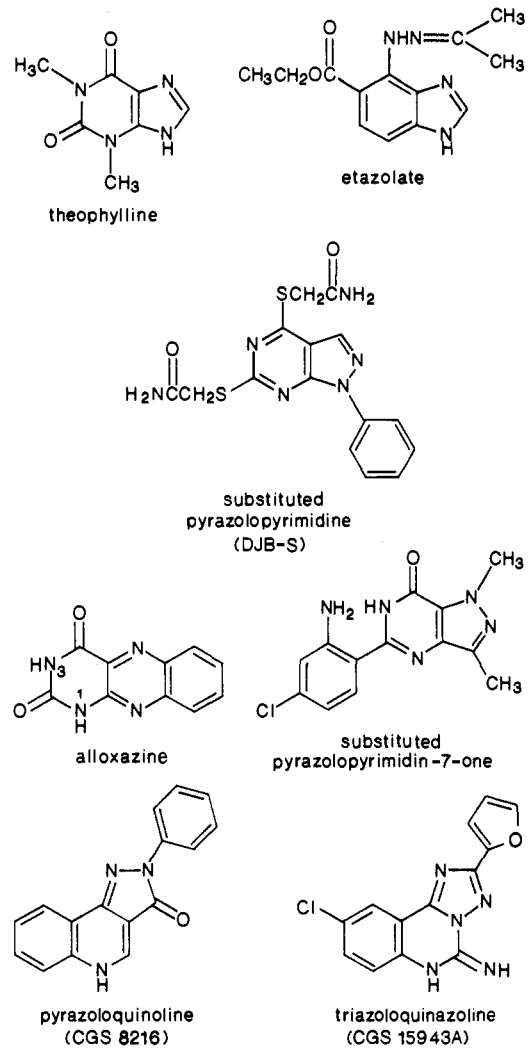


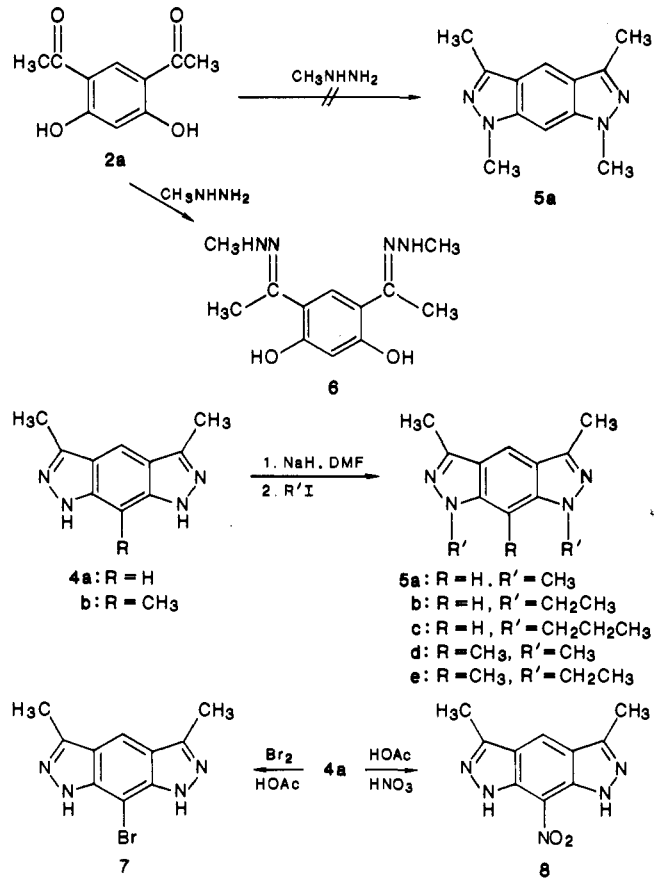
Figure 1. Non-xanthine adenosine antagonists.

in vitro and in vivo for bronchodilatory effects. Structure–activity relationships that become apparent may prove useful in the production of antagonists with greater potency and/or selectivity.

Chemistry

The preparation of 1,7-dihydro-3,5-dimethylbenzo[1,2-*c*:5,4-*c*']dipyrazole (**4a**) and its 8-methyl homologue **4b** is shown in Scheme I. Fries rearrangement of resorcinol diacetate (**1a**) with ferric chloride provided 4,6-diacetylresorcinol (**2a**),[31] albeit in moderate yield. Brief treatment of 4,6-diacetylresorcinol (**2**) in 2-methoxyethanol with hydrazine hydrate gave bishydrazone **3**. Attempts to convert isolated **3** to benzodipyrazole **4a** were not successful. Benzodipyrazole **4a** was best prepared directly from **2a** by treatment with hydrazine hydrate in 2-methoxyethanol for an extended period of time. The 8-methyl homologue of **4a** (**4b**) was prepared in similar fashion. In this case, a double Fries rearrangement of 2-methylresorcinol diacetate (**1b**) can give only one product, i.e., 2-methyl-3,5-diacetylresorcinol (**2b**), and aluminum chloride effected this conversion in 63% yield.[32] This result suggests that the low yield of Fries rearrangement of **1a** to the desired **2a** may result from acetyl migration to the 2-position. Subsequent treatment with hydrazine hydrate in 2-methoxyethanol at reflux for 84 h gave benzodipyrazole **4b** in 81% yield.

