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

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

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

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

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

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

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

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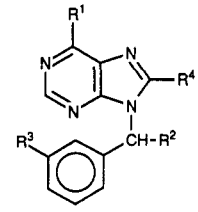
Table I. Benzodiazepine Receptor and Conflict Responding Activity of 9-(1-Phenylethyl)purines



no.	R^1	R^2	R^3	R^4	IC_{50},[a] μM	% change in conflict responding[b]
1[c]	$N(CH_3)_2$	H	H	H	13.0	−17 ± 44
2[c]	$N(CH_3)_2$	H	NH_2	H	0.9	+4 ± 9
3[d]	$N(CH_3)_2$	H	H	Br	3.0	
4[d]	$N(CH_3)_2$	H	NH_2	Br	0.11 ± 0.01	+2 ± 7
5[d]	$N(CH_3)_2$	H	NHCHO	Br	0.011 ± 0.002	+9 ± 9
6[c]	$N(CH_3)_2$	CH_3 (*S*)	H	H	2.1	+5 ± 7
7[c]	$N(CH_3)_2$	CH_3 (*R*)	H	H	100.0	
8	$N(CH_3)_2$	CH_3 (*R,S*)	NH_2	H	0.16	−22 ± 18
9	$N(CH_3)_2$	CH_3 (*R,S*)	NH_2	Br	0.52	+5 ± 17
10[c]	$N(CH_3)_2$	H	OH	H	1.2	+2 ± 10
11[c]	$N(CH_3)_2$	H	$OCOCH_3$	H	0.44	+2 ± 9
12[c]	OH	H	H	H	19.0	
13[c]	SCH_3	H	H	H	3.3	+13 ± 7
14	$N(CH_3)_2$	CH_3 (*R,S*)	OH	H	0.48	−7 ± 6
15	OH	CH_3 (*R,S*)	OH	H	2.2	
16	SH	CH_3 (*R,S*)	OH	H	(64%)	
17	SCH_3	CH_3 (*R,S*)	OH	H	1.2	
18	$N(CH_3)_2$	CH_3 (*R,S*)	$OCOCH_3$	H	0.38	
19	OH	CH_3 (*R,S*)	$OCOCH_3$	H	(68%)	
20	SCH_3	CH_3 (*R,S*)	$OCOCH_3$	H	1.7	
chlordiazepoxide					0.2	+67 ± 10[e]
diazepam					0.006 ± 0.001	

[a] The IC_{50}s were determined by the method described in ref 7 and are the concentration of compound that decreased specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors by 50%. The values in parentheses are percent inhibition of [^{3}H]diazepam binding by 100 μM compound. The mean ± SEM are given for the most active compounds. [b] Compounds were tested in Long–Evans rats as described in ref 7 on a modified Geller–Seifter conflict schedule. Compounds were administered by oral gavage in a 0.5% methylcellulose suspension at 25 mg/kg. [c] Data taken from ref 7. [d] Data taken from ref 8. [e] Chlordiazepoxide was administered at 20 mg/kg.

agent with anxiolytic activity. The BZR-binding activity and in vivo Geller–Seifter conflict activity of these analogues are described herein.

Chemistry

9-Benzylpurines **8**, **9**, and **14–20** were prepared from acetophenone **22** or **23** as outlined in Scheme I. Preparation of **8** and **9** commenced with reductive amination[11] of 3-nitroacetophenone (**22**) to give **24**, which was reacted with 4,6-dichloro-5-aminopyrimidine[12] to provide intermediate **26**. Pyrimidine **26** was cyclized with triethyl orthoformate to provide 6-chloropurine **28**, which was aminated with dimethylamine to give **30**. Purine **30** was brominated with bromine in sodium acetate buffer–tetrahydrofuran to give **31**.[8] Nitro compounds **30** and **31** were reduced with Pd on carbon or Raney nickel to give **8** and **9**, respectively.

Phenol derivatives **14–20** were prepared from 3-hydroxyacetophenone (**23**). Reductive amination[11] of **23** gave **25**,[13] which was converted to **29** in two steps as described for **28**. Dimethylamination of **29** provided **14**, acidic hydrolysis gave a low yield of **15**, and thiation with thiourea led to **16**. Methylation of **16** with methyl iodide provided **17**. Phenols **14**, **15**, and **17** were conveniently acetylated with acetic anhydride and 4-(dimethylamino)-pyridine in pyridine to give **18–20**.

