6-Substituted-9-(3-formamidobenzyl)-9*H*-purines. Benzodiazepine Receptor Binding Activity

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A series of 6-substituted-9-(3-formamidobenzyl)purines were synthesized and studied for benzodiazepine receptor (BZR) binding activity. Most of the target compounds were prepared by reaction of 6-chloro-9-(3-formamidobenzyl)-9*H*-purine (**17**) with the appropriate amine, alcohol or other nucleophilic reagent. Alternatively, the 6-cyclopropylaminopurine **5** was synthesized *via* the nitrobenzyl precursor **22**, and the 6-alkylthiopurines **14** and **15** were prepared by alkylation of the appropriate purine with 3-formamidobenzyl bromide. Purines with a variety of 6-substituents retained potent BZR binding properties, although certain bulky 6-substituents led to compounds with diminished activity. None of the compounds exhibited significant activity on a modified Geller-Seifter Conflict schedule.

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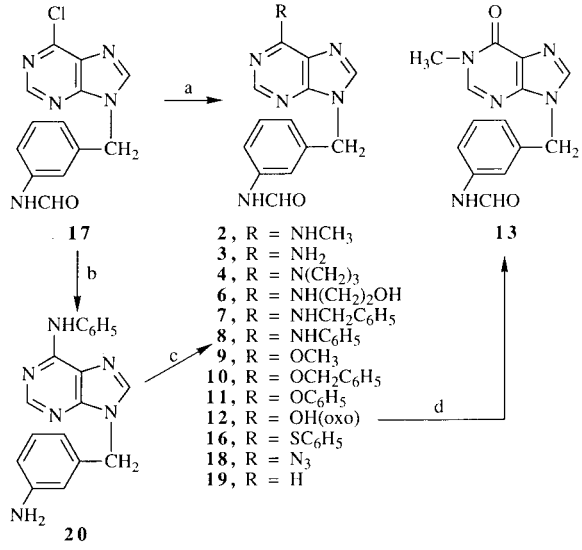
The benzodiazepines (BZs), which exhibit diverse therapeutic applications [1], exert their pharmacological activities through high-affinity binding sites or receptors [2-4]. Compounds of diverse structure bind to the benzodiazepine receptor (BZR) [5]; some compounds possess BZ-like activity, but others are characterized as antagonists or inverse agonists [5,6]. We recently reported the potent BZR binding activity of a series of 9-benzylpurines [7-9]. The most active is the 8-bromo-9-(3-formamidobenzyl)-purine **1a** (Table II), which has an IC_{50} of 0.011 μM; **1a** has an affinity for the BZR comparable to that of diazepam [8]. However, **1a** and several congeners are not active in the Geller-Seifter Conflict test [8,10,11]. *In vitro* experiments (modified with GABA) suggested these purines are antagonists like Ro 15-1788, not agonists like diazepam [8,12-14]. To further explore the effect of structural changes on BZR binding activity and *in vivo* Geller-Seifter Conflict activity, we prepared a series of 6-substituted analogues of **1a**. The synthesis and pharmacological evaluation of these compounds are described.

Scheme I[a]



[a] Reagents: (a) see experimentals; (b) $NH_2C_6H_5$, EtOH, 80 °C; (c) ethyl formate, 110 °C; (d) 1N NaOH, dimethyl sulfate.

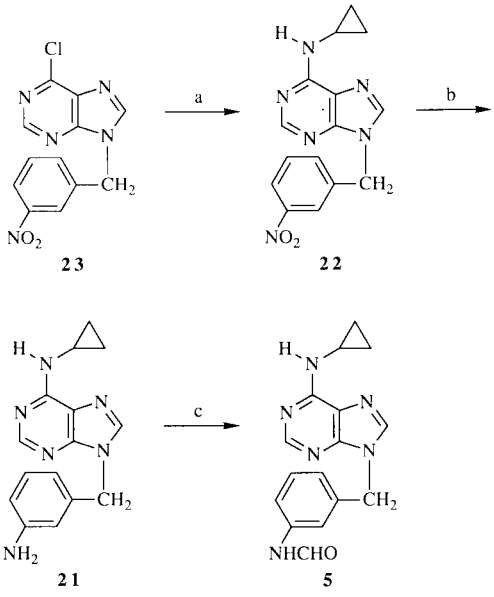
Chemistry.

The 6-substituted 9-(3-formamidobenzyl)purines **2**, **4**, **6-13**, **16**, **18**, and **19** were prepared from 6-chloropurine **17** [8] as outlined in Scheme I. Amination of **17** with excess methylamine, azetidine, 2-hydroxyethylamine, or benzyl amine in alcoholic or aqueous solution at room temperature gave **2**, **4**, **6**, and **7**, respectively. Under conditions required for replacement of the 6-chloro group of **17** with aniline, concurrent hydrolysis of the 3-formamido moiety occurred to give **20**. Heating **20** with ethyl formate at 110° gave **8** in good yield.