Scheme II



Attempts to prepare a 1,7-dialkylated version of **4a** (**5a**) by treatment of **2a** with methylhydrazine led only to bishydrazone **6** (Scheme II). An alternate approach to **5a** proved successful. Dialkylation of **4a** with methyl iodide gave **5a**; the diethyl analogue **5b** was prepared in similar fashion. Likewise, compounds **5c–e** were prepared from **4b**.

Electrophilic substitution of benzodipyrazole **4a** was easily accomplished, since the C-9 position is activated by both N-1 and N-9. Thus, bromination and nitration of **4a** gave compounds **7** and **8**, respectively.

Only a few benzo[1,2-*c*:5,4-*c*']dipyrazoles have been reported,[33] some of which contain oxidized (quinone)[34-36] or

(21) Jacobson, K. A.; Ukena, D.; Padgett, W.; Daly, J. W.; Kirk, K. L. *J. Med. Chem.* **1987**, *30*, 211.
(22) Ukena, D.; Shamim, M. T.; Padgett, W.; Daly, J. W. *Life Sci.* **1986**, *39*, 743.
(23) Haleen, S. J.; Steffen, R. P.; Hamilton, H. W. *Life Sci.* **1987**, *40*, 555.
(24) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59.
(25) Glennon, R. A.; Tejani-Butt, S. M.; Padgett, W.; Daly, J. W. *J. Med. Chem.* **1984**, *27*, 1364.
(26) Psychoyos, S.; Ford, C. J.; Phillips, M. A. *Biochem. Pharmacol.* **1982**, *31*, 1441.
(27) Davies, L. P.; Chow, S. C.; Skerrit, J. H.; Brown, D. J.; Johnston, G. A. R. *Life Sci.* **1984**, *34*, 2117.
(28) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 91.
(29) Williams, M.; Francis, J.; Ghai, G.; Braunwalder, A.; Psychoyos, S.; Stone, G. A.; Cash, W. D. *J. Pharm. Exp. Ther.* **1987**, *241*, 415.
(30) Daly, J. W.; Hong, O.; Padgett, W. L.; Shamim, M. T.; Jacobson, K. A.; Ukena, D. *Biochem. Pharmacol.* **1988**, *37*, 655.
(31) Baker, W. *J. Chem. Soc.* **1934**, 71.
(32) Worden, L. R.; Burgstahler, A. W.; Kaufman, K. D.; Weis, J. A.; Schaaf, T. K. *J. Heterocycl. Chem.* **1969**, *6*, 191.

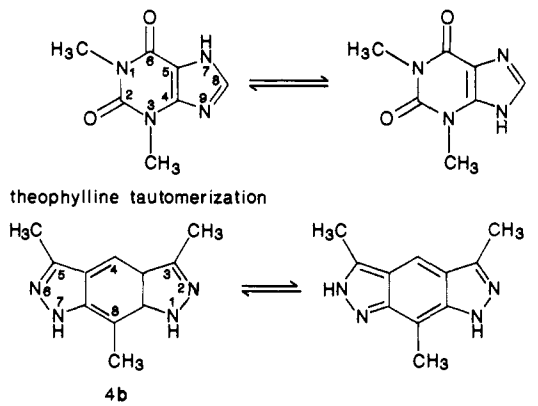


Figure 2. Structural similarities between theophylline and benzodipyrazole **4b**.

reduced[37] central rings. In addition, isomeric benzodipyrazoles have been reported, namely, benzo[1,2-*c*:4,3-*c*']dipyrazoles,[38-40] benzo[1,2-*c*:4,5-*c*']dipyrazoles,[38,41,42] and a benzo[1,2-*c*:3,4-*c*']dipyrazole.[43]

Biological Results and Discussion

The benzodipyrazoles were tested for A_1- and A_2-adenosine receptor binding, effects on smooth muscle tissue in vitro, and bronchodilator activity in vivo.

Receptor Binding. Affinity for A_1-adenosine receptors was evaluated in a binding assay with [^{3}H](phenylisopropyl)adenosine and rat cerebral cortical membranes. At the A_2-adenosine receptor, affinity was defined by the ability to oppose *N*-ethyladenosine-5'-uronamide stimulation of human platelet adenylate cyclase. In both assays, adenosine deaminase was used to inactivate endogenous adenosine. Rolipram, a potent phosphodiesterase inhibitor, was used in the A_2 assay to eliminate the effects of any phosphodiesterase inhibition by the test compounds. Both systems are suitable models for determining adenosine receptor affinity; rat brain A_1 binding data parallel data on A_1 receptors inhibitory to rat fat cell adenylate cyclase, and human platelet A_2 data parallel data on A_2 receptors stimulatory to rat PC12 adenylate cyclase.[22]

The benzodipyrazoles in which R_1 = H (**4a**, **4b**, and **8**) are the most potent and A_1-selective (Table I). The nitro analogue (**8**) is about 3 times as potent as theophylline at A_1 receptors but less potent at A_2 receptors. The methyl analogue (**4b**) is about 6 times as potent at A_1 receptors and more than twice as potent at A_2 receptors.

