J. Med. Chem. 1987, *30*, 91-96

Synthesis and Structure-Activity Relationships of Pyrazolo[4,3-*d*]pyrimidin-7-ones as Adenosine Receptor Antagonists

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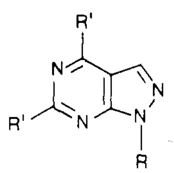
Received March 27, 1986

A series of 21 1,3-dialkylpyrazolo[4,3-*d*]pyrimidin-7-ones substituted in the 5-position with various phenyl substituents has been synthesized and found to have affinity for the adenosine A_1 receptor. The potency pattern due to substituents of the phenyl ring was found to parallel that found in a previously reported[4] 1,3-dialkyl-8-phenylxanthine series. A quantitative structure-activity relationship was developed between these two series that correctly predicted the potencies of six additional 5-substituted pyrazolo[4,3-*d*]pyrimidines that were synthesized during the course of the analysis. With use of the correlation as a guide, one additional 5-phenylpyrazolo[4,3-*d*]pyrimidine containing a 4-[[(dimethylamino)ethyl]amino]sulfonyl substituent to improve aqueous solubility was prepared. On the basis of the high correlation between adenosine binding affinities of analogously substituted xanthines and pyrazolo-[4,3-*d*]pyrimidines and the close superposition of the heterocyclic rings and substituents that is apparent from molecular models of these two series (Figure 2), it is hypothesized they fit the receptor in an analogous fashion.

Alkylxanthines are known as prototype antagonists for adenosine receptors. Many of their physiological functions such as changes in conductance in the heart, CNS stimulatory activity, and effects on the lung and trachea have recently been ascribed to their ability to block these receptors and antagonize endogenous adenosine, rather than to their ability to inhibit phosphodiesterase.[1]

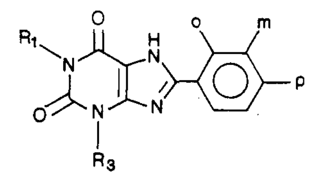
There are relatively few reports of compounds other than modified xanthines that function as adenosine receptor antagonists. Carbamazepine and (bromobenzoylmethyl)adamantylamine have been described as adenosine antagonists.[2] The pyrazolo[3,4-*d*]pyrimidines (I) are the only general class of compound that have been reported



I

to have adenosine receptor affinity.[3] Of the 10 compounds in this series, the most potent (I, R = phenyl, R′ = $SCHMeCONH_2$) had a K_i value of 0.37 μM (IC_{50} = 1.03 μM), which is 15 times less potent than the most potent pyrazolo[4,3-*d*]pyrimidine reported in this paper. These pyrazolo[3,4-*d*]pyrimidines antagonize adenosine-stimulated adenylate cyclase in guinea pig brain slices as well and thus are functional antagonists.

From our earlier quantitative structure-activity relationship (QSAR) study, parameters necessary for optimal adenosine A_1 receptor binding among a series of 1,3-dialkyl-8-phenylxanthines (II) were determined (eq 1).[4]



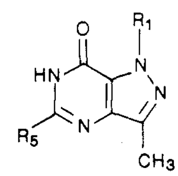
II

$$\log (1000/IC_{50}) = -0.99\ (\pm 0.13)HACCEPT_m + 0.81\ (\pm 0.18)\pi R_3 - 1.16\ (\pm 0.18)MR_o - 0.88\ (\pm 0.20)\sigma_o - 1.57\ (\pm 0.24)ACID - 1.17\ (\pm 0.24)HBOND + 2.22 \quad (1)$$

$$n = 56,\ r^2 = 0.83,\ F = 40,\ s = 0.40$$

Binding affinity was found to increase with increasing lipophilicity (or size) of the R_1/R_3 alkyl groups (πR_3) and to decrease with phenyl substitution by strongly acidic groups (ACID), meta phenyl substitution by proton-accepting groups ($HACCEPT_m$), or ortho phenyl substitution by groups capable of forming strong hydrogen bonds to the imidazole N7-H (HBOND). A critical relationship was found between binding affinity and size (MR_o) and electronic effects (σ_o) of the ortho phenyl substituents: high affinity was observed for analogues containing small, electron-releasing groups such as H, 2-OH, and $2-NH_2$. Affinity was relatively insensitive to changes in para phenyl substitution. The last finding was used in the design of derivatives with greater water solubility containing large sulfonamide groups at the para phenyl position.

At the time the QSAR work on the xanthines was being pursued in our laboratories, a series of pyrazolo[4,3-*d*]-pyrimidin-7-ones (III) substituted in the 5-position with



III

various aryl and alkyl groups was also under development. These compounds were tested for their ability to bind to adenosine receptors and were found to possess affinity for the A_1 receptor. Furthermore, the qualitative SAR of the 5-phenyl substituents for this series seemed to parallel that seen for the 8-phenyl substituents on the xanthine series. Because of this, a more detailed study of the correlation between receptor binding SARs of these two series was undertaken. If a quantitative correlation between the potencies of analogously substituted 8-phenylxanthines

(1) (a) Fredholm, N. N. *Trends Pharmacol. Sci.* **1980**, *1*, 129. (b) Daly, J. W.; Bruns, R. F.; Snyder, S. H. *Life Sci.* **1981**, *28*, 2083. (c) Evans, D. B.; Schenden, J. A.; Bristol, J. A. *Life Sci.* **1982**, *31*, 2425.

(2) (a) Marangos, P.; Post, R. M.; Patel, J.; Zander, K.; Parma, A.; Weiss, S. *Eur. J. Pharmacol.* **1983**, *93*, 175. (b) Maszaros, J.; Keleman, K.; Kecskeneti, V.; Szegi, J. *Eur. J. Pharmacol.* **1984**, *98*, 265.

(3) (a) Davies, L. P.; Chow, S. C.; Skerritt, J. H.; Brown, D. J.; Johnston, G. A. R. *Life Sci.* **1984**, *34*, 2117. (b) Davies, L. P.; Brown, D. J.; Chow, S. C.; Johnston, G. A. R. *Neurosci. Lett.* **1983**, *41*, 189.

(4) 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.