Biological Results and Discussion

The parent 9-benzylpurine **1** inhibits specific binding of [^{3}H]diazepam to rat brain receptors with an IC_{50} = 13 μM (Table I).[7] Introduction of a *m*-amino group (**2**) or substitution at the 8-position with bromo (**3**) gives 14- and 4-fold increases in receptor affinity, respectively. When both substituents are introduced into **1**, the increased BZR affinity is additive, which results in a 100-fold lower IC_{50} for **4**. Binding potency is further enhanced with *m*-formamido analogue **5**.[8]

Earlier studies showed that introduction of a methyl group on the benzylic methylene of **1** gives a 6-fold increase in receptor affinity if the methyl is of the *S* configuration as in **6**; *R* enantiomer **7** is almost 50-fold less active.[7] Introduction of an α-methyl on **4** or **5** could increase BZR-binding potency, if the effect of a third substituent is additive. The racemic α-methyl analogue **8** was over 5-fold more active than parent **2**. If only one enantiomer of **8** has good binding affinity, the effects of the α-methyl group and the *m*-amino substituent are additive. However, addition of an α-methyl group to the *m*-amino-8-bromopurine **4** to give **9** resulted in a 4-fold loss in BZR affinity. The trisubstituted analogue **9** was over 13-fold less active than expected if the α-methyl effect had been additive as with **8**.

Examination of Corey–Pauling–Koltun[14] molecular models of **4**, **8**, and **9** revealed that the conformational freedom of the 9-[1-(3-aminophenyl)ethyl] moiety of **9**, relative to those of **4** and **8**, is considerably restricted due to unfavorable steric interactions between the 8-bromo and

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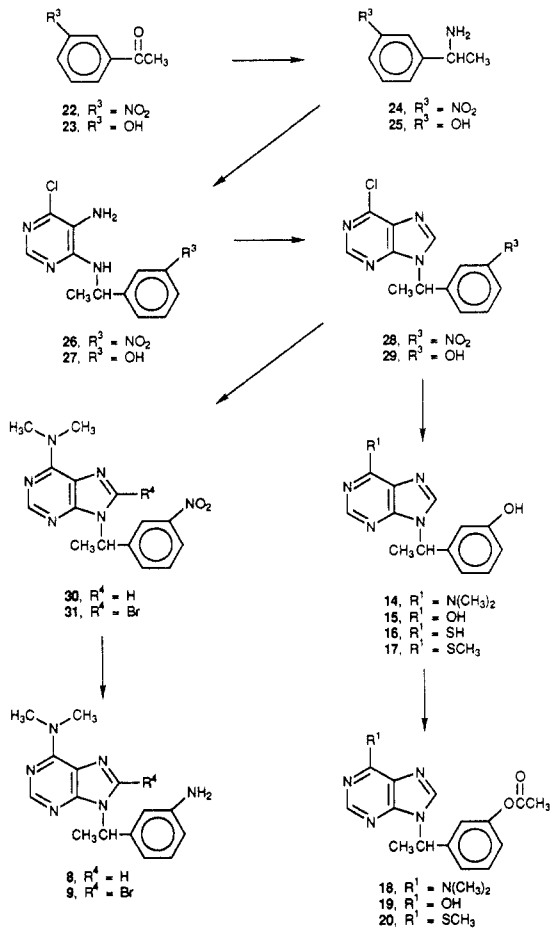
Scheme I



9-(1-phenylethyl) substituents of **9**. This observation was further expanded by construction of **4**, **8**, and **9** with MacroModel.[15] The structures were energy minimized with Amber,[15] which generated two minimum-energy conformations for each compound. The energy-minimized structures of **4** and **8** were subjected to a "nonflexible superimposition" routine of MacroModel. With an exact superimposition of the purine rings, the aminophenyl moieties of **4** and **8** occupied essentially the same area in space in both sets of energy-minimized structures. Superimposition of the energy-minimized structures of **8** and **9** showed that the aminophenyl moiety of **9** occupied a slightly different area in space, relative to those of **4** and **8**. This is apparently due to repulsive, steric interactions between the 8-bromo and 9-(1-phenylethyl) substituents, which results in a conformation that is not optimal for interaction of the aminophenyl moiety with the BZR.