In contrast to the xanthines (Table II), the benzodipyrazoles become less active when they are N-alkylated (**5a-e**). This has been noted previously with alloxazine (Figure 1), another competitive inhibitor of adenosine.[7] However, like the xanthines, the potency ranking of the *N*-alkyl groups is propyl > ethyl > methyl. This could be related to the increase in lipophilicity that accompanies an increase in alkyl substituent size.

There are notable similarities between the benzodipyrazoles and the xanthines, particularly if tautomerization is considered (Figure 2). In view of this, N-alkylation of the former would be expected to decrease activity by decreasing their resemblance to the latter (by introducing bulk where it does not occur in the xanthine series) as well as by preventing tautomerization. The R' groups of **5a-e** could be considered analogous to a xanthine N^9 substituent, which drastically reduces potency.[7] The R groups of **4b**, **5d**, and **5e**, on the other hand, could be considered analogous to a xanthine N^3 substituent; in either series of compounds, methylation at this point enhances activity (Tables I and II). The C-5 benzodipyrazole methyl group may bind to the same region of the receptor as a xanthine N^1 substituent. Finally, the benzodipyrazole N^7 is quite similar to the xanthine carbonyl oxygen at position 2, especially in the analogues capable of tautomerization.

In Vitro Assay. Contractions in cylindrical segments of guinea pig trachea were induced by potassium chloride, histamine, acetylcholine, and 5-hydroxytryptamine. The benzodipyrazoles, tested for relaxant activity against each spasmogen, produced a concentration-dependent reduction in smooth muscle tone.

Although the group of benzodipyrazoles was quite active in reversing the effects of the spasmogens, there were no strong correlations in the potency rankings of the compounds among the four systems or between these systems and the binding assays. This suggests that adenosine receptor antagonism may not be the only mechanism by which the benzodipyrazoles cause smooth muscle relaxation; varying degrees of phosphodiesterase inhibition, for example, could be involved.

The activity of the benzodipyrazoles compared favorably with that of aminophylline. Five of the compounds (**4a**, **4b**, **8**, **5b**, and **7**) were more active than aminophylline in all four systems, three (**5c**, **5d**, and **5e**) were more active in three of the systems, and one (**5a**) was more active in two of the systems.

In Vivo Assay. In the conscious guinea pig, exposure to aerosolized histamine induces bronchospasm and subsequently collapse. The benzodipyrazoles were administered to guinea pigs intraperitoneally and evaluated for their ability to delay collapse.

Three analogues with high affinities for adenosine receptors (**4a**, **4b**, and **7**) were also the three with the greatest activities in vivo (quantified as the area under the curve: hours postdose versus seconds delay in collapse). All of the benzodipyrazoles were found to be more active in vivo than aminophylline.

Conclusions. The benzodipyrazoles are competitive inhibitors of adenosine; the 8-nitro and 8-methyl analogues (**8** and **4b**, respectively) are both more potent and more A_1-selective than theophylline. The in vitro and in vivo activities within this series generally exceed those of aminophylline.

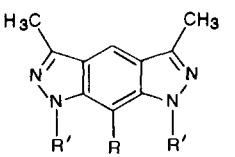
By analogy to the xanthines, where alterations at various positions can markedly enhance potency or selectivity, it is likely that benzodipyrazoles with greater potency and selectivity can be synthesized. This approach could lead to agents having greater therapeutic utility and reduced side effects.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra

(33) Mangiavacchi, S.; Calistri, M. T. *Ann. Chim. (Rome)* **1973**, *63*, 303, 883.
(34) Eistert, B.; Riedinger, J.; Kueffner, G.; Lazik, W. *Chem. Ber.* **1973**, *106*, 727.
(35) Eistert, B.; Pfleger, K.; Arackal, T. J.; Holzer, G. *Chem. Ber.* **1975**, *108*, 693.
(36) Elliot, A. J.; Gibson, M. S. *J. Org. Chem.* **1980**, *45*, 3677.
(37) Valentour, J. C. *Diss. Abstr. Int. B* **1971**, *32*, 191.
(38) Vasely, V.; Medvedina, A. *Collect. Czech. Chem. Commun.* **1935**, *7*, 228; **1937**, *9*, 176.
(39) Nef, J. U. *Liebigs Ann. Chem.* **1890**, *258*, 261.
(40) Ruchardt, C.; Hassmann, V. *Liebigs Ann. Chem.* **1980**, *6*, 908.
(41) Spiteller, G.; Schmidt, G.; Budzikiewicz, H.; Wessely, F. *Monatsh. Chem.* **1960**, *91*, 456.
(42) Wessely, F.; Budzikiewicz, H.; Janda, H. *Monatsh. Chem.* **1960**, *91*, 456.
(43) Gillis, B. T.; Valentour, J. C. *J. Heterocycl. Chem.* **1970**, *7*, 1131.