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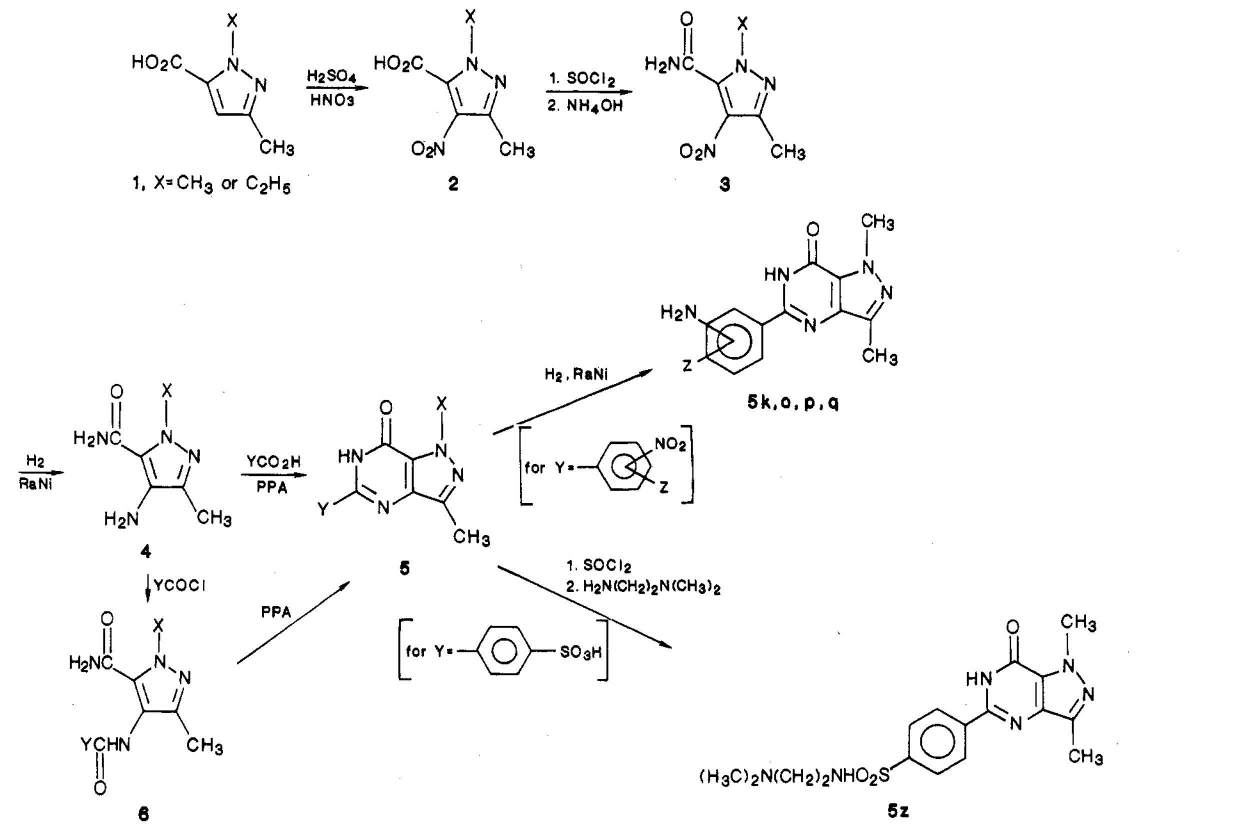
Scheme I



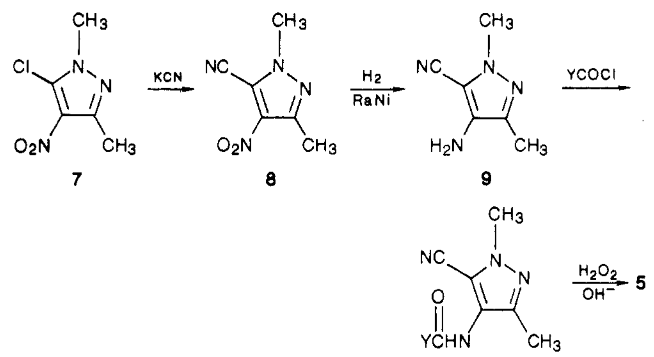
and 5-phenylpyrazolo[4,3-*d*]pyrimidin-7-ones could be demonstrated, then the prior (Q)SAR developed for the xanthines could be directly applied to the latter series, thus reducing the number of compounds to be synthesized in order to optimize potency and achieve good aqueous solubility. This communication describes the synthesis and adenosine A_1 receptor binding of a series of 5-substituted pyrazolo[4,3-*d*]pyrimidin-7-ones and the quantitative comparison of the SAR of this series with that of the 8-substituted xanthines.

Chemistry. The 5-substituted pyrazolo[4,3-*d*]pyrimidin-7-ones (**5a–z**, Table I) are prepared by the reactions given in Schemes I and II. In Scheme I, the appropriately substituted pyrazole[5] (**1**) is nitrated with a mixture of sulfuric acid and fuming nitric acid at 80–100 °C. Heating of the nitration mixture above 140 °C causes decarboxylation. The requisite amide **3** is synthesized from the acid by chlorination with thionyl chloride followed by workup in ammonium hydroxide. The nitropyrazole amide is then catalytically reduced to the amine **4** by use of Raney nickel. The substituted pyrazoloamino amide **4** is cyclized to **5** by stirring with the appropriately substituted carboxylic acid in polyphosphoric acid for 4–24 h at 140 °C. Often the overall yield is improved by employing a two-step procedure in which bis-amide **6** is preformed from the appropriate acid chloride and the isolated product subsequently cyclized in polyphosphoric acid.

Pyrazolo[4,3-*d*]pyrimidin-7-ones containing nitro-substituted 5-phenyl rings were converted to the corresponding amines by catalytic reduction using Raney nickel. The *p*-benzenesulfonic acid analogue [4-(1,3-dimethyl-7-oxopyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonic acid; compound **5r**] was further derivatized to the *N*-[2-(dimethylamino)ethyl-*p*-benzenesulfonamide by chlorination by $SOCl_2$ followed by displacement with *N*,*N*-dimethyl-1,2-ethanediamine.

(5) Habraken, C.; Moore, J. A. *J. Org. Chem.* **1965**, *30*, 1982.