The *m*-hydroxy (**10**), *m*-acetoxy (**11**), 6-oxo (**12**), and 6-(methylthio) (**13**) analogues of **1** have good affinity for the BZR.[7] The α-methyl analogues of these compounds were also tested for binding affinity (see **14–20**). However, no significant additivity of substituent effects was found with this set of compounds.

Several compounds were tested for activity on a modified Geller–Seifter conflict schedule.[7,9,10] Under conditions where chlordiazepoxide (CDP) produced significant dose-related increases in responding, none of the purines tested at 25 mg/kg po produced any significant change in conflict responding (Table I). This lack of activity in the Geller–Seifter conflict test is explained by the finding that this class of BZR binding agents are antagonists rather than agonists of the BZR.[8] In a series of experiments with **4**, **5**, and 10 μM γ-aminobutyric acid (GABA), the IC_{50} values for BZR binding were unaltered, suggesting that, like Ro 15-1788, these benzylpurines are antagonists of the BZR.[8,16-18] In contrast to agonists of the BZR, antagonists are not active in the Geller–Seifter conflict test in vivo.

Conclusion

9-(3-Aminobenzyl)-8-bromopurine **4** and 9-[1-(3-aminophenyl)ethyl]purine **8** bind to the BZR with 100- and 80-fold increases in receptor affinity relative to parent **1**. However, further combination of receptor-binding-enhancing substituents to give **9** did not lead to enhanced BZR affinity. This lack of additivity probably results from restriction of **9**, by repulsive, steric interactions, to a conformation that is not optimal for binding to the BZR.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian XL-100-15-FT, a Varian T-60, or a Hitachi Perkin-Elmer R-24 spectrometer with Me_4Si as an internal standard. Ultraviolet absorption spectra were measured on a Unicam SP 800 or Cary 118 UV–vis spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. TLCs were developed on Whatman 200 μm MK6F plates of silica gel with fluorescent indicator. Preparative flash chromatography[19] was performed on silica gel 60 (40–63 μm, E. Merck No. 9385). The analytical samples gave combustion values for C, H, N within 0.4% of the theoretical values. Elemental analyses were performed by Atlantic Microlab, Inc.

9-[1-(3-Aminophenyl)ethyl]-6-(dimethylamino)-9*H*-purine Dihydrochloride (8). A mixture of **30** (1.0 g, 3.3 mmol), 5% Pd on carbon (0.20 g), and glacial AcOH (100 mL) was shaken in the presence of hydrogen at 2–3 atm for 15 min. The catalyst was removed by vacuum filtration, and the solution was spin evaporated in vacuo. The residue was diluted with concentrated HCl (10 mL) and EtOH (50 mL) and spin evaporated. Additional EtOH as added and the solvent was spin evaporated to give a solid that was recrystallized from EtOH–Et_2O to give 0.953 g (90%) of **8**·2HCl. The analytical sample was obtained by recrystallization from EtOH: yield, 0.394 g (38%); mp 192–195 °C; TLC (EtOAc); UV (pH 7 buffer + 9.5% EtOH) λ_{max} 278 nm (ϵ 21 400); NMR (DMSO-d_6) δ 1.98 (d, 3 H, CH_3, J = 7.1 Hz), 3.58 (br s, 6 H, $N(CH_3)_2$), 6.00 (q, 1 H, CH, J = 7.1 Hz), 7.4 (m, 4 H, Ar), 8.35 (s, 1 H, purine H), 8.71 (s, 1 H, purine H), 9.76 (br s, 4 H, NH_2). Anal. ($C_{15}H_{18}N_6$·2HCl·0.03EtOH) C, H, N.