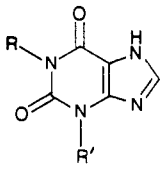
Table I. Biological Activity of 3,5-Dimethylbenzo[1,2-*c*:5,4-*c*']dipyrazoles



compd	R	R'	A_1 receptor rat cerebral cortex: K_i,[a] μM	A_2 receptor human platelets: K_i,[b] μM	reversal of spasmogen-induced contractns of guinea pig cylindrical tracheal segments: ED_{50}[c] KCl (20 mM)	histamine (1×10^{-5} M)	acetylcholine (1×10^{-5} M)	5-hydroxytryptamine (2×10^{-6} M)	prolongation of time to collapse in guinea pigs treated with aerosolized histamine[f]
4a	H	H	8.0 (6.8–9.3)	25.6 (15.6–42)	0.2 (0.1–0.4)	0.14 (0.02–0.30)	0.4 (0.2–0.7)	0.2 (0.07–0.30)	727 (592–862)
8	NO_2	H	1.9 (1.6–2.2)	14.4 (12.5–16.6)	0.99 (0.37–2.6)	0.64 (0.33–0.98)	2.5 (1.7–4.9)	0.15 (0.05–0.25)	
7	Br[d]	H			0.2 (0.18–0.28)	0.3 (0.25–0.35)	0.2 (0.11–0.33)	0.09 (0.07–0.11)	488 (389–586)
4b	CH_3	H	1.2 (1.0–1.6)	4.8 (2.2–10.4)	0.83 (0.51–1.2)	2.4 (0.27–6.5)	0.85 (0.59–1.2)	0.46 (0.29–0.55)	587 (468–706)
5a	H	CH_3	>100 (38%)	80 (46–139)	15 (10.6–24.4)	7.5 (2.9–13.6)	1.9 (1.6–2.2)[e]	1.7 (0.7–2.7)	443 (297–588)
5b	H	C_2H_5	27 (24–32)	56 (46–69)	0.84 (0.58–1.1)	0.52 (0.23–0.82)	1.1 (0.92–1.4)[e]	0.13 (0.03–0.2)	263 (188–338)
5c	H	n-C_3H_7	10.5 (9.4–11.8)	32 (26–39)	3.3 (2.4–4.6)	4.1 (2.7–6.7)	6.9 (3.9–34)[e]	1.3 (0.63–2.3)	395 (297–493)
5d	CH_3	CH_3	54 (44–65)	170 (110–260)	24 (19–35)	3.8 (2.8–5.3)	0.96 (0.72–1.3)[e]	2.0 (1.11–3.2)	314 (201–427)
5e	CH_3	C_2H_5	20 (13.6–29)	53 (46–62)	5.1 (3.2–7.7)	0.41 (0.26–0.74)	0.43 (0.30–0.57)	0.10 (0.04–0.36)[e]	369 (268–470)
aminophylline					4.6 (3.2–9.5)	4.2 (3.0–5.7)	5.7 (2.5–9.1)	2.1 (1.5–2.6)	246 (161–402)
theophylline			7.4 (5–11)	13.8 (4.0–17.9)					

[a] Binding of 1 nM [^{3}H]PIA was measured at 37 °C. Values are geometric means with 95% confidence limits, $n = 3$, or are the percent inhibition at 300 μM. [b] Inhibition of NECA-stimulated adenylate cyclase was measured in human platelet membranes. Values are geometric means with 95% confidence limits. [c] 10^{-5} M, 95% confidence limits. [d] HBr salt. [e] Carbachol (7.4×10^{-7} M) used in place of acetylcholine. [f] Area under the curve (h s); values are means ± 1 SE.

Table II. Effect of N-Alkylation on Potency of Xanthines at A_1- and A_2-Adenosine Receptors



xanthine substituents		K_i, μM		
R	R′	A_1[a]	A_2[b]	A_2[c]
H	H			130 ± 20
CH_3	H	9 ± 3[d]		6.6 ± 0.3
CH_3	CH_3	14 ± 3	14 ± 2	4.8 ± 0.8
CH_3CH_2	CH_3CH_2	3.3 ± 0.2	3.0 ± 1.2	1.2 ± 0.2
CH_2=$CHCH_2$	CH_2=$CHCH_2$	10 ± 2.1	5 ± 2	0.82
CH_3CH_2-CH_2	CH_3CH_2-CH_2	0.7 ± 0.3	2.7 ± 0.8	0.68 ± 0.03
$(CH_3)_2$-$CHCH_2$	$(CH_3)_2$-$CHCH_2$	0.5 ± 0.2	1.7 ± 0.7	

[a] Binding of 1 nM [^{3}H]CHA to rat cerebral cortical membranes was measured at 37 °C. Values are means ± SEM for two to five separate determinations, each determination being done in triplicate.[19] These values are comparable to K_i values obtained versus binding of [^{3}H]PIA to rat cerebral cortical membranes. [b] Inhibition of 2-CADO-stimulated adenylate cyclase was measured in guinea pig cerebral cortical slices. Values are means ± SEM for two to five separate determinations, each determination being done in triplicate.[19] [c] Inhibition of adenosine-stimulated accumulation of cyclic AMP was measured in VA13 fibroblasts. Values are means ± SE.[7] [d] Unpublished results from our laboratory obtained versus binding of [^{3}H]PIA to rat cerebral cortical membranes.

were recorded with a Perkin-Elmer Model 727B spectrophotometer, NMR spectra with Varian EM-360A and Varian XL-300 (multinuclear probe) spectrometers, and MS at 70 eV with a Finnigan Model 4500 (electron impact and chemical ionization) mass spectrometer. Combustion analyses fell within 0.4% of the calculated values.