Scheme II

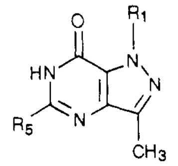


In Scheme II, nitrochloropyrazole[6] **7** is treated with potassium cyanide to give the nitrocyanopyrazole **8**, followed by catalytic reduction to the amine with use of Raney nickel. The resulting substituted cyanoaminopyrazole **9** is then stirred with an appropriately substituted acid chloride in an inert solvent with base to afford the cyanopyrazolo amide **10**, followed by oxidative cyclization with basic hydrogen peroxide at 80–100 °C to give the final product.

Biological Evaluation. The xanthine derivatives (Table II) were previously evaluated[4] for adenosine A_1 receptor affinity by measuring inhibition of *N*-[^{3}H]cyclohexyladenosine binding to bovine brain membranes. Values used in the present paper are IC_{50}'s (nM), which can be converted to K_i values (nM) by dividing by 1.76. The pyrazolopyrimidines (Table I) were tested in a modified assay using a rat brain membrane preparation (see Experimental Section). Previously, it was demonstrated

(6) (a) U.S. Patent 4 469 868. (b) U.S. Patent 3 121 092. (c) U.S. Patent 3 939 141. (d) Dewald, H. D.; Nordin, I. C.; L'Italien, Y. J.; Parcell, R. F.; *J. Med. Chem.* **1973**, *16*, 1346 (synthesis of 1-ethyl-3-methyl pyrazoles).

Table I. Pyrazolo[4,3-*d*]pyrimidin-7-ones



compd	R_1	R_5	mp, °C	method	yield, %	purification solvent	formula	anal.	IC_{50}[a]
5a[10]	CH_3	H	299–301						20 100
5b	CH_3	$CH_2C_6H_5$	233–234	B	81	H_2O	$C_{14}H_{14}N_4O{\cdot}0.125H_2O$	C, H, N	1 310
5c	CH_3	4-pyridyl	321–322	A	28	EtOH	$C_{12}H_{11}N_5O$	C, H, N	950
5d	CH_3	3-pyridyl	309–309.5	B	70	EtOH/H_2O	$C_{12}H_{11}N_5O{\cdot}0.1H_2O$	C, H, N	3 090
5e	C_2H_5	C_6H_5	218–221	B	60	*e*	$C_{14}H_{14}N_4O{\cdot}0.5H_2O$	C, H, N	670
5f	CH_3	C_6H_5	273–274	A, C	45, 26	EtOH	$C_{13}H_{12}N_4O{\cdot}0.25H_2O$	C, H, N	1 020
5g	CH_3	C_6H_4-4-Cl	314–316	B	92	*e*	$C_{13}H_{11}N_4ClO{\cdot}0.25H_2O$	C, H, N, Cl	410
5h	CH_3	C_6H_4-4-CH_3	271–272	B	53	EtOH/H_2O	$C_{14}H_{14}N_4O{\cdot}0.1H_2O$	C, H, N	400
5i	CH_3	C_6H_4-4-NO_2	>340	C	68	*e*	$C_{13}H_{11}N_5O_3{\cdot}0.25H_2O$	C, H, N	880
5j	CH_3	C_6H_4-3-NO_2	334–336	C	57	EtOH	$C_{13}H_{11}N_5O_3$	C, H, N	1 200
5k	CH_3	C_6H_4-3-NH_2	287–289	D	61	EtOH	$C_{13}H_{13}N_5O$	C, H, N[b]	1 540
5l	CH_3	C_6H_4-2-OCH_3	222–223	B	36	EtOH	$C_{14}H_{14}N_4O_2$	C, H, N	10 000
5m	CH_3	C_6H_3-3,4-$(OCH_3)_2$	259–260	A	19	EtOH	$C_{15}H_{16}N_4O_3$	C, H, N	1 920
5n	CH_3	C_6H_3-2,4-$(OCH_3)_2$	246–247	A	16	EtOH	$C_{15}H_{16}N_4O_3$	C, H, N	5 430
5o	CH_3	C_6H_3-2-NH_2,4-Cl	309–311	D	66	EtOH	$C_{13}H_{12}N_5ClO{\cdot}0.25H_2O$	C, H, N, Cl[c]	310
5p	CH_3	C_6H_4-2-NH_2	290–292	D	83	EtOH	$C_{13}H_{13}N_5O$	C, H, N	230
5q	CH_3	C_6H_4-4-NH_2	340–341	D	61	EtOH	$C_{13}H_{13}N_5O$	C, H, N	380
5r	CH_3	C_6H_4-4-SO_3H	>360	A	77	EtOH/H_2O	$C_{13}H_{12}N_4O_4S$	C, H, N, S	2 300
5s	CH_3	C_6H_3-3,4-Cl_2	>360	B	94	EtOH	$C_{13}H_{10}N_4Cl_2O$	C, H, N, Cl[d]	890
5t	CH_3	C_6H_3-3,5-$(OCH_3)_2$	255–256	B	24	EtOH	$C_{15}H_{16}N_4O_3{\cdot}0.5H_2O$	C, H, N	7 060
5u	CH_3	C_6H_4-3-OCH_3	263–264	C	14	EtOH	$C_{14}H_{14}N_4O_2$	C, H, N	1 470
5v	C_2H_5	$CH_2C_6H_5$	209–210	B	63	EtOH	$C_{15}H_{16}N_4O$	C, H, N	820
5w	C_2H_5	4-pyridyl	257–260	B	53	EtOH	$C_{13}H_{13}N_5O$	C, H, N	250
5x	CH_3	C_6H_4-4-CF_3	286–289	A	69	EtOH	$C_{14}H_{11}N_4F_3O{\cdot}0.5H_2O$	C, H, N	463
5y	CH_3	C_6H_3-2-NO_2,4-Cl	254–257	A	28	EtOH	$C_{13}H_{10}N_5ClO_3$	C, H, N, Cl	8 500
5z	CH_3	C_6H_4-4-$SO_2NH(CH_2)_2N(CH_3)_2$	236–238		7	MeOH	$C_{17}H_{22}N_6O_3S{\cdot}1.5H_2O$	C, H, N, S	68

[a] Nanomolar, using a rat brain membrane preparation. [b] N: calcd, 27.43; found, 27.02. [c] Cl: calcd, 12.05; found, 10.75. [d] Cl: calcd, 21.67; found, 20.84. [e] The compounds were satisfactory as isolated directly from reaction mixture.