9-[1-(3-Aminophenyl)ethyl]-8-bromo-6-(dimethylamino)-9*H*-purine Dihydrochloride (9). This compound was prepared from **31** as described for the preparation of 9-(3-aminobenzyl)-8-bromo-6-(dimethylamino)-9*H*-purine dihydrochloride.[8] The product was crystallized from EtOH and H_2O with 1 mL of 12 M HCl to give 2.25 g (45%) of **9**: mp 225–227 °C; TLC (EtOAc or MeOH–CH_2Cl_2 1:9); NMR (DMSO-d_6) δ 2.06 (d, 3 H, J = 7.5 Hz, CH_3C), 3.53 (br s, 6 H, $N(CH_3)_2$), 6.06 (q, 1 H, J = 7.5 Hz, CH), 7.3–7.5 (m, 4 H, ArH), 8.33 (s, 1 H, purine H), 10.8 (br s, 3 H, NH_3^+). Anal. ($C_{15}H_{17}N_6Br$·2HCl) C, H, N.

6-(Dimethylamino)-9-[1-(3-hydroxyphenyl)ethyl]-9*H*-purine (14). A solution of **29** (2.37 g, 8.64 mmol), EtOH (50 mL), and 40% aqueous dimethylamine (25 mL) was stirred at ambient temperature for 1 h. The solution was spin evaporated in vacuo, and the residue was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The organic layer was washed with H_2O (2 × 10 mL), filtered through glass wool, and spin evaporated in vacuo. The

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solid residue was recrystallized from EtOH, and the product was washed with EtOAc to give **14** as a white solid: yield, 1.45 g (60%); mp 163–164 °C. A second recrystallization from 2-PrOH gave the analytical sample: mp 163–164.5 °C; TLC (EtOAc); UV (0.1 N HCl + 10% EtOH) λ_{max} 270 nm (ϵ 20 300); UV (0.1 N NaOH + 10% EtOH) λ_{max} 278.5 nm (ϵ 21 300); NMR (DMSO-d_6) δ 1.90 (d, 3 H, J = 7 Hz, CH_3), 3.47 (s, 6 H, $N(CH_3)_2$), 5.80 (q, 1 H, J = 7 Hz, CH), 6.6–7.3 (complex m, 4 H, Ar), 8.23 (s, 1 H, purine H), 8.38 (s, 1 H, purine H), 9.37 (s, 1 H, OH). Anal. ($C_{15}H_{17}N_5O$) C, H, N.

1,9-Dihydro-9-[1-(3-hydroxyphenyl)ethyl]-6*H*-purin-6-one (15). A solution of **29** (2.37 g, 8.64 mmol) and 1 N HCl (50 mL) was heated on a steam bath for 1 h, 12 M HCl (10 mL) was added, and the solution was refluxed for 1 h. The reaction was cooled, filtered through glass wool, and spin evaporated in vacuo. The residue was dispersed in H_2O (50 mL), and the pH was adjusted to 8 with 5% aqueous $NaHCO_3$. The solvent was decanted, and the residue was crystallized from EtOH (charcoal) to give 0.50 g (22%) of **15**, mp 230–240 °C dec. Several recrystallizations from EtOH gave the analytical sample as white granules: yield, 0.167 g (7%); mp 240–243 °C; TLC (EtOAc–EtOH 1:1); UV (1 N HCl) λ_{max} 251 nm (ϵ 11 700); UV (pH 7.0 buffer) λ_{max} 250 nm (ϵ 13 000); UV (1 N NaOH) λ_{max} 246.5 nm (ϵ 16 500); NMR (DMSO-d_6) δ 1.89 (d, 3 H, J = 7.2 Hz, CH_3), 5.72 (q, 1 H, J = 7.2 Hz, CH), 6.6–7.2 (complex m, 4 H, Ar), 8.02 (br s, 1 H, purine 2-H), 8.33 (s, 1 H, purine 8-H), 9.43 (s, 1 H, OH), 12.28 (br s, 1 H, NH). Anal. ($C_{13}H_{12}N_4O_2$) C, H, N.