1,5-Diacetyl-2,4-dihydroxybenzene (2a). A mixture of 50.0 g (0.257 mol) of resorcinol diacetate (**1a**) (Pfaltz and Bauer) and 22 g of anhydrous $FeCl_3$ was heated, under nitrogen, over a 30-min period to 180 °C and then held at 180 °C for 3 h. The dark semisolid was mixed with 200 mL of water and 50 mL of concentrated HCl for 30 min. The mixture was cooled and the dark solid collected, washed with water, air-dried, and subjected to Soxhlet extraction with $CHCl_3$ (600 mL) for 3 h. The dark solution was concentrated to leave 15.5 g of dark solid which was recrystallized from EtOH (300 mL) to yield 7.80 g (16%) of **2a**: mp 179–181 °C (lit.[31] mp 182 °C); ^{1}H NMR (Me_2SO-d_6) δ 12.43 (s, 2, both OH groups), 8.40 (s, 1, H ortho to acetyl groups), 6.37 (s, 1, H ortho to OH groups); MS (70 eV, CI, CH_4), m/z 195 (M^+ + 1), 223 (M^+ + 29), 235 (M^+ + 41). Anal. ($C_{10}H_{10}O_4$) C, H.

1,1′-(4,6-Dihydroxy-1,3-phenylene)bis(ethanone) Dihydrazone (3). A solution of 1.00 g (5.15 mmol) of **2a** in 25 mL of 2-methoxyethanol (warm) was treated with 5 mL of hydrazine hydrate (MCB; 85%). The solution immediately turned amber and was allowed to cool. TLC (9:1 $CHCl_3$–MeOH) of the solution showed the absence of starting material and a single material different from **4a**. The yellow needles that formed on cooling were collected and oven-dried to afford 0.600 g (52%) of **3**: mp 250 °C (turns orange), >310 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.47 (s, 1, H ortho to OH groups), 6.40 (s, 4, both NH_2 groups), 6.22 (s, 1, H meta to OH groups), 3.40 (s, 2, both OH groups), 2.20 (s, 6, both CH_3 groups); MS (70 eV, CI, CH_4), m/z 223 (M^+ + 1), 251 (M^+ + 29), 263 (M^+ + 41). Anal. ($C_{10}H_{14}N_4O_2$) C, H, N.

1,7-Dihydro-3,5-dimethylbenzo[1,2-*c*:5,4-*c*′]dipyrazole (4a). A solution of 16.0 g (82.4 mmol) of 4,6-diacetylresorcinol (**2a**) in 400 mL of hydrazine hydrate and 150 mL of 2-methoxyethanol was heated at reflux for 14 h and cooled, and the resulting white prisms were collected, washed with water, and oven-dried to give 18.8 g (90%) of **4a**: mp >315 °C (2-methoxyethanol); NMR (Me_2SO-d_6) δ 12.20 (br s, 2, both NH groups, D_2O exchangeable), 8.00 (d, J = 1 Hz, 1, C4-H), 7.20 (d, J = 1 Hz, 1, C8-H), 2.57 (s, 6, both CH_3 groups); HRMS (EI, 1.5 eV), m/z 186.0904 (molecular ion corresponding to ($C_{10}H_{10}N_4$).

1,3-Diacetoxy-2-methylbenzene (1b). A solution of 100 g (0.806 mol) of 2-methylresorcinol (Aldrich) and 2 g of NaOAc·$3H_2O$ in 1 pint (473 mL) of Ac_2O was heated at reflux for 15 h. The solution was concentrated to a small volume and poured over ice. The mixture was extracted with ether, and the extracts were washed with saturated $NaHCO_3$ until effervescence ceased. The extracts were dried (Na_2SO_4) and concentrated in vacuo to leave 186 g of oil. The oil was purified by Kugelrohr distillation. The first fraction gave 78.0 g of a 1:1 mixture of desired diacetate and HOAc. Cooling gave prisms, which were collected, washed with water, and air-dried to afford 36.9 g of pure **1b**. The second fraction provided an additional 94.0 g of **1b**. Total yield of **1b** was 131 g (78%): mp 42–43.5 °C (lit.[32] mp 42–44 °C; ^{1}H NMR ($CDCl_3$) δ 7.30–6.80 (m, 3, aromatic), 2.30 (s, 6, both $COCH_3$ groups), 2.00 (s, 3, $ArCH_3$).

