Blood Pressure Measurements in Renal Hypertensive Rats. The testing of compounds for antihypertensive activity in renal hypertensive rats was performed by Pharmakon Laboratories in Waverly, PA. The testing method they employed is described below.

Hypertension of renal origin was produced in rats by placing a silver clip around the left renal artery near the aorta and leaving the contralateral kidney intact. Several weeks later, the rats were cannulated for blood pressure monitoring by the method of Weeks and Jones.[8] Rats with mean blood pressure greater than 160 mmHg were used for the studies. Four rats received the test compound orally in a 0.25% methylcellulose aqueous solution at 5 mL/kg. Two rats were administered the 0.25% methylcellulose aqueous solution alone at 5 mL/kg orally and served as the controls. Systolic, diastolic, and mean blood pressure and heart rate were monitored prior to dosing and hourly for 8 h and at 24 h after test or control article administration.

Registry No. **2**, 96792-01-7; **3**, 96792-02-8; **4**·HOAc, 96792-14-2; **5**, 63808-36-6; **6**, 33876-20-9; **7**, 96792-04-0; **8**, 74075-31-3; **9**, 96792-05-1; **10**, 73858-58-9; **11**, 96792-06-2; **12**, 33878-70-5; **13**, 96792-07-3; **14**, 96792-08-4; **15**, 96792-09-5; **16**, 96792-10-8; **17**, 96792-12-0; **17** (free base), 96792-11-9; $(BOC)_2O$, 24424-99-5; ACE, 9015-82-1; *N*-(benzyloxycarbonyl)-L-proline amide, 34079-31-7; *N*-(*tert*-butyloxycarbonyl)-L-proline, 15761-39-4; hydroxylamine hydrochloride, 5470-11-1; succinimidyl 5-(*S*)-[[N^{ϵ}-(*tert*-butyloxycarbonyl)-N^{α}-(cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoate, 96792-13-1.

Synthesis of Xanthines as Adenosine Antagonists, a Practical Quantitative Structure–Activity Relationship Application

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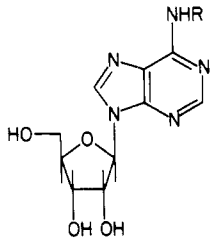
A set of 56 8-phenylxanthines, previously tested for adenosine antagonism (adenosine A_1 receptor affinity), was analyzed by quantitative structure–activity relationship (QSAR) techniques. The resulting QSAR revealed that (1) the most potent receptor binders had already been made in this series and thus suggested the termination of synthesis of compounds with additional phenyl substituents to increase potency and (2) potency was much more strongly affected by changes in ortho than para phenyl substitution. On the basis of this study, an additional 20 compounds were synthesized that contained primarily para substituents designed to increase aqueous solubility. High potency was maintained among the resulting sulfonamide derivatives (as predicted by the QSAR), and aqueous solubility was dramatically increased. Furthermore, in vitro antagonism of an adenosine receptor mediated physiological effect was demonstrated.

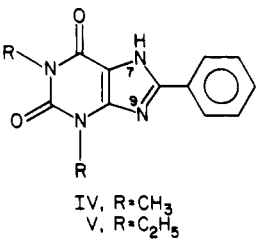
Xanthines have long been known to cause a variety of physiological effects. The central nervous system (CNS) stimulatory properties of caffeine have been utilized for centuries. In addition, tachycardia[1] and bronchodilation[2] are responses elicited by this class of compounds. Inhibition of phosphodiesterase in the heart, brain, and lungs has been postulated[1,3] as the mechanism by which xanthines elicit these effects.

Recently, the role of xanthines as antagonists of adenosine (I) binding has emerged as an alternate explanation for these effects. In vitro, xanthines antagonize a number



I, R=H
II, R=cyclohexyl
III, R=(*R*)-1-methyl-2-phenylethyl

of effects produced by adenosine and the adenosine deaminase resistant analogues N^6-cyclohexyladenosine (II) and (*R*)-N^6-(1-methyl-2-phenylethyl)adenosine ((*R*)-PIA, III).[4] The effects of adenosine and these analogues are mediated by extracellular adenosine receptors that can be divided into two subtypes, A_1 and A_2, which inhibit and stimulate, respectively, adenylate cyclase. Because of the relatively low affinity (micromolar) of caffeine and theophylline at adenosine receptors, 8-phenyltheophylline (IV) and 1,3-diethyl-8-phenylxanthine (V) were developed.[5] They possess at least 25-fold higher affinities at adenosine receptors and act as antagonists in vitro.[5a]



IV, R=CH_3
V, R=C_2H_5

Other pharmacological studies using xanthines as adenosine antagonists support this explanation.[6] Xanthine antagonism of endogenous adenosine in the brain has

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(1) Ritchie, J. M. In "The Pharmacological Basis of Therapeutics"; Goodman, L. S., Gilman, A., Eds.; Macmillan: New York, 1968; pp 367–378.
(2) (a) Jones, T. R.; Lefcoe, N. M.; Hamilton, J. T. *Can. J. Physiol. Pharm.* **1980**, *58*, 1356. (b) Karlsson, J. A.; Kjellin, G.; Persson, C. G. A. *J. Pharm. Pharmacol.* **1982**, *34*, 788.
(3) (a) "Kaffee and Coffein"; Eichler, O., Ed.; Springer: Berlin, 1976. (b) Piafsky, K. H.; Ogilvie, R. I. *N. Engl. J. Med.* **1975**, *299*, 1218.
(4) (a) Schwabe, U. *Trends Pharmacol. Sci.* **1981**, 299. (b) Murphy, K. M. M.; Snyder, S. H. *Mol. Pharmacol.* **1983**, *22*, 250.
(5) (a) Bruns, R. F. *Biochem. Pharmacol.* **1981**, *30*, 325. (b) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547.
(6) (a) Fredholm, B. B. *Trends Pharmacol. Sci.* **1980**, *1*, 129. (b) Daly, J. W.; Bruns, R. F.; Snyder, S. H. *Life Sci.* **1981**, *28*, 2083.

0022-2623/85/1828-1071$01.50/0

been studied electrophysiologically,[7] behaviorally,[8] and biochemically.[9] Xanthines antagonize the cardiac depressant effects of adenosine and adenosine agonists[10] at much lower concentrations than are required to inhibit phosphodiesterase,[6] thus suggesting adenosine antagonism rather than phosphodiesterase inhibition as their primary mechanism of action.

Quantitative structure–activity relationship (QSAR) studies have been reported for xanthine derivatives in a number of different pharmacological areas. 9-Phenylguanines have been studied extensively as inhibitors of xanthine oxidase[11] and other enzymes.[12] 6-Mercaptoxanthines have been studied for bronchodilator activity.[13] Other xanthine analogues have been examined for phosphodiesterase inhibition and cytotoxicity[14] as well as inhibition of human erythrocytic hypoxanthine phosphoribosyltransferase.[15] Purine derivatives have been examined for prediction of pK_a values and anticancer activity.[16] No QSAR studies have been reported, however, for xanthines as adenosine antagonists.