1,9-Dihydro-9-[1-(3-hydroxyphenyl)ethyl]-6*H*-purine-6-thione (16). A solution of **29** (4.66 g, 17.0 mmol), thiourea (1.64 g, 21.5 mmol), and EtOH (100 mL) was refluxed with stirring for 2 h. The solids were collected, washed with EtOH, and dried to give 3.11 g (67%) of **16** that was a single spot on TLC. Recrystallization of a sample from EtOH gave the analytical sample: mp 266–270 °C (dec); TLC (MeOH–$CHCl_3$ 1:9); UV (1 N HCl) λ_{max} 327 nm (ϵ 19 200); UV (0.1 N NaOH) λ_{max} 310 nm (ϵ 24 100); NMR (DMSO-d_6) δ 1.91 (d, 3 H, J = 7.3 Hz, CH_3), 5.74 (q, 1 H, J = 7.3 Hz, CH) 6.6–7.2 (complex m, 4 H, Ar), 8.16 (s, 1 H, purine H), 8.53 (s, 1 H, purine H), 9.40 (br s, 1 H, OH), 13.55 (br, 1 H, NH). Anal. ($C_{13}H_{12}N_4OS$) H, N; C: calcd, 57.33; found, 56.89.

9-[1-(3-Hydroxyphenyl)ethyl]-6-(methylthio)-9*H*-purine (17). Methyl iodide (0.8 mL) was added to a vigorously stirred solution of **16** (2.73 g, 10.0 mmol) in H_2O (30 mL) and 1 N NaOH (20 mL). After 1.5 h the pH of the solution was adjusted to 5 with 50% aqueous AcOH. The solvent was decanted, and the residual gum was dissolved in EtOAc (100 mL), dried ($MgSO_4$), and spin evaporated in vacuo. The residue was crystallized from EtOAc–hexanes to give 2.44 g (85%) of **17**, mp 154–155 °C, that contained a few percent of **16**. A 0.5-g sample of **17** was purified by flash chromatography on a 2-cm column using EtOAc–cyclohexane 2:1 to give 0.325 g (65%) of **17**: mp 169–170 °C; TLC (EtOAc–cyclohexane 1:1), NMR (DMSO-d_6) δ 1.96 (d, 3 H, J = 7.2 Hz, CH_3), 2.67 (s, 3 H, SCH_3), 5.86 (q, 1 H, J = 7.2 Hz, CH), 6.6–7.3 (complex m, 4 H, Ar), 8.71 (s, 2 H, purine Hs), 9.43 (s, 1 H, OH). Anal. ($C_{14}H_{14}N_4OS$) C, H, N.

9-[1-(3-Acetoxyphenyl)ethyl]-6-(dimethylamino)-9*H*-purine (18). A solution of **14** (0.77 g, 2.7 mmol), CH_2Cl_2 (30 mL), pyridine (1 mL), acetic anhydride (1 mL), and 4-(dimethylamino)pyridine (0.21 mg) was stirred at ambient temperature for 15 h. The reaction was diluted with CH_2Cl_2 (70 mL) and washed with 5% aqueous $NaHCO_3$ (2 × 25 mL) and H_2O (2 × 25 mL). The solution was filtered through glass wool, added to silica gel 60, and spin evaporated in vacuo. The residual solids were introduced on a column (2 cm × 20 cm) of silica gel 60 wetted with cyclohexane. The column was eluted with EtOAc–cyclohexane 3:1 using flash chromatography. The fractions that contained product were combined and spin evaporated in vacuo. The residual oil was triturated with H_2O to give a solid that was collected and recrystallized from cyclohexane to give 0.388 g (44%) of **18**: mp 106–108 °C; TLC (EtOAc–cyclohexane 3:1); NMR (DMSO-d_6) δ 2.25 (s, 3 H, $C(O)CH_3$), 8.19 (s, 1 H, purine H), 8.42 (s, 1 H, purine H). Anal. ($C_{17}H_{19}N_5O_2$) C, H, N.

9-[1-(3-Acetoxyphenyl)ethyl]-1,9-dihydro-6*H*-purin-6-one (19). This compound was prepared from **15** as described for preparation of **18**, except that the column was eluted with EtOAc–EtOH 10:1. The product was recrystallized from EtOH to give 0.236 g (56%) of **19**: mp 228–230 °C; TLC (EtOAc–EtOH 10:1); NMR (DMSO-d_6) δ 2.25 (s, 3 H, $C(O)CH_3$), 8.01 (d, 1 H, J = 3.1 Hz, purine 2-H), 8.36 (s, 1 H, purine 8-H), 12.28 (br s, 1 H, NH). Anal. ($C_{15}H_{14}N_4O_3$) C, H, N.