Compound **17** was converted to the 6-methoxy derivative **9** with sodium methoxide and to the benzyloxy and phenoxy derivatives **10** and **11** with sodium hydroxide and either benzyl alcohol or phenol. The 6-phenylthio analogue **16** was prepared from **17** with benzenethiol and sodium hydroxide. The 6-oxo, 6-azido, and 6-hydrogen analogues **12**, **18**, and **19** were prepared from **17** by sodium azide displacement, formic acid hydrolysis, and catalytic hydrogenolysis, respectively. Catalytic reduction of the azido moiety in **18** gave the 6-amino analogue **3**. Methylation of **12** with dimethylsulfate in aqueous sodium hydroxide gave **13**.

The 6-cyclopropyl amino analogue **5** was prepared in three steps from 6-chloro-9-(3-nitrobenzyl)-9*H*-purine [15] (**23**) by Scheme II. Amination of **23** with cyclopropyl amine gave **22**. The 3-nitrobenzyl derivative **22** was reduced by

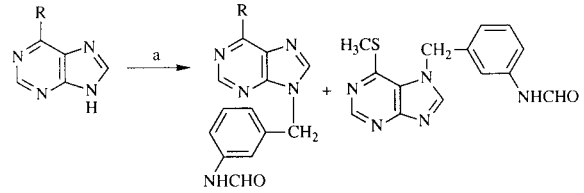
Scheme II[a]



[a] Reagents: (a) $NH_2C_3H_5$, EtOH; (b) H_2, PtO_2, AcOH; (c) ethyl formate, Et_3N, reflux.

catalytic hydrogenation to give **21**, which was converted to **5** by heating with ethyl formate.

Scheme III[a]



24, R = SCH_3
25, R = $SCH_2C_6H_5$

14, R = SCH_3
15, R = $SCH_2C_6H_5$

26

[a] Reagents: (a) 3-formamidobenzyl bromide, K_2CO_3, DMF.

The thio analogues **14**, **26**, and **15** were prepared from 6-methylthio **24** and 6-benzylthio **25** purine by alkylation with 3-formamidobenzyl bromide [8] in potassium carbonate and DMF (Scheme III). Chromatography was used to isolate and purify the 9-substituted compounds **14** and **15**. The 7-(3-formamido)benzylpurine **24** was also isolated from the column.

Biological Results and Discussions.

The compounds in Table II were tested for activity in the BZR binding assay by measuring inhibition of specific binding of 1.5 n*M* [^{3}H]diazepam to rat brain receptors [7]. The IC_{50} values represent the concentration of compound that decreased specific binding by 50%. The increased potency of a compound as an inhibitor of [^{3}H]diazepam binding was assumed to reflect its increased affinity for the receptor.

The 8-bromo-6-dimethylaminopurine **1a** inhibits specific binding of [^{3}H]diazepam to rat brain receptors with an IC_{50} = 0.011 μM [8]. This compound is nearly as potent as diazepam (IC_{50} = 0.006 μM) and has an affinity for the BZR 1000-fold greater than the parent, 9-benzyl-6-(dimethylamino)-9*H*-purine [7,8]. The 8-bromo substituent of **1a** increases affinity for the BZR by only 3-fold over **1b**. Therefore, to evaluate the effect of various 6-substituents on activity, we studied a series of analogues of **1b**, which were more easily synthesized.

The prototype *meta*-formamidobenzyl purine **1b** (R = $N(CH_3)_2$) inhibits specific binding of [^{3}H]diazepam with an IC_{50} = 0.034 μM. Several different 6-amino substituents were well tolerated if steric bulk was small; compounds **2** (R = $NHCH_3$), **3** (R = NH_2), **4** (R = $N(CH_2)_3$), **5** (R = NHC_3H_5), and **6** (R = $NHCH_2CH_2OH$) had IC_{50}s ranging from 0.040 to 0.069 μM. The bulky $NHCH_2C_6H_5$ (**7**) and NHC_6H_5 (**8**) subsituents were significantly less active with IC_{50}s of 0.58 and 10.23 μM, respectively.

With one exception, the 6-oxygen containing analogues, **9-13**, were significantly less active than **1b**. The 6-benzyloxy analogue, **10**, was quite active with an IC_{50} = 0.055 μM. The 6-thio analogues **14-16** also had good activity with IC_{50}s ranging from 0.056 to 0.17 μM.

The 6-chloro **17** and 6-azido **18** purines were active at 0.18 and 0.22 μM, respectively. Removal of the 6-substituent to give **19** (R = H) resulted in a compound with an IC_{50} = 0.081 μM.