The general methods of synthesis[17] and a qualitative structure–activity analysis (SAR)[18] of alkyl- and arylxanthines as antagonists of adenosine A_1 receptors have been reported. Adenosine receptor binding affinity was measured by inhibition of N^6-[^{3}H]cyclohexyladenosine binding to bovine brain membranes. In the SAR study, substitutions on the phenyl ring of 8-phenylxanthines were found to have a profound effect on the receptor binding potency of the compounds.[18]

Although several of these derivatives are quite potent in receptor binding, evaluation in in vivo models designed to detect CNS[19] and cardiovascular[20] effects revealed little or no activity. This was thought to be due to low aqueous solubility, leading to inadequate bioavailability. Indeed, when tritiated **16** (Table I) was administered orally to dogs, absorption was found to be less than 0.1%.[21]

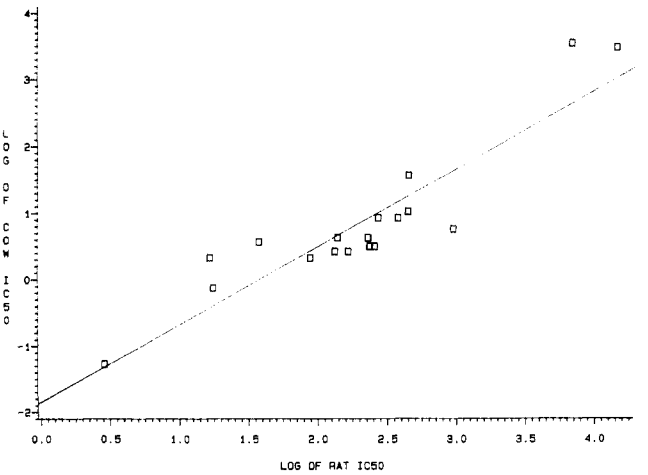

Figure 1. Plot of the logs of the cow vs. rat brain assay results.

In an attempt to define xanthine structures with maximum in vitro receptor binding potency, we have analyzed the compounds of ref 17c and 18 by QSAR techniques.[22] In particular, the QSAR study was used to determine positions on the phenyl ring where potency was the least sensitive to changes in substitution. Once defined, these positions were substituted with groups to increase aqueous solubility. Thus, with use of the QSAR study as a guide, an additional 20 xanthines were synthesized with substituents designed to maintain potency and to increase aqueous solubility. This report describes the underlying QSAR and the synthesis and A_1 receptor binding affinity for these additional compounds. Selected compounds were demonstrated to have adenosine antagonist activity in vitro.

Biological Evaluation

The initial set of xanthine derivatives (**1–56**, Table I) was evaluated for adenosine A_1 receptor affinity by measuring inhibition of N^6-[^{3}H]cyclohexyladenosine binding to bovine brain membranes.[5a,18] Values used in the present paper are IC_{50}'s, which can be converted to K_i values by dividing by 2.43. The new compounds (**57–76**, Table II) were tested in a modified assay[23] using a rat brain membrane preparation (see Experimental Section). To verify that the results from the two protocols could be quantitatively compared, a diverse set of 18 derivatives was selected from ref 18 (Table III) to provide a large range in receptor affinities and retested by using the modified assay procedure. Linear regression on the logarithms of the IC_{50}'s gave eq 1. The data are plotted in Figure 1.

$$\log IC_{50rat} = 0.71\ (\pm 0.08) \log IC_{50cow} + 1.7 \qquad (1)$$

$$n = 18,\ r^2 = 0.83,\ F = 80,\ s = 0.37$$

Because of the high correlation between assay results from these two protocols, QSAR's developed with the IC_{50cow} data can be reasonably applied to the IC_{50rat} results. As is evident from eq 1, affinities of 8-substituted xanthines are about 50-fold higher at the bovine than the rat A_1 receptor.

Quantitative Structure–Activity Relationships

The set of 56 xanthine derivatives considered for this study (**1–56**), their potencies in the bovine membrane assay (IC_{50cow}), and the pertinent physicochemical parameters and indicator variables appear in Table I. Potency

(7) (a) Dunwiddie, T. V.; Hoffer, B. J.; Fredholm, B. B. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *316*, 326. (b) Motley, S. J.; Collins, G. G. S. *Neuropharmacology* **1983**, *22*, 1081.
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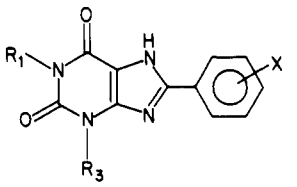
(9) Sattin, A.; Rall, T. W. *Mol. Pharmacol.* **1970**, *6*, 13.
(10) Evans, D. B.; Schenden, J. A.; Bristol, J. A. *Life Sci.* **1982**, *31*, 2425.
(11) (a) Silipo, C.; Hansch, C. *Farmaco, Ed. Sci.* **1974**, *30*, 35. (b) Grieco, C.; Silipo, C.; Vittoria, A. *Ibid.* **1977**, *33*, 382.
(12) Silipo, C.; Hansch, C. *J. Med. Chem.* **1976**, *19*, 62.
(13) Bowden, K.; Wooldridge, K. R. H. *Biochem. Pharmacol.* **1973**, *22*, 1015.
(14) Lien, E. J.; Mayer, K.; Wang, P. H.; Tong, G. L. *Acta Pharm. Jugosl.* **1979**, *29*, 181.
(15) (a) Olaru, N.; Simon, Z. *Rev. Roum. Biochim.* **1981**, *18*, 51. (b) Olaru, N.; Simon, Z. *Ibid.* **1981**, *18*, 131.
(16) (a) Neiman, Z.; Quinn, F. R. *J. Pharm. Sci.* **1981**, *70*, 425. (b) Neiman, Z.; Quinn, F. R. *Ibid.* **1982**, *71*, 618.
(17) (a) Goldner, H.; Dietz, G.; Carstens, E. British Patent 982 079, May 1, 1962. (b) Traube, W. *Chem. Ber.* **1900**, *33*, 3035. (c) Belgian Patent 898 946, June 18, 1984.
(18) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2077.
(19) CNS symptom etiology was examined in male Swiss-Webster mice (×4) at five doses. Drugs were administered intraperitoneally in a mixed solvent system (60% Emulphor, 6% glacial acetic acid, 30% Me_2SO, and 4% H_2O), and the mice were observed over 30 min.
(20) Evans, D. B.; Weishaar, R. E.; Kaplan, H. R. *Pharmacol. Ther.* **1982**, *16*, 303.
(21) Compound **16** was tritiated by catalytic reduction of the diallyl analogue with tritium gas. After purification, the specific activity was found to be 100 Ci/mmol. After administration of 2 mg/kg po to two dogs, absorbtion was determined by scintillation counting of the plasma and urine.
(22) Martin, Y. C. "Quantitative Drug Design, A Critical Introduction"; Grunewald, G. L., Ed.; Marcel Dekker: New York, 1978; p 236.
(23) Hamilton, H. W.; Bristol, J. A. *J. Med. Chem.* **1983**, *26*, 1601.