9-[1-(3-Acetoxyphenyl)ethyl]-6-(methylthio)-9*H*-purine (20). This compound was prepared from **17** as described for preparation of **18** except that the column was eluted with EtOAc–cyclohexane 1:1 to give an oil that was induced to crystallize by trituration with pentane to give 0.69 g (31%) of **20** as a white solid: mp 102–103 °C; TLC (EtOAc–cyclohexane 3:1); NMR (DMSO-d_6) δ 2.25 (s, 3 H, $C(O)CH_3$), 2.65 (s, 3 H, SCH_3), 8.70 (s, 1 H, purine H), 8.75 (s, 1 H, purine H). Anal. ($C_{16}H_{16}N_4O_2S$) C, H, N.

1-(3-Nitrophenyl)ethylamine (24). A solution of **22** (33.0 g, 0.20 mol), ammonium acetate (150 g, 2.0 mol), and sodium cyanoborohydride[11] (8.8 g, 0.14 mol) in MeOH (600 mL, dried over 3A molecular sieves) was stirred with 3A molecular sieves (100 g) under N_2 for 3 days. The pH of the mixture was adjusted to 2 with 12 M HCl, the mixture was filtered through wire gauze supported filter paper, and the solids were washed with MeOH and H_2O. The filtrates and washes were combined and spin evaporated in vacuo. The residue was dissolved in H_2O (400 mL), and the pH was adjusted to 10 with NaOH, during which a large quantity of NH_3 was given off. The cooled solution was extracted with Et_2O (4 × 300 mL). The combined extracts were washed with brine, dried ($MgSO_4$), and spin evaporated in vacuo to a light yellow oil. The crude product was introduced on a column (3 cm × 20 cm) of silica gel 60 wetted with Et_2O. The column was eluted with Et_2O using flash chromatography. The appropriate fractions were combined and spin evaporated in vacuo to give 15.3 g (46%) of **24** as a homogeneous, light yellow oil; TLC (EtOAc–hexane 1:2 + 3 drops of di(2-propyl)amine); NMR ($CDCl_3$) δ 1.46 (d, 3 H, CH_3, J = 7.0 Hz), 1.86 (s, 2 H, NH_2), 4.32 (q, 1 H, CH, J = 7.0 Hz), 7.30–8.40 (complex m, 4 H, Ar).

5-Amino-4-chloro-6-[[1-(3-nitrophenyl)ethyl]amino]pyrimidine (26). A mixture of 5-amino-4,6-dichloropyrimidine (14.26 g, 87 mmol), **24** (14.8 g, 88 mmol), triethylamine (14.2 mL, 102 mmol), and 1-butanol (150 mL) was refluxed under N_2 for four days. The solvent was removed by spin evaporation in vacuo. The residual paste was stirred with water (250 mL), and the brown solid was collected by filtration: yield, 24.3 g (94%); mp 180–188 °C. The solid was dried and introduced onto a column (4 cm × 20 cm) of silica gel 60 wetted with EtOAc–hexane 1:1. The column was eluted with EtOAc–hexane in step gradients of 1:2 (1 L), 1:1 (1 L), and 2:1 (3 L). The appropriate fractions were combined and spin evaporated in vacuo to give 21.5 g (83%) of **26**, mp 190–192 °C; NMR (DMSO-d_6) δ 1.53 (d, 3 H, CH_3, J = 7.0 Hz), 5.19 (br s, 2 H, NH_2), 5.39 (q, 1 H, CH), 7.25 (br d, 1 H, NH, J = 7.0 Hz), 7.40–8.35 (complex m, 4 H, Ar), 7.66 (s, 1 H, pyrimidine H).