1,5-Diacetyl-2,4-dihydroxy-3-methylbenzene (2b). A solution of 35.0 g (0.168 mol) of **1b** in 60 mL of nitrobenzene was cooled in an ice bath and 46.7 g (0.350 mol) of anhydrous $AlCl_3$ was added with stirring. The mixture was heated at 75 °C in an oil bath under nitrogen for 3 h. During the first hour, foaming occurred (volume tripled) and then subsided. To the cooled glass was added ice chips and 50 mL of 1 N HCl (exothermic). The nitrobenzene was removed by steam distillation and the oily product in the aqueous medium crystallized on cooling. The brown solid was collected and recrystallized from EtOH to give 22.1 g (63%) of **2b** as white needles: mp 137–139 °C (lit.[32] mp 139–142 °C); ^{1}H NMR ($CDCl_3$) δ 13.10 (s, 2, both OH groups), 8.05 (s, 1, aromatic), 2.58 (s, 6, both $COCH_3$ groups), 2.03 (s, 3, $ArCH_3$); MS (70 eV, EI), m/z 208 (molecular ion).

1,7-Dihydro-3,5,8-trimethylbenzo[1,2-*c*:5,4-*c*′]dipyrazole (4b). A mixture of 14.0 g (67.2 mmol) of **2b**, 350 mL of hydrazine monohydrate, and 70 mL of 2-methoxyethanol was heated at reflux. Solution initially resulted, followed by precipitation to yield an orange mixture which persisted after 24 h. After 84 h the pale yellow solution was allowed to cool, and the white prisms were collected, washed with EtOH, and air-dried to give 10.9 g (81%) of **4b**: mp >300 °C; IR (KBr) 3460–2500 (NH), 1635 (C=N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 12.20 (s, 2 H, both NH groups), 7.80 (s, 1, aromatic), 2.60 (s, 3, C8-CH_3), 2.53 (s, 6, C3-CH_3 and C5-CH_3); MS (70 eV, EI), m/z 200 (molecular ion). Anal. ($C_{11}H_{12}N_4$) C, H, N.

1,7-Dihydro-1,3,5,7-tetramethylbenzo[1,2-*c*:5,4-*c*′]dipyrazole (5a). To a solution of 4.00 g (21.5 mmol) of **4a** in 100 mL of DMF under N_2 was added a slurry of 1.32 g (54.5 mmol) of dry NaH in 25 mL of DMF followed by a solution of 7.65 g (53.9 mmol) of CH_3I in 10 mL of DMF. After 114 h the solution was diluted with water and the precipitate was collected. Extraction of the filtrate with ether provided additional solid. Total yield of **5a** was 3.34 g (72%): mp 215–218 °C (*i*-PrOH–H_2O); ^{1}H NMR (Me_2SO-d_6) δ 7.95 (d, J = 1 Hz, 1, C4-H), 7.29 (d, J = 1 Hz, 1, C8-H), 3.88 (s, 6, both NCH_3 groups); MS (70 eV, CI, CH_4), m/z 215 (M^+ + 1), 243 (M^+ + 29), 255 (M^+ + 41). Anal. ($C_{12}H_{14}N_4$) C, H, N.

1,7-Diethyl-1,7-dihydro-3,5-dimethylbenzo[1,2-*c*:5,4-*c*′]dipyrazole (5b). To a solution of 4.00 g (21.5 mmol) of **4a** in 100 mL of DMF under N_2 was added a slurry of 1.32 g (54.5 mmol) of dry NaH in 25 mL of DMF followed by a solution of 8.50 g (54.5 mmol) of iodoethane in 10 mL of DMF. After 138 h the mixture was diluted with water and the precipitate was collected. Extraction of the filtrate with ether gave additional solid. Total yield of **5b** after recrystallization (*i*-PrOH–H_2O) was 2.97 g (57%): mp 128–129 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.93 (d, J = 1 Hz, 1, C4-H), 7.37 (d, J = 1 Hz, 1, C8-H), 4.29 (q, J = 6 Hz, 4, both CH_2 groups), 2.56 (s, 6, C3-CH_3 and C5-CH_3), 1.35 (t, J = 6 Hz, 6, both CH_2CH_3 groups); MS (70 eV, CI, CH_4), m/z 243 (M^+ + 1), 271 (M^+ + 29), 283 (M^+ + 41). Anal. ($C_{14}H_{18}N_4$) C, H, N.