Table I. Xanthines Used To Develop the QSAR



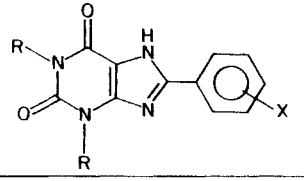
V

compd	R_1	R_3	X	HACCEPT$_m$	ACID	HBOND	σ_o	MR$_o$[a]	π_{R3}	IC$_{50cow}$[b]	potency[c] obsd	potency[c] calcd[d]	potency[c] residual
1	C_2H_5	C_2H_5	H	0	0	0	0.00	0.10	1.02	3.00	2.52	2.93	−0.41
2	CH_3	H	H	0	0	0	0.00	0.10	0.00	2.50	2.60	2.10	0.50
3	CH_3	CH_3	H	0	0	0	0.00	0.10	0.56	3.00	2.52	2.56	−0.04
4	$CH_2CH{=}CH_2$	CH_3	H	0	0	0	0.00	0.10	0.56	4.00	2.40	2.56	−0.16
5	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	H	0	0	0	0.00	0.10	1.85	0.70	3.15	3.61	−0.46
6	CH_3	CH_3	2-CH_3	0	0	0	−0.17	0.56	0.56	6.50	2.19	2.17	0.02
7	CH_3	CH_3	2-CO_2H	0	1	0	0.45	0.69	0.56	2500.00	−0.40	−0.09	−0.31
8	CH_3	CH_3	2-F	0	0	0	0.06	0.09	0.56	12.50	1.90	2.52	−0.62
9	CH_3	CH_3	2-NH_2	0	0	0	−0.66	0.54	0.56	5.50	2.26	2.63	−0.37
10	CH_3	CH_3	2-NH_2, 4-CH_3	0	0	0	−0.66	0.54	0.56	10.00	2.00	2.63	−0.63
11	$CH_2CH{=}CH_2$	CH_3	2-NH_2, 4-CH_3	0	0	0	−0.66	0.54	0.56	7.00	2.15	2.63	−0.48
12	C_2H_5	C_2H_5	2-NH_2, 4-Cl	0	0	0	−0.66	0.54	1.02	0.80	3.10	3.01	0.09
13	CH_3	CH_3	2-NH_2, 4-Cl	0	0	0	−0.66	0.54	0.56	0.35	3.46	2.63	0.83
14	$CH_2CH{=}CH_2$	CH_3	2-NH_2, 4-Cl	0	0	0	−0.66	0.54	0.56	2.00	2.70	2.63	0.07
15	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	2-NH_2, 4-Cl	0	0	0	−0.66	0.54	1.10	0.80	3.10	3.07	0.03
16	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	2-NH_2, 4-Cl	0	0	0	−0.66	0.54	1.85	0.05	4.30	3.69	0.61
17	CH_3	CH_3	2-NH_2, 4-NO_2	0	0	0	−0.66	0.54	0.56	2.50	2.60	2.63	−0.03
18	CH_3	CH_3	2-NO_2	0	0	0	0.78	0.74	0.56	80.00	1.10	1.13	−0.03
19	CH_3	CH_3	2-OCH_3	0	0	1	−0.27	0.79	0.56	350.00	0.46	0.83	−0.37
20	CH_3	CH_3	2-OH	0	0	1	−0.37	0.28	0.56	10.00	2.00	1.50	0.50
21	CH_3	CH_3	2,3-$(CHCH)_2$	0	0	0	0.00	1.75	0.56	80.00	1.10	0.65	0.45
22	CH_3	CH_3	2,4-$(NH_2)_2$	0	0	0	−0.66	0.54	0.56	8.00	2.10	2.63	−0.53
23	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	2,4-$(NH_2)_2$	0	0	0	−0.66	0.54	1.85	0.15	3.82	3.69	0.13
24	CH_3	CH_3	2,4-$(OCH_3)_2$	0	0	1	−0.27	0.79	0.56	200.00	0.70	0.83	−0.13
25	CH_3	CH_3	2,6-$(CH_3)_2$, 4-OH	0	0	0	−0.34	1.12	0.56	30.00	1.52	1.68	−0.16
26	CH_3	CH_3	3-Br	0	0	0	0.00	0.10	0.56	10.00	2.00	2.56	−0.56
27	CH_3	CH_3	3-CH_3	0	0	0	0.00	0.10	0.56	13.00	1.89	2.56	−0.67
28	CH_3	CH_3	3-CO_2H	1	1	0	0.00	0.10	0.56	1000.00	0.00	0.00	0.00
29	CH_3	CH_3	3-F	0	0	0	0.00	0.10	0.56	4.00	2.40	2.56	−0.16
30	CH_3	CH_3	3-$N(CH_3)_2$	1	0	0	0.00	0.10	0.56	80.00	1.10	1.57	−0.47
31	CH_3	CH_3	3-NH_2	1	0	0	0.00	0.10	0.56	10.00	2.00	1.57	0.43
32	CH_3	CH_3	3-NO_2	1	0	0	0.00	0.10	0.56	50.00	1.30	1.57	−0.27
33	CH_3	CH_3	3-OCH_3	1	0	0	0.00	0.10	0.56	20.00	1.70	1.57	0.13
34	CH_3	CH_3	3-OH	1	0	0	0.00	0.10	0.56	6.00	2.22	1.57	0.65
35	CH_3	CH_3	3,4-$(CHCH)_2$	0	0	0	0.00	0.10	0.56	5.50	2.26	2.56	−0.30
36	CH_3	CH_3	3,4-$(OCH_3)_2$	1	0	0	0.00	0.10	0.56	22.50	1.65	1.57	0.08
37	CH_3	CH_3	3,4-Cl_2	0	0	0	0.00	0.10	0.56	5.00	2.30	2.56	−0.26
38	CH_3	CH_3	3,5-$(OCH_3)_2$	2	0	0	0.00	0.10	0.56	500.00	0.30	0.58	−0.28
39	C_2H_5	C_2H_5	4-Br	0	0	0	0.00	0.10	1.02	1.00	3.00	2.93	0.07
40	CH_3	CH_3	4-Br	0	0	0	0.00	0.10	0.56	0.75	3.12	2.56	0.56
41	CH_3	CH_3	4-$CH(CH_3)_2$	0	0	0	0.00	0.10	0.56	2.50	2.60	2.56	0.04
42	CH_3	CH_3	4-CH_3	0	0	0	0.00	0.10	0.56	0.80	3.10	2.56	0.54
43	CH_3	CH_3	4-Cl	0	0	0	0.00	0.10	0.56	0.80	3.10	2.56	0.54
44	CH_3	CH_3	4-CO_2H	0	1	0	0.00	0.10	0.56	50.00	1.30	0.99	0.31
45	CH_3	CH_3	4-C_2H_5	0	0	0	0.00	0.10	0.56	0.80	3.10	2.56	0.54
46	CH_3	CH_3	4-C_6H_5	0	0	0	0.00	0.10	0.56	3.50	2.46	2.56	−0.10
47	CH_3	CH_3	4-F	0	0	0	0.00	0.10	0.56	3.50	2.46	2.56	−0.10
48	CH_3	CH_3	4-I	0	0	0	0.00	0.10	0.56	1.30	2.89	2.56	0.33
49	CH_3	CH_3	4-$N(CH_3)_2$	0	0	0	0.00	0.10	0.56	1.80	2.74	2.56	0.19
50	CH_3	CH_3	4-NH_2	0	0	0	0.00	0.10	0.56	1.75	2.76	2.56	0.20
51	CH_3	CH_3	4-NO_2	0	0	0	0.00	0.10	0.56	8.00	2.10	2.56	−0.46
52	CH_3	CH_3	4-$O(CH_2)_3CH_3$	0	0	0	0.00	0.10	0.56	4.00	2.40	2.56	−0.16
53	CH_3	CH_3	4-OCH_3	0	0	0	0.00	0.10	0.56	1.50	2.82	2.56	0.26
54	CH_3	CH_3	4-OC_2H_5	0	0	0	0.00	0.10	0.56	2.00	2.70	2.56	0.14
55	CH_3	CH_3	4-OH	0	0	0	0.00	0.10	0.56	2.00	2.70	2.56	0.14
56	CH_3	CH_3	4-SCH_3	0	0	0	0.00	0.10	0.56	2.00	2.70	2.56	0.14

[a] Scaled by 0.1. [b] Nanomolar. [c] Defined as the log (1000/IC$_{50cow}$) [d] Using eq 5.