that results from the two protocols were highly correlated, with affinities being roughly 50-fold higher at the bovine than the rat A_1 receptor.[4]

SAR Correlation. To determine if the effects on potency by 1- and 5-substituents of the pyrazolo[4,3-*d*]pyrmidin-7-ones paralleled those from the 3- and 8-positions, respectively, on the xanthines, a quantitative correlation of potencies of analogously substituted analogues in the two series was run. Initially, 15 pairs of analogues were available for comparison that possessed measured IC_{50} values (Table II, set 1). Linear regression analysis using logarithms of the IC_{50} values gave eq 2, where $IC_{50pyrim}$ and

$$\log(100\,000/IC_{50pyrim}) = 0.34\ (\pm 0.06)\log(100\,000/IC_{50xan}) + 0.59 \quad (2)$$

$$n = 15,\ r^2 = 0.74,\ F = 38,\ s = 0.27$$

IC_{50xan} are IC_{50}'s of the pyrazolo[4,3-*d*]pyrimidin-7-ones and xanthine analogues, respectively. The benzyl-substituted pyrazolo[4,3-*d*]pyrimidin-7-one (**5b**, Table II) is much more potent than predicted, suggesting that this analogue is binding in a somewhat different orientation. A benzyl group would present its phenyl ring at an angle to the pyrazolo[4,3-*d*]pyrimidin-7-one ring system different than that of the other aryl-substituted derivatives, possibly altering the fit to the receptor. Deleting this compound and rerunning the regression produced eq 3. Potencies calculated with this equation and the residuals appear in Table II.

$$\log(100\,000/IC_{50pyrim}) = 0.41\ (\pm 0.04)\log(100\,000/IC_{50xan}) + 0.29 \quad (3)$$

$$n = 14,\ r^2 = 0.91,\ F = 117,\ s = 0.17$$

Subsequent to these calculations, IC_{50} measurements were completed on six additional 1,5-disubstituted pyrazolo[4,3-*d*]pyrimidin-7-ones for which analogously substituted xanthines were known, thus affording the opportunity to test the predictivity of eq 3. These are shown on the bottom of Table II (set 2). Potencies of the additional analogues were well-predicted, with five of six within 1 standard deviation of the predictions from eq 3.

Equations 4 and 5 are the correlations that were respectively obtained by using the entire compound set and deleting the benzyl analogue (compound **5b**). The data

$$\log(100\,000/IC_{50pyrim}) = 0.37\ (\pm 0.05)\log(100\,000/IC_{50xan}) + 0.49 \quad (4)$$

$$n = 21,\ r^2 = 0.74,\ F = 53,\ s = 0.27$$

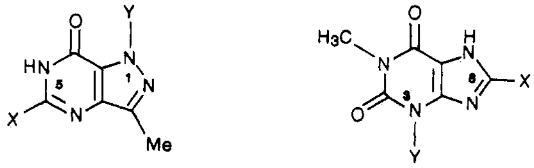
$$\log(100\,000/IC_{50pyrim}) = 0.43\ (\pm 0.04)\log(100\,000/IC_{50xan}) + 0.23 \quad (5)$$

$$n = 20,\ r^2 = 0.86,\ F = 116,\ s = 0.20$$

are plotted in Figure 1. In view of the high correlations, it is hypothesized that the pyrazolo[4,3-*d*]pyrimidin-7-ones and the xanthines are acting at the same receptor in a similar manner. The close overlap possible for both the rings and substituents between the two series is illustrated in Figure 2 with the 2-amino-4-chlorophenyl derivatives.

Subsequent to this analysis, one additional pyrazolo-[4,3-*d*]-pyrimidin-7-one (compound **5z**) containing a para phenyl substituent to improve aqueous solubility was prepared. High potency was maintained with compound **5z**, as expected, and aqueous solubility was increased. This compound is currently undergoing further testing. These pyrazolo[4,3-*d*]pyrimidin-7-ones thus represent novel

Table II. Binding Affinities of Analogously Substituted Pyrazolo[4,3-*d*]pyrimidines and Xanthines



compd[a]	X	Y	IC_{50xan}[b]	PCY_{xan}[c]	$IC_{50pyrim}$[d]	PCY_{pyrim}[c] obsd	calcd[e]	residual
				Set 1				
5a	H	CH_3	20 000	0.7	20 100	0.7	0.6	0.1
5b	$CH_2C_6H_5$	CH_3	1 500	1.8	1 310	1.9	1.0	0.9[f]
5c	4-pyridyl	CH_3	35	3.5	950	2.0	1.7	0.3
5d	3-pyridyl	CH_3	50	3.3	3 090	1.5	1.6	-0.1
5e	C_6H_5	C_2H_5	3.0	4.5	670	2.2	2.1	0.1
5f	C_6H_5	CH_3	3.0	4.5	1 020	2.0	2.1	-0.1
5g	C_6H_4-4-Cl	CH_3	0.8	5.1	410	2.4	2.4	0.0
5h	C_6H_4-4-CH_3	CH_3	0.8	5.1	400	2.4	2.4	0.0
5i	C_6H_4-4-NO_2	CH_3	8.0	4.1	880	2.1	2.0	0.1
5j	C_6H_4-3-NO_2	CH_3	50	3.3	1 200	1.9	1.6	0.3
5k	C_6H_4-3-NH_2	CH_3	10	4.0	1 540	1.8	1.9	-0.1
5l	C_6H_4-2-OCH_3	CH_3	350	2.5	10 000	1.0	1.3	-0.3
5m	C_6H_3-3,4-$(OCH_3)_2$	CH_3	23	3.6	1 920	1.7	1.8	-0.1
5n	C_6H_3-2,4-$(OCH_3)_2$	CH_3	200	2.7	5 430	1.3	1.4	-0.1
5o	C_6H_3-2-NH_2, 4-Cl	CH_3	0.4	5.4	310	2.5	2.5	0.0
				Set 2				
5p	C_6H_4-2-NH_2	CH_3	5.5	4.3	230	2.6	2.0	0.6[f]
5q	C_6H_4-4-NH_2	CH_3	1.8	4.7	380	2.4	2.2	0.2[f]
5r	C_6H_4-4-SO_3H	CH_3	22	3.6	2 300	1.6	1.8	-0.2[f]
5s	C_6H_3-3,4-Cl_2	CH_3	5.0	4.3	890	2.0	2.0	0.0[f]
5t	C_6H_3-3,5-$(OCH_3)_2$	CH_3	500	2.3	7 060	1.2	1.2	0.0[f]
5u	C_6H_4-3-OCH_3	CH_3	2.0	3.7	1 470	1.8	1.8	0.0[f]

[a] Compound number of pyrazolo[4,3-*d*]pyrimidine analogue. [b] Potency (nM) of xanthine analogue, using a bovine brain membrane preparation. [c] Defined as log $(100\,000/IC_{50})$. [d] Potency (nM) of pyrazolo[4,3-d]pyrimidine analogue, using a rat brain membrane preparation. [e] Using eq 3. [f] These compounds were not used in the development of eq 3.