6-Chloro-9-[1-(3-nitrophenyl)ethyl]-9*H*-purine (28). A mixture of **26** (21.0 g, 71.7 mmol) and ethanesulfonic acid (0.3 g, 2.7 mmol) in triethyl orthoformate (200 mL) was stirred at ambient temperature for 18 h. The solution was spin evaporated in vacuo. The dark tar was dissolved in $CHCl_3$ and washed successively with 5% aqueous $NaHCO_3$, H_2O, and brine. The $CHCl_3$ solution was dried ($MgSO_4$), filtered through a pad of Superfiltrol No. 19, and washed with $CHCl_3$. The combined filtrates were spin evaporated in vacuo to give a light yellow solid: yield 20.5 g (93%); mp 150–152 °C. Several recrystallizations from EtOH gave the analytical sample; yield 14.2 g (64%); mp 154–155 °C; TLC (EtOAc–hexane 1:2), UV (pH 7 buffer + 9.5% EtOH) λ_{max} 265.5 nm (ϵ 17 400); NMR (DMSO-d_6) δ 2.13 (d, 3 H, CH_3, J = 7.0 Hz), 6.21 (q, 1 H, CH, J = 7.0 Hz), 8.0 (complex m, 4 H, Ar), 8.77 (s, 1 H, purine H), 9.07 (s, 1 H, purine H). Anal. ($C_{13}H_{10}ClN_5O_2$) C, H, N.

6-Chloro-9-[1-(3-hydroxyphenyl)ethyl]-9*H*-purine (29). A mixture of 5-amino-4,6-dichloropyrimidine (8.03 g, 49.0 mmol), **25**[13] (6.00 g, 43.7 mmol), triethylamine (9.05 g, 89.6 mmol), and 1-butanol (100 mL) was refluxed with stirring for 22 h. The reaction was cooled, and the volatiles were removed by spin evaporation in vacuo. The residue was dissolved in EtOAc (600 mL)–EtOH (50 mL)–$CHCl_3$ (200 mL) and washed with H_2O (4 × 50 mL). The organic phase was filtered and spin evaporated in vacuo. The residue was dissolved in 1 N NaOH (400 mL) and filtered to remove the insoluble material. The pH of the filtrate was adjusted to 5 with 12 M HCl. The mixture was extracted

with CH_2Cl_2-EtOH 8:2 (2 × 400 mL) and filtered through glass wool. The solution was spin evaporated in vacuo to give 8.01 g (69%) of 5-amino-4-chloro-6-[[1-(3-hydroxyphenyl)ethyl]-amino]pyrimidine (**27**) as a foam that was a single spot on TLC (EtOAc-cyclohexane 1:1) and was used without further purification in the next step; UV (0.1 N HCl) λ_{max} 304 nm; UV (0.1 N NaOH) λ_{max} 292 nm.

A mixture of **27** (8.00 g, 30.2 mmol), ethanesulfonic acid (0.15 g, 1.3 mmol), and triethyl orthoformate (100 mL) was stirred at ambient temperature for 40 h. The dark solution was treated with charcoal, filtered through Celite, and spin evaporated in vacuo at 80 °C to give a quantitative yield of **29** as a glass, which was a single spot on TLC (EtOAc-cyclohexane 1:1) and was used without further purification in the next steps; UV (0.1 N HCl) λ_{max} 266.5 nm; UV (0.1 N NaOH) λ_{max} 267 nm; NMR (DMSO-d_6) δ 2.00 (d, 3 H, J = 7 Hz, CH_3), 5.95 (q, 1 H, J = 7 Hz, CH), 6.6-7.3 (complex m, 4 H, Ar), 8.77 (s, 1 H, purine H), 8.97 (s, 1 H, purine H), 9.42 (br s, 1 H, OH).

6-(Dimethylamino)-9-[1-(3-nitrophenyl)ethyl]-9*H*-purine (30). A solution of **28** (10.0 g, 33 mmol) and 10% dimethylamine in EtOH (100 mL) was stirred at ambient temperature for 24 h. The solution was spin evaporated in vacuo, and the residue was dissolved in EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$) and spin evaporated in vacuo to give a light yellow solid. Recrystallization from toluene gave 10.2 g (99%) of **30**, mp 136-138 °C. Another recrystallization from toluene gave the analytical sample: mp 137-138 °C; TLC (EtOAc); UV (pH 7 buffer + 9.5% EtOH) λ_{max} 274.5 nm (ϵ 25 800); NMR (DMSO-d_6) δ 2.02 (d, 3 H, J = 7.1 Hz, CH_3), 3.47 (s, 6 H, $N(CH_3)_2$), 6.03 (q, 1 H, J = 7.1 Hz, CH), 7.7 (complex m, 4 H, Ar), 8.21 (s, 1 H, purine H), 8.52 (s, 1 H, purine H). Anal. ($C_{15}H_{16}N_6O_2$) C, H, N.