(PTNCY$_{cow}$) is defined as log (1000/IC$_{50cow}$); values range from −0.4 to 4.3, with a standard error of replicate analyses of 0.15. MR was multiplied by 0.1 to place it on a scale similar to that of the other parameters. Several parameters and indicator variables other than those in Table I were also considered. These included $\sum\pi$, $\sum\sigma$, and $\sum$MR of the phenyl ring substituents; π, σ, MR, and hydrogen donating/accepting capability (HDONOR/HACCEPT) of substituents at specific positions on the phenyl ring; an indicator variable (ORTHO) to denote the capability of an ortho substituent to form a six-membered hydrogen-bonded ring with the imidazole N[9]; and π, MR, $\sum\pi$, and

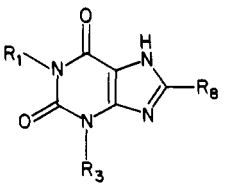
Table II. New Xanthines



compd	R	X	mp, °C	yield, %	purifcn solvent	formula	anal.	IC_{50rat}[a]	IC_{50cow}[b]	calcd IC_{50cow}[c]
16[d]	$(CH_2)_2CH_3$	2-NH_2, 4-Cl						5.0	0.04	
57	CH_3	4-SO_3H	>360	17	MeOH/H_2O	$C_{13}H_{10}N_4O_5SNa_2 \cdot H_2O$	C, H, N, H_2O	448	21.9	78.8 (12.6–501)
58	C_2H_5	4-$N(CH_3)_2$	326–328	26	EtOH	$C_{17}H_{21}N_5O_2$	H, N; C[e]	118	3.3	0.69 (0.11–4.3)
59	C_2H_5	3-SO_3H	>360	31	H_2O	$C_{15}H_{15}N_4O_5SK \cdot 2H_2O$	C, H, N, S	30700	8424	251 (39.8–1584)
60	C_2H_5	4-SO_3H	>360	10	H_2O	$C_{15}H_{16}N_4O_5S$	C, H, N, S	5540	755	25.7 (4.07–162)
61	C_2H_5	4-SO_2NH_2	350–353 dec	57	EtOH	$C_{15}H_{17}N_5O_4S$	C, H, N, S	47	0.91	0.69 (0.11–4.3)
62	C_2H_5	4-$SO_2NHCH_2CH(OH)CH_3$	283–285	64	EtOH	$C_{18}H_{23}N_5O_5S \cdot {}^1/_3H_2O$	C, H, N, S, H_2O	138	4.2	0.69 (0.11–4.3)
63	C_2H_5	3-$SO_2NH(CH_2)_2N(CH_3)_2$	250–251	18	EtOH	$C_{19}H_{26}N_6O_4S \cdot {}^1/_2H_2O$	C, H, S; N,[f] H_2O[g]	553	29.4	6.7 (1.07–42.6)
64	C_2H_5	4-$SO_2NH(CH_2)_2N(CH_3)_2$	264–265	48	EtOH	$C_{19}H_{26}N_6O_4S$	C, H, N, S	116	3.3	0.69 (0.11–4.3)
65	C_2H_5	4-$SO_2NH(CH_2)_3N(CH_3)_2$	291–292	12	EtOH	$C_{20}H_{28}N_6O_4S \cdot HCl \cdot {}^1/_2H_2O$	C, H, N, Cl, S, H_2O	70	1.6	0.69 (0.11–4.3)
66	C_2H_5	4-SO_2-morpholino	354–355 dec	54	EtOH	$C_{19}H_{23}N_5O_4S$	C, H, N, S	310	13.0	0.69 (0.11–4.3)
67	C_2H_5	4-SO_2-thiomorpholino	340–350 dec	97	EtOH	$C_{19}H_{23}N_5O_4S_2$	C, H, N, S	646	36.6	0.69 (0.11–4.3)
68	C_2H_5	4-SO_2-*N*-methylpiperazino	305–307	87	EtOH	$C_{20}H_{26}N_4O_4S$	C, H, N, S	190	6.5	0.69 (0.11–4.3)
69	$CH_2CH{=}CH_2$	4-SO_3H	340–350 dec	67	EtOH	$C_{17}H_{16}N_4O_5S$	C, H, N, S	7440	1144	22.0 (3.49–139)
70	$CH_2CH{=}CH_2$	4-$SO_2NH(CH_2)_3N(CH_3)_2$	310–330 dec	59	EtOH	$C_{22}H_{28}N_6O_4S \cdot HCl$	H, N, Cl, S; C[h]	263	10.3	0.59 (0.09–3.74)
71	$(CH_2)_2CH_3$	2-NH_2	276–277 dec	21	MeOH	$C_{17}H_{21}N_5O_2$	C, H, N	41	0.75	0.21 (0.03–1.35)
72	$(CH_2)_2CH_3$	4-SO_3H	>360	10	MeOH	$C_{17}H_{19}N_4O_5SK \cdot {}^1/_4H_2O$	C, H, N, S, H_2O	120	3.4	7.1 (1.12–44.7)
73	$(CH_2)_2CH_3$	4-$SO_2NH(CH_2)_2N(CH_3)_2$	270–272 dec	39	EtOH	$C_{21}H_{30}N_6O_4S$	C, H, N, S	6.5	0.05	0.19 (0.03–1.20)
74	$(CH_2)_2CH_3$	4-$SO_2NH(CH_2)_3N(CH_3)_2$	246–248 dec	31	EtOH	$C_{22}H_{32}N_6O_4S$	C, H, N, S	8.2	0.08	0.19 (0.03–1.20)
75	$(CH_2)_2CH_3$	4-$SO_2NH(CH_2)_4N(CH_3)_2$	198–204 dec	56	EtOH	$C_{23}H_{34}N_6O_4S \cdot HCl \cdot {}^1/_4H_2O$	C, H, N, Cl, S, H_2O	7.5	0.07	0.19 (0.03–1.20)
76	$(CH_2)_2CH_3$	4-$SO_2NH(CH_2)_3CO_2C_2H_5$	254–256	44	H_2O	$C_{23}H_{31}N_5O_4S$	C, H, N, S	18.5	0.24	0.19 (0.03–1.20)
theophylline								12700	2430	

[a] Nanomolar. [b] Nanomolar, transformed from IC_{50rat} values by using eq 1. [c] Using eq 5. The numbers in parentheses are plus or minus 2 standard deviations. [d] Reference 18. [e] C: calcd, 62.37; found, 61.50. [f] N: calcd, 18.94; found, 18.45. [g] H_2O: calcd, 2.03; found (KF), 1.14. [h] C: calcd, 51.91; found, 51.46.

Table III. Xanthines Used To Correlate Binding Results



compd	R_1	R_3	R_8	log (IC_{50cow})	log (IC_{50rat})
2	CH_3	H	C_6H_5	0.40	2.21
	CH_3	CH_3	H	3.48	4.15
	CH_3	CH_3	NO_2	3.54	3.84
	CH_3	CH_3	$CH(CH_2)_4$	0.30	1.21
	CH_3	CH_3	3-furyl	0.60	2.13
	CH_3	CH_3	4-pyridyl	1.54	2.64
3	CH_3	CH_3	C_6H_5	0.48	2.40
9	CH_3	CH_3	C_6H_4-2-NH_2	0.74	2.96
51	CH_3	CH_3	C_6H_4-4-NO_2	0.90	2.56
56	CH_3	CH_3	C_6H_4-4-SCH_3	0.30	1.93
46	CH_3	CH_3	C_6H_4-4-C_6H_5	0.54	1.56
22	CH_3	CH_3	C_6H_3-2,4-$(NH_2)_2$	0.90	2.42
17	CH_3	CH_3	C_6H_3-2-NH_2, 4-NO_2	0.40	2.11
10	CH_3	CH_3	C_6H_3-2-NH_2, 4-CH_3	1.00	2.63
1	C_2H_5	C_2H_5	C_6H_5	0.48	2.36
4	$CH_2CH{=}CH_2$	CH_3	C_6H_5	0.60	2.35
5	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	C_6H_5	−0.15	1.23
16	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	C_6H_3-2-NH_2, 4-Cl	−1.30	0.45