1,7-Dihydro-3,5-dimethyl-1,7-dipropylbenzo[1,2-*c*:5,4-*c*′]dipyrazole (5c). To a solution of 4.00 g (21.5 mmol) of **4a** in 100 mL of DMF under N_2 was added a slurry of 1.32 g (54.5 mmol) of dry NaH in 25 mL of DMF followed by 9.26 g (54.5 mmol) of iodopropane in 10 mL of DMF. After 138 h the mixture was diluted with water and the precipitate was twice recrystallized (*i*-PrOH–H_2O) to give 3.44 g (59%) of **5c**: mp 86.5–87.5 °C; ^{1}H

NMR (Me_2SO-d_6) δ 7.91 (d, J = 1 Hz, 1, C4-H), 7.38 (d, J = 1 Hz, 1, C8-H), 4.20 (t, J = 7 Hz, 4, both NCH_2 groups), 2.54 (s, 6, C3-CH_3 and C5-CH_3), 1.81 (q, J = 6 Hz, 4, both NCH_2CH_2 groups), 0.85 (t, J = 7 Hz, 6, both $NCH_2CH_2CH_3$ groups); MS (70 eV, CI, CH_4), m/z 271 (M^+ + 1), 299 (M^+ + 29), 312 (M^+ + 41). Anal. ($C_{16}H_{22}N_4$) C, H, N.

1,7-Dihydro-1,3,5,7,8-pentamethylbenzo[1,2-*c*:5,4-*c*']dipyrazole (5d). To a mixture of 1.45 g (60.0 mmol) of dry NaH and 100 mL of DMF under N_2 was added 4.00 g (20.0 mmol) of **4b**. After 30 min, 8.52 g (60.0 mmol) of CH_3I was added. After 112 h the solution was concentrated by Kugelrohr distillation. The residue was triturated with water and the resulting precipitate was collected to give 2.51 g (55%) of **5d**: mp 238.5–239.5 °C (*i*-PrOH–H_2O); ^1H NMR (Me_2SO-d_6) δ 7.58 (s, 1, C4-H), 4.20 (s, 6, both NCH_3 groups), 3.00 (s, 3, C8-CH_3), 2.52 (s, 6, C3-CH_3 and C5-CH_3); MS (70 eV, EI), m/z 228 (molecular ion). Anal. ($C_{13}H_{16}N_4$) C, H, N.

1,7-Diethyl-1,7-dihydro-3,5,8-trimethylbenzo[1,2-*c*:5,4-*c*']dipyrazole (5e). To a mixture of 0.910 g (37.5 mmol) of dry NaH and 100 mL of DMF under N_2 was added 3.00 g (15.0 mmol) of **4b**. After 30 min, 5.85 g (37.5 mmol) of iodoethane was added. After 110 h the solution was concentrated by Kugelrohr distillation and the residue was triturated with water. The resulting solid was collected and dried to give 2.55 g (66%) of **5e**: mp 153–154 °C (*i*-PrOH–H_2O); ^1H NMR (Me_2SO-d_6) δ 7.60 (s, 1, C4-H), 4.54 (q, J = 7 Hz, 4, both CH_2 groups), 2.93 (s, 3, C8-CH_3), 2.57 (s, 6, C3-CH_3 and C5-CH_3), 1.40 (t, J = 7 Hz, 6, both CH_2CH_3 groups); MS (70 eV, EI), m/z 256 (molecular ion). Anal. ($C_{15}H_{20}N_4$) C, H, N.

8-Bromo-1,7-dihydro-3,5-dimethylbenzo[1,2-*c*:5,4-*c*']dipyrazole (7). To a solution of 2.00 g (10.7 mmol) of **4a** in 50 mL of HOAc was added a solution of 2.00 g (12.5 mmol) of Br_2 in 20 mL of HOAc. A precipitate began forming as soon as the addition began. After 15 min of stirring the yellow solid was collected and oven-dried to yield 3.27 g (88%) of the hydrobromide salt of **7**: mp >310 °C; IR (KBr) 3300–2000 (NH), 1640 (C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.15 (s, 1, aromatic), 7.13 (broad signal, both NH groups), 2.58 (s, 6, both CH_3 groups); MS (70 eV, EI), m/z 264 (molecular ion).

A sample of the hydrobromide salt was dissolved in water and the free base was precipitated by adding $NaHCO_3$ solution. The solid was collected and recrystallized to afford pure **7**: mp >300 °C. Anal. ($C_{10}H_9BrN_4$) C, H, N.

1,7-Dihydro-3,5-dimethyl-8-nitrobenzo[1,2-*c*:5,4-*c*']dipyrazole (8). To 20 mL of concentrated H_2SO_4 was added 1.40 g (7.52 mmol) of **4a** (exothermic addition) and 12 mL of HNO_3 (exothermic addition). After 10 min of stirring, the bright yellow solution was carefully added to 200 mL of cold water. The resulting yellow solid was collected, washed with water, and oven-dried to yield 1.55 g (89%) of **8**: mp >310 °C (2-methoxyethanol); ^1H NMR (Me_2SO-d_6) δ 13.28 (br s, 2, both NH groups), 8.66 (s, 1, aromatic), 2.56 (s, 6, both CH_3 groups); MS (70 eV, EI), m/z 231 (molecular ion). Anal. ($C_{10}H_9N_5O_2$) C, H, N.