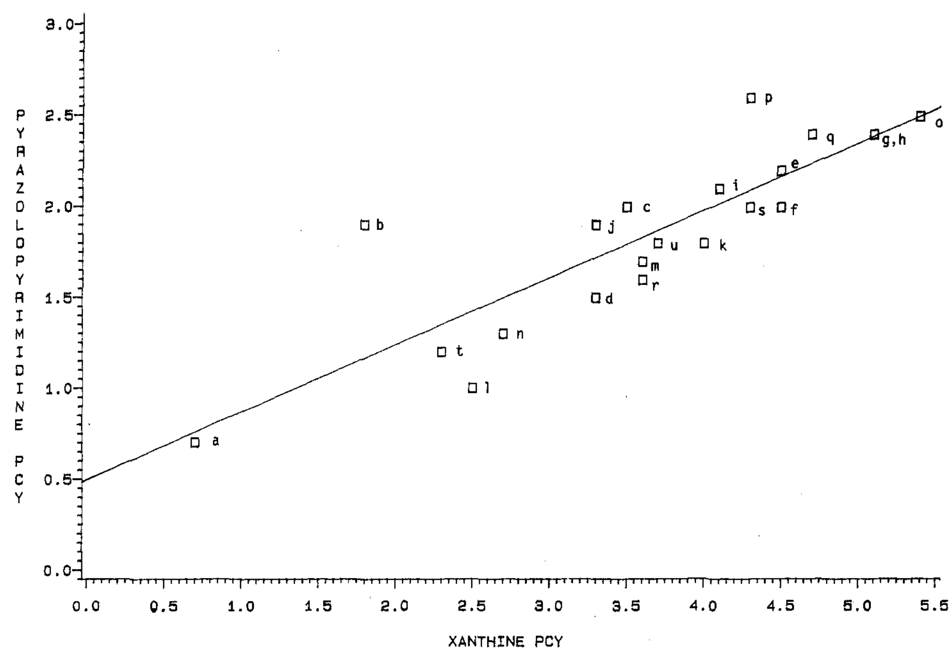


Figure 1. Plot of the potencies of analogously substituted pyrazolo[4,3-*d*]pyrimidin-7-ones and xanthines. Letters denote analogues of compound 5 (see Table II).

adenosine antagonists that are hypothesized to fit the receptor in an analogous fashion to the 8-phenylxanthines reported earlier.

Experimental Section

Chemistry. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were determined on a Digilab FTS-14 spectrometer, as KBr pellets unless noted otherwise. ^1H NMR spectra were run on a Varian Associates EM-390 instrument; chemical shifts are reported in parts per million (δ) relative to Me_4Si as an internal standard. Mass spectra were obtained with a Finnigan 4523 GC/MS instrument. Elemental analyses were performed by the Warner-Lambert/Parke-Davis Analytical Chemistry Section.

1,3-Dimethyl-4-nitro-1*H*-pyrazole-5-carboxylic Acid (2). To a mixture of 112 mL of concentrated H_2SO_4 and 42 mL of 90% HNO_3 at 70–80 °C was added portionwise 39.0 g (0.28 mol) of 1,3-dimethyl-1*H*-pyrazole-5-carboxylic acid[5] (1) with stirring, while

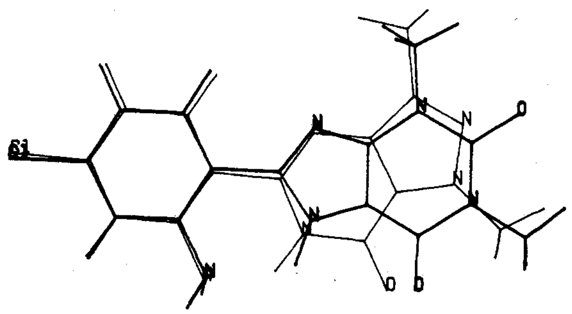


Figure 2. Superposition of 5-(2-amino-4-chlorophenyl)-1,6-dihydro-1,3-dimethyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (thin lines) and xanthine (thick lines) analogues showing overlap of functional groups.

the temperature was maintained at <90 °C. After 2.5 h, the reaction mixture was cooled to ambient temperature and poured into ice. The resulting precipitate was collected, dried, and recrystallized from ethanol to give 40.5 g (78%) of the product: mp 141–142 °C; IR 1729, 1518, 1385, 1243, 1161 cm^{-1}; NMR (Me_2SO-d_6) δ 9.3 (bs, 1 H), 3.85 (s, 3 H), 2.35 (s, 3 H).

1,3-Dimethyl-4-nitro-1*H*-pyrazole-5-carboxamide (3). A mixture of 40.0 g (0.22 mol) of 1,3-dimethyl-4-nitro-1*H*-pyrazole-5-carboxylic acid (2) and 100 mL of $SOCl_2$ was heated under reflux for 3.5 h. The mixture was evaporated to dryness in vacuo, and the resulting oil was dissolved in acetone and added to cold ammonium hydroxide with stirring. The resulting precipitate was collected and dried to give 21.3 g (53%) of product: mp 154–158 °C; IR 3400, 1675, 1520, 1350 cm^{-1}; NMR (Me_2SO-d_6) δ 8.4–8.1 (bd, 2 H), 3.75 (s, 3 H), 2.4 (s, 3 H).