Table IV. Eigenvalues and Rotated Factor Pattern for Initial Set of 28 Parameters

	factor 1	factor 2	factor 3	factor 4	factor 5	factor 6	factor 7
eigenvalue	8.25	4.33	3.36	2.63	2.45	2.07	1.20
explained variance, %	30	16	12	9	9	7	4
cumulative variance explained, %	30	46	58	67	76	83	87
parameter	factor 1	factor 2	factor 3	factor 4	factor 5	factor 6	factor 7
ORTHO	0.96	−0.04	0.21	0.00	0.06	−0.06	0.04
$HDONOR_o$	0.93	−0.08	0.18	0.03	0.08	−0.08	0.04
π_o	−0.90	0.05	−0.21	−0.06	0.28	0.06	0.01
$HACCEPT_o$	0.85	−0.14	0.11	0.04	0.20	−0.16	0.04
σ_o	−0.84	−0.04	−0.21	0.12	−0.09	0.05	−0.01
π_o^2	0.81	0.00	0.21	−0.07	0.45	−0.03	0.07
MR_{mp}	−0.14	0.95	−0.08	−0.03	0.01	0.21	0.04
MR_{mp}^2	−0.20	0.93	−0.09	0.00	0.03	0.11	0.04
ΣMR	0.16	0.91	−0.05	−0.10	0.22	0.10	0.06
MR_p	−0.01	0.86	−0.03	−0.08	−0.26	−0.32	0.14
$\Sigma\pi_{R1,3}$	0.18	−0.06	0.97	−0.04	0.00	−0.04	0.02
π_{R1}	0.22	−0.08	0.95	−0.01	0.00	−0.06	0.02
π_{R3}	0.13	−0.03	0.95	−0.07	0.01	−0.01	0.01
MR_{R1}	0.30	−0.08	0.83	0.08	0.00	−0.10	0.02
MR_{R3}	0.17	−0.02	0.93	−0.02	0.01	−0.02	0.01
$HDONOR_p$	0.06	−0.18	0.06	−0.83	0.04	−0.02	0.10
$HACCEPT_p$	0.04	0.12	−0.09	−0.81	−0.08	−0.10	0.10
σ_p	0.09	−0.13	−0.01	0.72	−0.05	0.07	0.11
MR_o^2	0.05	0.03	−0.02	−0.04	0.96	0.01	0.12
MR_o	0.34	−0.06	0.02	−0.06	0.89	−0.09	0.10
MR_m	−0.21	0.28	−0.08	0.09	0.35	0.76	−0.02
σ_m	−0.09	−0.11	−0.08	0.12	−0.22	0.71	0.09
$HDONOR_m$	−0.10	−0.11	−0.05	0.08	0.01	0.12	−0.85
π_m	−0.11	0.23	−0.01	0.08	0.36	0.25	0.77
$HACCEPT_m$	−0.11	0.07	−0.08	0.02	−0.08	0.70	−0.62
$\Sigma\pi$	−0.61	0.52	−0.13	0.42	0.05	−0.09	0.28
π_p	−0.03	0.66	0.01	0.64	−0.16	−0.24	0.05
$\Sigma\sigma$	−0.53	−0.14	−0.18	0.58	−0.16	0.30	0.08

$\sum$MR of substituents at R_1 and R_3 (see structure V, Table I). Electronic parameters for substituents at R_1 and R_3 were not included because only H or alkyl groups were present. Values for all parameters were taken from a recent compilation[24] and are given in the supplementary material.

The 28 parameters initially considered were clearly in excess of the maximum parameter to observation ratio advocated by Topliss[25] to avoid chance correlations. Therefore, a factor (principal component) analysis (Table IV; rotation of factors using the VARIMAX method)[26,27] was employed as a preprocessing step to reduce the number of parameters. Each factor contained a group of

(24) Hansch, C.; Leo, A. "Substituent Constants For Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979.

(25) Topliss, J. G.; Edwards, R. P. *J. Med. Chem.* **1979**, *22*, 1238.

(26) SAS Institute Inc. "SAS User's Guide: Statistics, 1982 Edition"; SAS Institute Inc., Cary, NC, 1982; p 309.

(27) (a) See ref 22, p 236. (b) Malinowski, E. R.; Howery, D. G. "Factor Analysis in Chemistry"; Wiley: New York, 1980.

Table V. Development of Equation 5

eq no.	equation	n	r^2	F	s
	$PTNCY_{cow}$ = −0.99 (±0.26) $HACCEPT_m$ + 2.38	56	0.20	13.9	0.82
	$PTNCY_{cow}$ = −0.89 (±0.24) $HACCEPT_m$ + 1.19 (±0.31) π_{R3} + 1.64	56	0.36	14.7	0.74
	$PTNCY_{cow}$ = −1.09 (±0.22) $HACCEPT_m$ + 1.19 (±0.28) π_{R3} − 1.14 (±0.28) MR_o + 1.96	56	0.51	18.2	0.65
2	$PTNCY_{cow}$ = −1.03 (±0.20) $HACCEPT_m$ + 0.91 (±0.26) π_{R3} − 1.45 (±0.26) MR_o − 1.09 (±0.29) σ_o + 2.08	56	0.62	20.6	0.58
3	$PTNCY_{cow}$ = −1.02 (±0.20) $HACCEPT_m$ + 0.93 (±0.27) π_{R3} − 1.42 (±0.28) MR_o + 0.68 (±0.23) ORTHO + 2.05	56	0.59	18.1	0.61
4	$PTNCY_{cow}$ = −0.96 (±0.16) $HACCEPT_m$ + 0.92 (±0.21) π_{R3} − 1.34 (±0.22) MR_o − 0.81 (±0.24) σ_o − 1.51 (±0.29) ACID + 2.15	56	0.75	30.2	0.48
5	$PTNCY_{cow}$ = −0.99 (±0.13) $HACCEPT_m$ + 0.81 (±0.18) π_{R3} − 1.16 (±0.18) MR_o − 0.88 (±0.20) σ_o − 1.57 (±0.24) ACID − 1.17 (±0.24) HBOND + 2.22	56	0.83	40.0	0.40

Table VI. Correlation Matrix

	$PTNCY_{cow}$	π_{R3}	$HACCEPT_m$	MR_o	σ_o	ACID	HBOND
$PTNCY_{cow}$	1.00						
π_{R3}	0.44	1.00					
$HACCEPT_m$	−0.45	−0.11	1.00				
MR_o	−0.25	0.10	−0.23	1.00			
σ_o	−0.37	−0.31	0.17	−0.35	1.00		
ACID	−0.51	−0.07	0.10	0.00	0.22	1.00	
HBOND	−0.31	−0.07	−0.09	0.24	−0.13	−0.06	1.00

correlated and chemically related parameters that were highly loaded (>0.7) in only that factor. With the exceptions noted below, only one parameter from each of these groups was selected for use in subsequent regression analyses. Continuous variables were chosen over indicator variables. Thus, σ_o, $\sum MR$, π_{R3}, σ_p, MR_m, MR_o, and π_m were selected. In addition, parameters that were loaded in more than one factor ($\sum\pi$, π_p, $\sum\sigma$, $HACCEPT_m$) were selected. Thus, the initial set of 28 parameters was reduced to a more manageable set of 11 for subsequent regression analyses.

Examination of Table I revealed the strong influence of ortho substitution on potency. Because of this, special attention was paid to the set of correlated parameters pertaining to ortho substituents (π_o, π_o^2, σ_o, $HDONOR_o$, $HACCEPT_o$, ORTHO). Each of these parameters was examined separately with the remaining 10 when formulating model equations.

Multiple regression analyses resulted in eq 2, the stepwise development of which is shown in Table V. Replacement of σ_o with ORTHO produced eq 3 (Table V), the next best equation as judged by r^2 values. An attempt was also made to discern if a nonlinear relationship in π_o existed, but replacing σ_o in eq 2 with π_o and π_o^2 did not produce a meaningful correlation since coefficients for both π_o and π_o^2 were positive.