***(R)*-N^6-(1-Phenyl-2-propyl)[^{3}H]adenosine ([^{3}H]PIA) in Rat Brain Membranes.** Membranes from rat cerebral cortex were prepared, and the binding of 1 nM [^{3}H]PIA to these membranes at 37 °C was assayed essentially as described; IC_{50} values were transformed into K_i values using a K_D for [^{3}H]PIA binding of 1.0 nM[44] and the Cheng–Prusoff equation.[45]

Activity of Human Platelet Adenylate Cyclase. Human platelet membranes were prepared, and adenylate cyclase activity was determined essentially as described.[46] Briefly stated, the medium contained 0.1 mM [α-^{32}P]ATP (0.3 μCi/tube), 1 μM GTP, 1 mM $MgCl_2$, 0.1 mM cyclic AMP, 1 μg/mL adenosine deaminase, 0.1 mM Rolipram [4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone, ZK 62,711], 1 mM EGTA, 5 mM creatine phosphate as the Tris salt, 0.4 μg/mL creatine kinase, 2 mg/mL bovine serum albumin, and 50 mM Tris·HCl, pH 7.4, in a total volume of 100 μL. Incubations were initiated by the addition of 5–15 μg of membrane protein and were conducted for 10 min at 37 °C. Reactions were stopped by the addition of 0.4 mL of 125 mM zinc acetate and 0.5 mL of 144 mM Na_2CO_3. Cyclic AMP was purified as described.[46]

EC_{50} values for *N*-ethyladenosine-5′-uronamide (NECA) were obtained from concentration–response curves in the absence or presence of the benzodipyrazole in three experiments. K_i values for the benzodipyrazole were then calculated by using the Schild equation.[47]

Smooth Muscle Relaxation in Vitro. After male guinea pigs were stunned and bled, their tracheae were removed. Each trachea was cut into two cylindrical segments 8 mm in length. Each segment was then placed between stainless steel hooks, supended in a 10-mL tissue bath containing Burns' modified Tyrode's solution at 37 °C, and attached to a force transducer (Gould Instruments) for recording isometric tension. The amount of tension was recorded through a Buxco analog computer. The preparation was allowed to equilibrate for 60 min under a resting tension of 8 g. The modified Tyrode's solution (8.0 g/L NaCl, 0.2 g/L KCl, 0.26 g/L $CaCl_2·2H_2O$, 0.02 g/L $MgCl_2·6H_2O$, 1.0 g/L $NaHCO_3$, and 0.05 g/L $NaH_2PO_4·H_2O$) was aerated with a mixture of 95% O_2 and 5% CO_2.

Tissues were precontracted with one of four contractile agents (KCl 20 mM, histamine 1×10^{-5} M, acetylcholine 1×10^{-5} M, or 5-hydroxytryptamine 2×10^{-6} M) at a bath concentration that produced 70–80% of the maximal response. The maximal response had been determined previously with concentration–contraction curves. The test compound was then added to the bath until the precontraction was completely reversed; relaxation beyond that point would have represented a decrease in the base-line tone of the tissue. The relaxation caused by each concentration of the test compound was expressed as a percentage of that obtained with 3.2×10^{-7} M isoproterenol (assumed to produce 100% relaxation), and these percentages were used to calculate the ED_{50}. Each tissue sample was assigned treatment according to a balanced incomplete block design.

Reversal of Bronchospasm in Vivo. Male Hartley–Duncan guinea pigs weighing 150–300 g were placed in individual plexiglass chambers (7 in. × 3 in. × 5 in.). With a DeVilbiss No. 180 nebulizer at a pressure of 20 psi, an 0.2% solution of histamine dihydrochloride was aerosolized into each chamber until the animal collapsed. The time in seconds from the start of the aerosol until collapse occurred was measured for each animal. Animals not responding within 180 s were excluded from further testing. After reviving, the animals were dosed intraperitoneally with test compound. The test compounds were solubilized by titration with 0.1 N HCl of a stirred, aqueous suspension of compound in a warm water bath. Compounds **5d** and **5e** required, in addition, a small supplemental volume of PEG 200 for dissolution. The animals were later replaced in the chambers and reexposed to histamine until they collapsed, or for a maximum of 360 s.

The times of retest were 0.25, 0.5, 1, 2, 3, and 4 h after dosing. Separate groups of animals were dosed for each period tested, three to five animals per group. The dosage used was 30% of the LD_{50} in guinea pigs (acute 72-h toxicity).

The area under the curve with a low-to-high variation was computed for each compound with the mean delay in collapse plus or minus a standard error.

Acknowledgment. We thank Elaine Meng for assistance in the preparation of this manuscript and Gregory L. Karrick for assistance in the synthesis of compounds.

Registry No. **1a**, 108-58-7; **1b**, 35236-42-1; **2a**, 2161-85-5; **2b**, 22304-66-1; **3**, 115705-35-6; **4a**, 115705-36-7; **4b**, 115705-37-8; **5a**, 115705-38-9; **5b**, 115705-39-0; **5c**, 115705-40-3; **5d**, 115705-41-4; **5e**, 115705-42-5; **7**, 115705-43-6; **7**·HBr, 115705-44-7; **8**, 115705-45-8; 2-methylresorcinol, 608-25-3.

(44) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4089.

(45) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

(46) Ukena, D.; Boehme, E.; Schwabe, U. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 36.

(47) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.