4-Amino-1,3-dimethyl-1*H*-pyrazole-5-carboxamide (4). To a solution of 50.0 g (0.27 mol) of 1,3-dimethyl-4-nitro-1*H*-pyrazole-5-carboxamide (3) in 500 mL of methanol was added 2.0 g of 50% aqueous Raney nickel, and the mixture was reduced under a hydrogen atmosphere until an 8.15-lb drop in pressure was noted. The reaction mixture was filtered, the filtrate concentrated to dryness in vacuo, and the residue recrystallized from ethyl acetate to yield 24.8 g (60%) of the product: mp 154–155 °C; IR 3370, 1650, 1620 cm^{-1}; NMR (Me_2SO-d_6) δ 7.4 (bs, 2 H), 4.0 (bs, 2 H), 3.8 (s, 3 H), 2.0 (s, 3 H).

1,3-Dimethyl-4-nitro-1*H*-pyrazole-5-carbonitrile (8). To a solution of 55.0 g (0.31 mol) of 5-chloro-1,3-dimethyl-4-nitro-1*H*-pyrazole[6] (7) in 400 mL of CH_3CN was added 17.4 g (0.27 mol) of potassium cyanide, followed by 0.5 g of KI and 5 mL of DMF. After 22 h of reflux, the reaction mixture was cooled and filtered. The filtrate was concentrated to dryness in vacuo and added to 500 mL of water. The resulting solid was collected and recrystallized twice from *i*-PrOH to yield 44.5 g (85%) of product: mp 96–98 °C; IR 2200, 1543, 1356 cm^{-1}.

4-Amino-1,3-dimethyl-1*H*-pyrazole-5-carbonitrile (9). A solution of 27.0 g (1.64 mol) of 1,3-dimethyl-4-nitro-1*H*-pyrazole-5-carbonitrile (8) in 1 L of MeOH was reduced catalytically using Raney nickel. The reaction mixture was filtered, and the filtrate was concentrated to dryness in vacuo. The resulting solid (153.8 g, 69%), mp 109–111 °C, was used without further purification: IR 3420, 3360, 2220 cm^{-1}; NMR ($CDCl_3$) δ 3.8 (s, 3 H), 3.5–3.0 (bs, 2 H), 2.2 (s, 3 H).

Preparation of Pyrazolo[4,3-*d*]pyrimidin-7-ones (5a–y, Table I). Method A. A mixture of 5.0 g (32.4 mmol) of 4-amino-1,3-dimethyl-1*H*-pyrazole-5-carboxamide (4) and 4.0 g (32.4 mmol) of benzoic acid was added to 50 g of polyphosphoric acid at 80 °C. The mixture was heated at 140 °C for 6 h, cooled, and poured into ice water with rapid stirring. The resulting precipitate was collected and recrystallized from ethanol to give 3.5 g (45%) of 1,3-dimethyl-5-phenylpyrazolo[4,3-*d*]pyrimidin-7-one (5f): mp 273–274 °C; IR 3200, 3100, 1680, 1560, 1310, 1280, 690 cm^{-1}; NMR (Me_2SO-d_6) δ 12.4–12.2 (bs, 1 H), 8.2–7.95 (m, 2 H), 7.6–7.4 (m, 3 H), 4.15 (s, 3 H), 2.43 (s, 3 H).

Compounds containing pyridyl groups (5c,d,w) were isolated from the aqueous polyphosphoric acid reaction mixture by neutralization with 6 N NaOH. The resulting precipitates were collected and recrystallized to give the products.

Method B. A mixture of 5.0 g (29.7 mmol) of 4-amino-1-ethyl-3-methyl-1*H*-pyrazole-5-carboxamide[6d] (4, X = Et), 3.0 g (29.7 mmol) of triethylamine, and 4.2 g (29.7 mmol) of benzoyl chloride in 25 mL of $CHCl_3$ was stirred for 8 h. The solution was washed with H_2O and concentrated to dryness in vacuo to give 8.0 g (98%) of 4-(benzoylamino)-1-ethyl-3-methyl-1*H*-pyrazole-5-carboxamide (6, X = Et, Y = C_6H_5). This was used in the next step without further purification.

To 150 g of polyphosphoric acid at 80 °C was added 5.1 g (18.8 mmol) of the above intermediate. After stirring for 4 h at 140 °C, the reaction mixture was poured over ice and the resulting precipitate collected and recrystallized from ethanol to give 2.9 g (60%) of 1-ethyl-3-methyl-5-phenylpyrazolo[4,3-*d*]pyrimidin-7-one (5e): mp 218–221 °C.

Method C. A solution of 1.0 g (7.3 mmol) of 4-amino-1,3-dimethyl-1*H*-pyrazole-5-carbonitrile (9), 1.0 g (7.3 mmol) of benzoyl chloride, and 0.7 g (7.3 mmol) of triethylamine in 25 mL of CH_2Cl_2 was stirred for 14 h. The reaction mixture was washed with 5% aqueous HCl, dried over $MgSO_4$, concentrated in vacuo to a pale-orange solid, and recrystallized from a 90:10 $CHCl_3$/MeOH mixture to give 1.0 g (56%) of *N*-(4-cyano-1,3-dimethyl-1*H*-pyrazol-4-yl)benzamide (10, Y = C_6H_5): mp 212–213 °C; IR 3190, 2250, 1660 cm^{-1}; NMR (Me_2SO-d_6) δ 10.1 (bs, 1 H), 8.0–7.8 (m, 2 H), 7.6–7.3 (m, 3 H), 3.9 (s, 3 H), 2.1 (s, 3 H).

To a solution of 0.2 g of NaOH in 30 mL of H_2O at 40 °C was added 0.8 mL of 30% H_2O_2, followed by 0.8 g (3.2 mmol) of the above intermediate. The reaction mixture was stirred at 80 °C for 4.5 h, cooled, and made acidic with glacial HOAc. The white precipitate was collected and recrystallized from ethanol to give 0.6 g (46%) of 1,3-dimethyl-5-phenylpyrazolo[4,3-*d*]pyrimidin-7-one (5f): mp 269–271 °C.