Among the most poorly estimated potencies were those for the 2-, 3-, and 4-CO_2H derivatives. They were consistently less potent than calculated with eq 2. The effect was strongest at the ortho and weakest at the para positions. This could be related to the fact that these compounds were highly ionized at the pH of the test system (7.7). Dropping these analogues and rerunning the regression improved the correlation slightly (r^2 = 0.65) but did not significantly alter the coefficients associated with the physicochemical parameters. Substituting parameter values for the carboxylate anions gave an inferior correlation. However, addition of an indicator variable (ACID) denoting substitution of the phenyl ring by strongly acidic groups significantly improved the fit (eq 4, Table V).

Also poorly fit were the 2-OCH_3 and the 2,4-$(OCH_3)_2$ analogues, and to a lesser extent, the 2-OH analogue. A possible explanation for this is that hydrogen bonding between the *o*-methoxyl and hydroxyl oxygens and the fused imidazole NH is competing with or preventing a critical interaction between this NH and the adenosine receptor, thus lowering the potency. The *o*-amino analogues do not show decreased potency, perhaps because the amino can hydrogen bond to the N^9 of the imidazole and thus leave the imidazole NH free to interact with the receptor. This hypothesis is supported by a molecular modeling study, to be reported in a separate communication. Solution IR work to demonstrate differences in hydrogen bonding was not feasible due to the insolubility of the *o*-amino compound.

Table VII. Physical Properties of Selected Xanthines

compd	log P	pK_a[a]	solubility, mg/mL: 0.1 N HCl	solubility, mg/mL: 0.1 M, pH 7.4 phosphate buffer
1	2.93[b]	9.1	0.001	0.006
16	4.02[b]	8.4	0.0004	<0.0001
60	−0.97[c]	<2, 8.0	>8	>57
64	2.57[b]	6.8, 8.0	11.5	0.11
71	3.54[b]	9.1	0.004	<0.001
72	−0.03[c]	<2, 8.0	1.3	>24
73	3.32[b]	6.0, 8.0	19.9	0.05

[a] 67% DMF. [b] HPLC correlation method[31] using 55% MeOH/45% pH 7.4 phosphate buffer. [c] Shake flask procedure using octanol/pH 7.4 phosphate buffer; quantitation by HPLC.

Addition of an indicator variable (HBOND) denoting ortho substitution of the phenyl ring by OR (R = H, Me) gave eq 5, which demonstrates the highest correlation found. Table VI is a correlation matrix for the parameters included in eq 5. Potencies calculated with this equation and the residuals appear in Table I.

An alternate explanation for some of the outliers is that an additional factor, overall lipophilicity of the phenyl moiety, is operative. Adding a positive term in $\sum\pi$ to eq 2 would result in a lower calculated potency for the hydrophilic derivatives (CO_2H, OCH_3) and a higher calculated potency for the hydrophobic naphthyl analogue, consistent with the observed potencies. However, inclusion of this term in eq 2 is not statistically justified (partial F test). It may be that π does not adequately represent the lipophilicity of certain phenyl ring substituents due to the proximity of the fused imidazole.[28] A solution to this would be to measure the log P of a variety of analogues; however, samples were not available. The log P data shown in Table VII for the new xanthines supports this argument. The hydrophilic SO_3H analogues (compounds

(28) Lewis, S. J.; Mirrlees, M. S.; Taylor, P. J. *Quant. Struct.–Act. Relat.* **1983**, *2*, 1.

60 and 72) are clearly less potent than the sulfonamides (compounds 64 and 73).

Conclusions from the QSAR Study

The QSAR study reveals that there is a critical relationship between potency and both the electron-releasing properties and the size of the ortho substituents on the phenyl ring. Thus, from eq 5 and Table I, small, electron-donating substituents increase potency. The consequence is that H, OH, and NH_2 are the best ortho substituents available among those commonly considered for use in pharmaceuticals. Moreover, the equation predicts that these substituents will produce compounds with essentially equivalent potency. These compounds are, in fact, among the most potent analogues (for example, see compounds 3, 9, and 20, Table I).

Potency is much more strongly dependent on the nature of the ortho substituents than the para substituents. No statistically significant relationships were found between potency and any parameter combinations that included variables related to the para substituents on the 8-phenyl ring when the entire compound set was used. Although variations in potency are seen on changing para substitution (for example, increased affinity with substitution by chloro or bromo), the magnitude of these differences was insufficient to have an impact on the QSAR. Indeed, from Table I, with the exception of compounds with proton-accepting groups in the meta position and the 4-CO_2H analogue, all unsubstituted ortho, 2-OH, and 2-NH_2 derivatives were quite potent (IC_{50cow} values from 0.1 to 13), *regardless* of para substitution.

Equation 5 and related equations also reveal that higher values for π_{R3} and MR_{R3} are associated with higher potencies. However, since π_{R3} and MR_{R3} are highly correlated ($r > 0.9$), it cannot be discerned by using this compound set which is the operative parameter. Also, the lipophilicity or size of R_3 appears to be more strongly correlated with potency than these properties of R_1, because utilization of π_{R3} or MR_{R3} in the regressions invariably produced higher correlations than using π_{R1} or MR_{R1}. There is some uncertainty associated with this conclusion, however, due to the high correlations between π and MR for R_1 and R_3 (see factor 3 in Table IV).

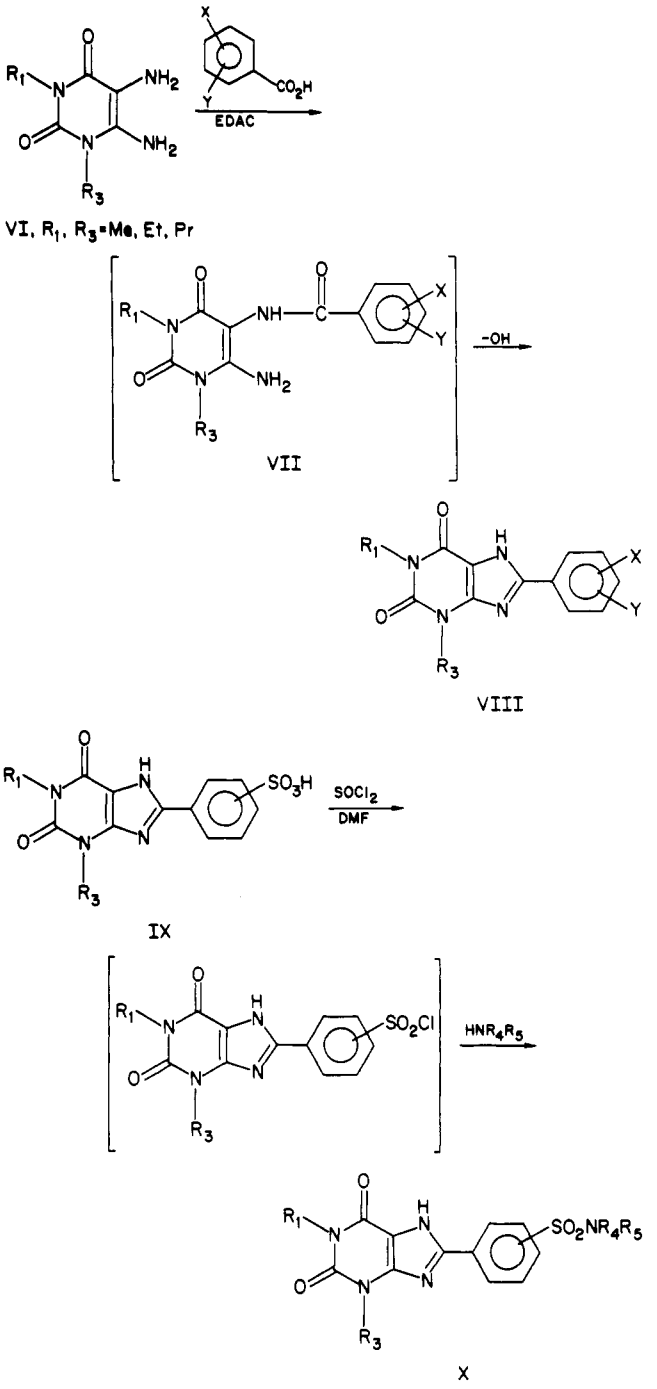
The results from the present QSAR study refine and extend the conclusions reached in the prior SAR analysis.[18]