8-Bromo-6-(dimethylamino)-9-[1-(3-nitrophenyl)ethyl]-9*H*-purine (31). This compound was prepared from **30** as described for preparation of 8-bromo-6-(dimethylamino)-9-(3-nitrobenzyl)-9*H*-purine.[8] The product was crystallized from EtOH to give 3.5 g (80%) of **31**, mp 168-169 °C; TLC (EtOAc-Hexane 1:1); NMR (DMSO-d_6) δ 2.12 (d, 3 H, J = 7.25 Hz, CH_3C), 3.4 (br s, 6 H, $N(CH_3)_2$), 6.11 (q, 1 H, J = 7.25 Hz, CH), 7.6-7.8 (m, 2 H, ArH), 8.19 (s, 1 H, purine H), 8.15-8.25 (m, 2 H, ArH). Anal. ($C_{15}H_{15}N_6BrO_2$) C, H, N.

Benzodiazepine-Binding Assay. The compounds in Table I were assayed for BZR-binding activity by the method described in ref 7. The IC_{50}s are the concentration at which specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors was decreased by 50%. Increased potency of the compound as an inhibitor of [^{3}H]diazepam binding was assumed to reflect increased affinity of the agent for the receptor.

Pharmacology. Conflict Responding Test. The compounds in Table I that were tested for conflict responding were tested as described in ref 7. This paradigm was a modification of a Geller-Seifter conflict schedule[9,10] in which chlordiazepoxide (CDP) produced significant dose-related increases in responding. At 10 and 20 mg/kg, CDP increased responding by 46 and 67%, respectively.

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Structure-Activity Relationships of Antineoplastic Agents in Multidrug Resistance

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Clinical resistance to many antineoplastic agents is a major cause of treatment failure. The well-documented phenomenon addressed as multidrug resistance (MDR) allows cells to withstand exposure to lethal doses of drugs with dissimilar chemical structures, modes of action, and physicochemical properties. In one of the earliest studies on MDR, Biedler and Riehm in an attempt to explain the cross-resistance profile of actinomycin D resistant Chinese hamster cells suggested that molecular weight was an important determinant. Our statistical analysis of their data validates their claim and indeed strongly demonstrates that cross resistance is enhanced by the increased size and hydrophobicity of the antitumor agent. Our preliminary studies with methotrexate-resistant L1210 cells indicates that cross resistance is increased in the case of moderate-sized, hydrophilic drugs. These two studies and others suggest that current chemotherapy regimens may be improved by treating resistant cells with antineoplastic agents displaying physicochemical characteristics opposite to that of the original inducing agent.

Resistance of tumor cells to multiple cytotoxic agents is one of the major causes of treatment failure in cancer chemotherapy. Malignancies that exhibit de novo resistance seem to be associated with previous exposure to carcinogens, e.g. lung cancer. Acquired resistance generally results from exposure of drug-sensitive malignant cells to various antineoplastic agents. Many experimental cell lines selected for resistance to actinomycin D, colchicine, vincristine, adriamycin, and trimetrexate have demonstrated multidrug resistance to a variety of antitumor agents with dissimilar chemical structures, modes of action, and physicochemical properties.[1-4] This general phenomenon of "pleiotropic drug resistance" is now addressed as multidrug resistance (MDR).

In a pioneering study, Beidler and Riehm were the first to describe the MDR phenomenon.[4] They found that exposure of several sublines of Chinese hamster cells to increasing concentrations of actinomycin D resulted in resistance to a broad range of structurally varied agents (Table I). Their results indicated that cross resistance was correlated with the molecular weights of the drugs.

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