Preparation of 5-(Aminophenyl)-1,3-dimethylpyrazolo[4,3-*d*]pyrimidin-7-ones (5k,o,p,q, Table I). Method D. A mixture of 4.3 g (15 mmol) of 1,3-dimethyl-5-(4-nitrophenyl)-pyrazolo[4,3-*d*]pyrimidin-7-one (5i), 100 mL of H_2O, 0.7 g of NaOH, and 0.4 g of Raney nickel was reduced under a hydrogen atmosphere for 6.5 h. Sufficient acetone was added to the reaction mixture to dissolve the resulting precipitate. The reaction mixture was filtered to remove the catalyst and the filtrate was partially concentrated in vacuo and adjusted to pH 6 with 0.1 N HCl. The resulting precipitate was collected, dried, and recrystallized from ethanol to give 2.4 g (61%) of 5-(4-aminophenyl)-1,3-dimethyl-pyrazolo[4,3-*d*]pyrimidin-7-one (5q): mp 340–341 °C.

4-(1,3-Dimethyl-7-oxopyrazolo[4,3-*d*]pyrimidin-5-yl)-*N*-[2-(dimethylamino)ethyl]benzenesulfonamide (5z). To a suspension of 2.7 g (8.3 mmol) of 4-(1,3-dimethyl-7-oxo-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonic acid (5r) in 300 mL of DMF (previously dried over 4A molecular sieves) at 0 °C was added 2.0 g (16.6 mmol) of $SOCl_2$, and the mixture was stirred for 1.5 h. The resulting solution was treated dropwise with 10 mL of *N*,*N*-dimethyl-1,2-ethanediamine, while the temperature was maintained at <6 °C. The reaction mixture was then heated at 50 °C for 1 h and cooled overnight. The resulting precipitate was collected, and the filtrate was concentrated in vacuo, added to ice water, stirred for 0.5 h, and filtered again. The resulting gray paste was stirred in hot MeOH for 1 h and filtered hot, and the filtrate was chilled at 0 °C. The precipitate that formed was collected and dried to give 0.2 g (7%) of the product: mp 236–238 °C; NMR (Me_2SO-d_6) δ 8.3 (s, 1 H), 8.2 (s, 1 H), 7.9 (s, 1 H), 7.8 (s, 1 H), 4.15 (s, 3 H), 2.85 (d of d, 2 H), 2.4 (s, 3 H), 2.25 (d of d 2 H), 2.1 (s, 6 H).

Preparation of 1,3-Dialkyl-8-phenylxanthines (I) (Table II). The synthesis of these compounds and the starting materials was reported previously.[4] All compounds had satisfactory ^1H NMR, IR, MS, and elemental analyses.

Pharmacology. Receptor Binding. N^6-[^3H]Cyclohexyladenosine binding[7] in rat brain was performed with use of triplicate incubations for 60 min at 25 °C in 2 mL of 50 mM Tris·HCl buffer (pH 7.7) with 20 mg wet weight of rat brain membranes (whole brain minus brainstem and cerebellum), 1 nM *N*-[^3H]-cyclohexyladenosine (30 Ci/mmol), and 0.1 unit/mL of adenosine deaminase.

Data Processing. Regression analyses and the plot were run on an IBM 3081 machine using the SAS program package.[8] In

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eq 1–5, the figures in parentheses are the standard errors of the regression coefficients. For a given equation, n is the number of compounds, r is the correlation coefficient, F is a significance test, and s is the standard error of the estimate.

Molecular Modeling. Figure 2 was generated by using the SYBYL Molecular Modeling Package[9] running on a VAX 11/780.

Acknowledgment. We thank Robert F. Bruns and Gina H. Lu for performing the receptor binding assays.

Registry No. 1(X = CH_3), 5744-56-9; 2(X = CH_3), 3920-37-4; 2(X = CH_3, acid chloride), 37141-71-2; 3(X = CH_3), 78208-58-9; 4(X = CH_3), 59023-32-4; 4(X = C_2H_5), 89239-62-3; **5a**, 51222-27-6; **5b**, 104393-21-7; **5c**, 104393-30-8; **5d**, 104393-22-8; **5e**, 104393-44-4; **5f**, 104393-31-9; **5g**, 104393-23-9; **5h**, 104393-24-0; **5i**, 104393-37-5; **5j**, 104393-38-6; **5k**, 104393-40-0; **5l**, 104393-25-1; **5m**, 104393-32-0; **5n**, 104393-33-1; **5o**, 104393-41-1; **5p**, 104393-42-2; **5q**, 104393-43-3; **5r**, 104393-34-2; **5s**, 104393-26-2; **5t**, 104393-27-3; **5u**, 104393-39-7; **5v**, 104393-28-4; **5w**, 104393-29-5; **5x**, 104393-35-3; **5y**, 104393-36-4; **5z**, 104393-45-5; **6b**, 104393-46-6; **6d**, 104393-47-7; **6e**, 104393-53-5; **6g**, 104393-48-8; **6h**, 104393-49-9; **6l**, 104393-50-2; **6s**, 104393-51-3; **6t**, 104393-52-4; **6v**, 104393-54-6; **6w**, 104393-55-7; **7**, 13551-73-0; **8**, 32183-13-4; **9**, 32183-14-5; **10f**, 104393-59-1; **10i**, 104393-56-8; **10j**, 104421-41-2; **10u**, 104393-57-9; **II**(R_1 = CH_3, R_3 = CH_3, 8 = H), 58-55-9; **II**(R_1 = R_3 = CH_3, 8 = $CH_2C_6H_5$), 2879-15-4; **II**(R_1 = R_3 = CH_3, 8 = 4-pyridyl), 1088-64-8; **II**(R_1 = R_3 = CH_3, 8 = 3-pyridyl), 1029-62-5; **II**(R_1 = CH_3, R_3 = C_2H_5, 8 = C_6H_5), 104393-58-0; **II**(R_1 = R_3 = CH_3, 8 = C_6H_5), 961-45-5; **II**(R_1 = R_3 = CH_3, *p*-Cl), 29064-02-6; **II**(R_1 = R_3 = CH_3, *p*-CH_3), 57196-70-0; **II**(R_1 = R_3 = CH_3, *p*-NO_2), 1094-63-9; **II**(R_1 = R_3 = CH_3, *m*-NO_2), 78146-59-5; **II**(R_1 = R_3 = CH_3, *m*-NH_2), 85872-65-7; **II**(R_1 = R_3 = CH_3, *o*-OCH_3), 85872-55-5; **II**(R_1 = R_3 = CH_3, *m*-OCH_3, *p*-OCH_3), 93214-85-8; **II**(R_1 = R_3 = CH_3, *o*-OCH_3, *p*-OCH_3), 93214-92-7; **II**(R_1 = R_3 = CH_3, *o*-NH_2, *p*-Cl), 85872-60-2; **II**(R_1 = R_3 = CH_3, *o*-NH_2), 18830-58-5; **II**(R_1 = R_3 = CH_3, *p*-NH_2), 85872-66-8; **II**(R_1 = R_3 = CH_3, *p*-SO_3H), 80206-91-3; **II**(R_1 = R_3 = CH_3, *m*-Cl, *p*-Cl), 54013-58-0; **II**(R_1 = R_3 = CH_3, *m*-OCH_3, 5-OCH_3), 93214-89-2; **II**(R_1 = R_3 = CH_3, *m*-OCH_3), 85872-64-6; $C_6H_5CO_2H$, 65-85-0; 3,4-$(OCH_3)_2C_6H_3CO_2H$, 93-07-2; 2,4-$(OCH_3)_2C_6H_3CO_2H$, 91-52-1; 4-$H_3CC_6H_4CO_2H$, 99-94-5; 4-$HO_3SC_6H_4CO_2H$, 636-78-2; 2-$O_2NC_6H_4CO_2H$, 552-16-9; 2-O_2N-4-$ClC_6H_4C_6H_3CO_2H$, 6280-88-2; C_6H_5COCl, 98-88-4; $(H_3C)_2NCH_2CH_2NH_2$, 108-00-9; 4-pyridylbenzoic acid, 55-22-1.