Since the QSAR analysis indicated that a variety of substituents could be placed at the para position of the phenyl ring without affecting potency, we prepared 17 additional xanthines (Table II) containing para groups designed to increase aqueous solubility. Basic sulfonamide groups were chosen because of their amphoteric nature; compounds containing these groups were expected to be soluble across a wide pH range and thus possibly better absorbed. In addition, some of the compounds prepared provided further tests of whether potency increases with 1,3-disubstitution by *n*-propyl over ethyl or methyl groups (compounds 58 and 71, Table II) and whether potency decreases when the phenyl ring is substituted with strongly acidic groups (compounds 57, 59, 60, 69, and 72, Table II) or meta substituted by proton-accepting groups (compounds 59 and 63, Table II).

Chemistry

The synthesis of these compounds is outlined in Scheme I.[17,29] The appropriate 5,6-diaminouracil VI was treated with a substituted benzoic acid to give the amide VII. In the case of the sulfonic acids, the water-soluble ethyl[3-(dimethylamino)propyl]carbodiimide (EDAC) was used as a condensing agent to facilitate amide formation. The amino amide was then cyclized by heating in base to afford either the desired 8-(substituted phenyl)xanthine VIII directly or the penultimate 8-phenylsulfonic acid IX. In the latter case, the sulfonic acid was converted to the sulfonyl chloride at low temperature with $SOCl_2$/DMF and then subjected without isolation to an excess of amine to yield the 8-(sulfamoylphenyl)-1,3-dialkylxanthines X.

Scheme I



Biological Results and QSAR Conclusions for New Compounds

The binding results on the additional xanthines were in accord with the QSAR conclusions. High potency was indeed maintained within the series, potency increased with 1,3-disubstitution by *n*-propyl over ethyl and methyl groups (compare compounds 73 and 64). Potency de-

(29) Bristol, J. A.; Badger, E. W. U.S. Patent 4 445 758, June 5, 1984.

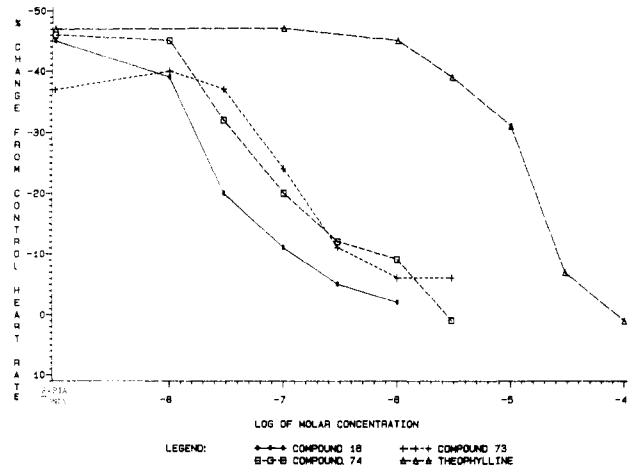


Figure 2. Effects of compounds **16**, **73**, **74**, and theophylline on heart rate.

creased with meta substitution of the phenyl ring by proton-accepting groups (compare compounds **63** and **64**). Reduced potency also resulted with phenyl substitution by strongly acidic groups (compounds **57**, **59**, **60**, **69**, and **72**). More importantly, the aqueous solubility of these new compounds was dramatically increased (Table VII).

Potencies of the new xanthines as calculated by eq 5 appear in Table II. Since most of the newly synthesized compounds purposely contained only para substituents designed to increase water solubility (exceptions are compounds **59**, **63**, and **71**) and thus are predicted to be equipotent using eq 5, the correlations were not rerun with the new compounds included. Indeed, with seven exceptions (the low-affinity SO_3H-substituted compounds **59**, **60**, and **69**; compounds **66–68**, which contain a heterocyclic ring in their phenyl substituents; and compound **70**), the potencies of the new compounds fell within 2 standard deviations of the predictions from eq 5 (Table II). Most importantly, the affinities of the tightest binders were well predicted.

Although the error of eq 5 (s, 0.40) was not particularly low relative to the error of the biological assay (0.15), the QSAR served three useful, practical purposes. First, it indicated that the most potent receptor binders had already been made in this series and thus suggested the termination of synthesis of compounds with additional phenyl substituents to increase potency. Second, it greatly facilitated the design of derivatives with increased water solubility and high potency. Third, it was found to be directly (quantitatively) applicable to substituents on the phenyl ring and the alkyl portions of a related series containing a different heterocycle and thus directed the substitution patterns. This work will be reported in a future communication.

In order to demonstrate in vitro adenosine antagonism, compounds **16**, **73**, **74**, and theophylline were examined in an isolated Langendorff heart model (see Experimental Section). In this assay, isolated perfused rat hearts are stimulated with (*R*)-PIA (III), a known adenosine receptor agonist. This produces bradycardia, an adenosine A_1 receptor mediated event.[10] The hearts are then challenged with increasing doses of the putative antagonist to determine effectiveness and thereby show in vitro adenosine receptor antagonism. The results are shown in Figure 2. In each case a reversible dose-dependent reversal of the bradycardic response to (*R*)-PIA was seen, indicating that these compounds do indeed function as adenosine A_1 antagonists in vitro as well as having receptor affinity. Futhermore, the increased affinity relative to theophylline is reflected in the shift to the left of the dose–response curves of compounds **16**, **73**, and **74**. This is consistent with the receptor binding data shown in Table II for these four analogues.

These 8-phenylsulfonamides thus represent novel soluble xanthines with high affinity for adenosine receptors in the brain which produce functional antagonism in the heart. They also illustrate the utility of QSAR in defining sites of substitution in order to enhance physicochemical properties.

Experimental Section

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. ^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.

Starting Materials. 1,3-Dialkyl-5,6-diaminouracils were synthesized by using the method of Blicke and Godt.[30] Substituted benzoic acids were obtained from Eastman Organic and were used without further purification. Ethyl[3-(dimethylamino)propyl]carbodiimide was obtained from JBL Chemical Co. and used directly. Reagents and solvents were distilled and dried as noted prior to use.

The synthesis of these compounds[17,18,29] and the starting materials[30] has been reported. All compounds had satisfactory ^1H NMR, IR, MS, and elemental analyses. An example of the general procedure is given by the synthesis of **73**.

4-(2,3,6,7-Tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonic Acid (72, Table II). A mixture of 5,6-diamino-1,3-dipropyl-2,4(1*H*,3*H*)-pyrimidinedione (VI, R = Pr; 16.0 g, 0.070 mol), 4-carboxybenzenesulfonic acid monopotassium salt (17.0 g, 0.071 mol) and water (250 mL) was prepared and the pH adjusted to 5.0 by the addition of a 10% KOH solution. To this solution was added ethyl[3-(dimethylamino)propyl]carbodiimide (13.6 g, 0.071 mol) in one portion, and the pH was maintained at 5.0 ± 0.5 by the dropwise addition of 4 N HCl. When the pH stabilized, the reaction mixture was treated with 30% aqueous KOH (100 mL), boiled under reflux for 10 min, treated with activated carbon, and filtered while warm. The resulting solution was chilled in ice, treated with 12 N HCl (1350 mL), and filtered. The solid was dried, recrystallized from MeOH, and dried to vacuo at 78 °C to give 5.1 g (17%) of 4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonic acid potassium salt, mp >360 °C. The free sulfonic acid was obtained by stirring with 12 N HCl and filtering the resulting solid to give, after drying, 4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonic acid (IX): mp >360 °C; ^1H NMR (Me_2SO-d_6) δ 11.6 (br s, 2 H), 8.05 (d, J = 9 Hz, 2 H), 7.67 (d, J = 9 Hz, 2 H); IR (KBr) 2980, 1720, 1680, 1340, 1170 cm^{-1}.

***N*-[2-(Dimethylamino)ethyl]-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonamide (73, Table II).** A mixture of 4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)benzenesulfonic acid (IX; 3.9 g, 0.010 mol) and DMF distilled from CaO (100 mL) at 0 °C was treated with $SOCl_2$ (2.4 g, 0.020 mol) and allowed to warm to ambient temperature with vigorous stirring. To the resulting slurry was added *N*,*N*-dimethyl-1,2-ethanediamine (4.4 g, 0.050 mol) in one portion. The resulting solution was concentrated to dryness in vacuo and the residue was suspended in water, filtered, recrystallized from EtOH, and dried in vacuo at 78 °C to give 0.60 g (12.5%) of product: mp 270–272 °C dec; ^1H NMR (Me_2SO-d_6) δ 8.32 (d, J = 9 Hz, 2 H), 7.92 (d, J = 9 Hz, 2 H), 3.96 (m, 4 H), 2.90 (t, J = 7.5 Hz, 2 H), 2.31 (t, J = 7 Hz, 2 H), 2.10 (s, 6 H), 1.70 (m, 4 H), 0.85 (d of t, J = 7 and 3 Hz, 6 H); IR (KBr) 2960, 1702, 1650,

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1160 cm^{-1}; MS, *m/e* 461. Anal. ($C_{21}H_{30}N_6O_4S$) C, H, N, S.

Pharmacology. Receptor Binding. N^6-[^{3}H]Cyclohexyladenosine binding[5b] 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^6-[^{3}H]cyclohexyladenosine (30 Ci/mmol), and 0.1 unit/mL of adenosine deaminase.

In Vitro Adenosine Antagonism. Two rate hearts were isolated and perfused in parallel by the Langendorff method[10] at physiological temperature, pH, and pressure. After 30 min, (*R*)-PIA (1.5×10^{-8} M) was introduced into the oxygenating reservoir, decreasing heart rate and increasing coronary flow (40–50% each). After stabilization, the drug was administered at increasing log doses, and the effect on heart rate and coronary flow was measured. Reversal of (*R*)-PIA-induced heart rate and coronary flow effects are interpreted as receptor antagonism. For each drug, the experiment was run in triplicate, thus giving a total of six observations at each dose. The results are shown in Figure 2.

Data Processing. Correlations, regressions, and factor analyses were run on an IBM 3081 machine using the SAS program package.[26] In eq 1–5, the figures in parentheses are the standard errors of the regression coefficinets. 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.

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Registry No. 1, 75922-48-4; 2, 2850-37-5; 3, 961-45-5; 4, 93214-97-2; 5, 85872-53-3; 6, 85884-03-3; 7, 78164-01-9; 8, 85872-56-6; 9, 18830-58-5; 10, 93215-02-2; 11, 93215-01-1; 12, 96445-28-2; 13, 85872-60-2; 14, 93214-98-3; 15, 93215-00-0; 16, 85872-51-1; 17, 85884-04-4; 18, 78146-60-8; 19, 85872-55-5; 20, 85872-57-7; 21, 973-69-3; 22, 85872-59-9; 23, 85872-63-5; 24, 93214-92-7; 25, 78146-61-9; 26, 85872-54-4; 27, 85872-61-3; 28, 85872-52-2; 29, 85872-67-9; 30, 93214-82-5; 31, 85872-65-7; 32, 78146-59-5; 33, 85872-64-6; 34, 85872-68-0; 35, 93214-90-5; 36, 93214-85-8; 37, 54013-58-0; 38, 93214-89-2; 39, 93214-91-6; 40, 63325-99-5; 41, 93214-86-9; 42, 57196-70-0; 43, 29064-02-6; 44, 85872-58-8; 45, 93214-87-0; 46, 93214-88-1; 47, 57281-09-1; 48, 93214-84-7; 49, 54013-59-1; 50, 85872-66-8; 51, 1094-63-9; 52, 93214-99-4; 53, 967-42-0; 54, 93214-83-6; 55, 85872-69-1; 56, 93215-04-4; 57, 80206-91-3; 58, 96445-29-3; 59, 89073-61-0; 60, 89073-47-2; 61, 89073-54-1; 62, 96445-30-6; 63, 96445-31-7; 64, 89073-49-4; 65, 96445-38-4; 65·HCl, 89073-51-8; 66, 89073-53-0; 67, 89073-55-2; 68, 89073-52-9; 69, 96445-32-8; 70, 96445-39-5; 70·HCl, 96445-33-9; 71, 96445-34-0; 72, 89073-57-4; 73, 89073-58-5; 74, 96445-35-1; 75, 96445-40-8; 75·HCl, 96445-36-2; 76, 96445-37-3; IV ($R_1 = R_3 = (CH_2)_2CH_3$), 81250-34-2; IX ($R_1 = R_3 = (CH_2)_2CH_3$, 4-SO_3H), 89073-57-4; 4-$HO_2CC_6H_4SO_3H$·K, 5399-63-3; $H_2N(CH_2)_2N(CH_3)_2$, 108-00-9.

Supplementary Material Available: Values for the 28 parameters used as input to the factor analysis (5 pages). Ordering information is given on any current masthead page.

Vinblastin-23-oyl Amino Acid Derivatives: Chemistry, Physicochemical Data, Toxicity, and Antitumor Activities against P388 and L1210 Leukemias

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The dimeric alkaloids vinblastine (VLB) and vincristine (VCR) differ structurally only in the functional group on the dihydroindole nitrogen. The semisynthetic derivative vindesine (VDS) differs slightly from VLB by having an amide group instead of an ester group. However, these minor distinctions are responsible for profound differences in the oncolytic spectrum, potency, and toxicity of these compounds. Vinblastin-23-oyl amino acid derivatives were synthesized by linking amino acid carboxylic esters to the vinblastin-23-oyl moiety through an amide linkage. Studies were extended to explore the influence of the nature of the amino acid, the ester alkyl chain lengths, the stereoisomerism of the amino acid, or the reacetylation of the hydroxyl group (position O-4) of the vindoline moiety. The present study deals with the synthesis of 21 vinblastin-23-oyl amino acid derivatives, some of their physicochemical data, the acute toxicity in mice, and therapeutic activities of these derivatives against the P388 and L1210 leukemias in comparison with VDS, VBL, and VCR.

The antitumor alkaloids vinblastine (VLB) and vincristine (VCR)[1] are extracted from the periwinkle plant *Catharanthus roseus* G. Don.[2] They possess a basic structure comprising an indole and a dihydroindole nucleus linked together.

Mitotic arrest and cytotoxicity are the principal biological actions cited.[3] Furthermore, their antitumor activities and toxic side effects are clearly related to certain structural features of the alkaloids. VLB differs in molecular structure from VCR in that it contains a methyl group instead of a formyl group and this minor structural difference leads to a different antitumor spectrum, potency, and toxicity.[4] Deacetylvinblastine amide (VDS)[4] is a semisynthetic derivative of VLB and it differs slightly from VLB by having an amide group in place of the ester

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