(8) *SAS User's Guide: Statistics, 1982 Edition*; SAS Institute, Inc.: Cary, NC, 1982.
(9) Commercially available from Tripos Associates, Inc., St. Louis, MO 63117.
(10) We thank Dr. Horace DeWald for graciously supplying a sample of this compound.

Substituted Arylmethyl Phenyl Ethers. 1.[1] A Novel Series of 5-Lipoxygenase Inhibitors and Leukotriene Antagonists

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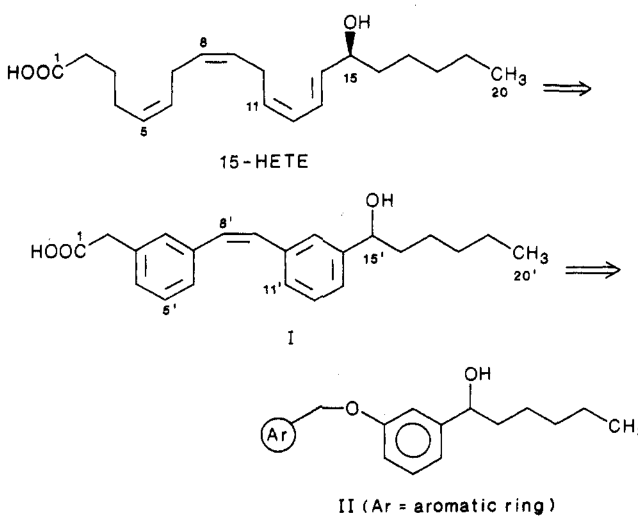
A series of new substituted arylmethyl phenyl ethers has been prepared. These compounds were tested as inhibitors of 5-lipoxygenase (5-LO) in rat neutrophils, in vitro antagonists of leukotriene-induced contraction of guinea pig (GP) lung parenchymal strips, and inhibitors of slow reacting substance of anaphylaxis (SRS-A) mediated bronchospasm in the GP in vivo. Most representatives of this new class of potential antiallergic/antiinflammatory agents showed potent inhibition of 5-LO activity in rat PMNs. The most potent compound, 2-[[3-(1-hydroxyhexyl)phenoxy]-methyl]quinoline (**33**), had an I_{50} of 0.12 μM in the rat PMN 5-LO assay and an I_{50} of 3.6 μM in the leukotriene-induced contraction of GP lung parenchymal strips, and it also showed 91% inhibition of SRS-A-mediated bronchospasm in the GP in vivo at 10 mg/kg, administered intraduodenally. *Some of the compounds in this series were also leukotriene* antagonists in vitro, and several of them showed in vivo activity against SRS-A-mediated bronchospasm in the GP.

The biosynthesis of prostaglandins (PG) from arachidonic acid (AA) is well-established.[2] Inhibition of this pathway may explain the therapeutic effects of nonsteroidal antiinflammatory agents in rheumatic diseases.[3] There is interest now in another aspect of the oxidative metabolism of arachidonic acid, i.e., the production of leukotrienes (LT) via the 5-lipoxygenase (LO) pathway.[4] Since LTC_4 and LTD_4 are potent bronchoconstrictors of human bronchi,[5] and LTB_4 is a powerful chemotactic factor for leukocytes,[6] inhibitors of 5-LO and/or antagonists of LTC_4 may be of therapeutic value in the treatment of asthma and inflammatory diseases.

Scheme I[a]



[a] ⟹ denotes stepwise structural evolution.

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(1) Preliminary accounts of this work were presented earlier. (a) Coutts, S.; Khandwala, A.; Van Inwegen, R.; Chakraborty, U.; Musser, J.; Bruens, J.; Jariwala, N.; Dalley-Meade, V.; Ingram, R.; Pruss, T.; Jones, H.; Neiss, E.; Weinryb, I. In *Prostaglandins, Leukotrienes, and Lipoxins*; Bailey, J. M., Ed.; Plenum: New York, 1985; p 627. (b) Gordon, R. J.; Travis, J.; Godfrey, H. R.; Sweeney, D.; Wolf, P. S.; Pruss, T. P.; Neiss, E.; Musser, J.; Chakraborty, U.; Jones, H.; Leibowitz, M. *Prostaglandins and Leukotrienes 1984*; Washington, DC, Abstr. 266.
(2) Samuelsson, B. *Fed. Proc. Am. Soc. Exp. Biol.* **1972**, *31*, 1442.
(3) Simon, L. S.; Mills, J. A. *New Engl. J. Med.* **1980**, *302*, Part 1, 1179, Part 2, 1237.

0022-2623/87/1830-0096$01.50/0