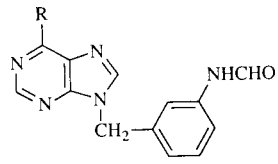
Thus, the structure-activity relationships of this set of 6-substituted purines show that a 6-substituent has only a minimal effect on improving the BZR binding activity of 9-(3-formamidobenzyl)purines. However, certain bulky substituents such as $NHCH_2C_6H_5$, **7**, NHC_6H_5, **8**, and OC_6H_5, **11**, led to a loss of activity, indicating limited bulk tolerance for certain large groups.

Eleven of the 6-substituted purines were tested for activity on a modified Geller-Seifter Conflict schedule [7,10,11]. Under conditions were chlordiazepoxide (CDP) produced significant dose-related increases in responding during the conflict portion of the operant schedule, none of the purines tested at 25 mg/kg p.o. produced any significant change in conflict responding (Table II).

Several of these 6-substituted-9-(3-formamidobenzyl)-purines bind to the BZR with potency comparable to the benzodiazepines. However, no *in vivo* activity was observed when they were tested in the Geller-Seifter Conflict test; increased responding is expected for BZ agonists [5,6]. In contrast to agonists of the BZR, antagonists are not active in the Geller-Seifter Conflict test. These *in vivo* data, coupled with *in vitro* GABA experiments on a series of 8-substituted-9-(benzyl)purines, [8] lend support to the concept that the 9-(benzyl)purines are antagonists of the BZR.

Table I

Physical Properties of 6-Substituted-9-(3-formamido)benzyl-9*H*-purines

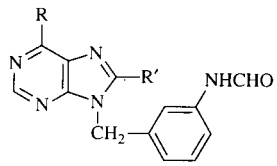


Compound No.	R	Method	Yield %	Mp °C	Molecular Formula	Analysis % Calcd. (Found) C	H	N
2	$NHCH_3$	A [a]	75	190-192 [b]	$C_{14}H_{14}N_6O$	59.56 (59.40)	5.00 (5.09)	29.77 (29.61)
4	$N(CH_2)_3$	A [c]	72	185-186 [d]	$C_{16}H_{16}N_6O$	62.32 (62.32)	5.23 (5.25)	27.26 (27.19)
5	NHC_3H_5	C [e]	32	163-164 [f]	$C_{16}H_{16}N_6O$	62.32 (62.18)	5.23 (5.20)	27.26 (27.25)
6	$NH(CH_2)_2OH$	A [g]	47	160-162 [h]	$C_{15}H_{16}N_6O_2$	57.68 (57.48)	5.16 (5.20)	26.91 (26.83)
7	$NHCH_2C_6H_5$	A	69	191-192.5 [i]	$C_{20}H_{18}N_6O$	67.02 (67.08)	5.06 (5.10)	23.45 (23.42)
8	NHC_6H_5	C	80 [d]	156-158	$C_{19}H_{16}N_6O \cdot 1/2H_2O$	64.58 (64.45)	4.85 (4.86)	23.78 (23.76)
14	SCH_3	B	37 [b]	206-207	$C_{14}H_{13}N_5OS$	56.17 (56.22)	4.38 (4.28)	23.40 (23.25)
15	$SCH_2C_6H_5$	B	34	150-150.5 [d]	$C_{20}H_{17}N_5OS$	63.98 (63.74)	4.56 (4.51)	18.65 (18.61)

[a] The alcoholic alkylamine was formed by dissolving a 5.75-fold excess of methylamine hydrochloride and a 2.3-fold excess of sodium hydroxide in ethanol. [b] Recrystallized from ethyl acetate. [c] After 18 hours the reaction was spin evaporated *in vacuo*. The residual solid was dissolved in ethyl acetate, washed with water, and evaporated to give the crude product. [d] Recrystallized from ethyl acetate. [e] Refluxing for 3 days with ethyl formate and 2 equivalents of triethylamine was used instead of heating in a bomb. [f] Recrystallized from dichloromethane-hexanes. [g] Reaction was complete after 3 hours. [h] Recrystallized from ethanol-water. [i] Recrystallized from ethanol.

Table II

Benzodiazepine Receptor Binding Activity of 6-Substituted-9-(3-formamido)benzyl-9H-purines



No.	R	R'	IC_{50}, μM [a]	% Change [b] in Conflict Responding
1a [c]	$N(CH_3)_2$	Br	0.011±0.002	+9±9
1b [c]	$N(CH_3)_2$	H	0.034±0.012	-2±21
2	$NHCH_3$	H	0.065	-11±9
3	NH_2	H	0.061	0±9
4	$N(CH_2)_3$	H	0.040	-7±14
5	NHC_3H_5	H	0.061	-8±14
6	$NHCH_2CH_2OH$	H	0.069	-21±10
7	$NHCH_2C_6H_5$	H	0.58	
8	NHC_6H_5	H	10.23	
9	OCH_3	H	0.14	-8±8
10	$OCH_2C_6H_5$	H	0.055	-13±9
11	OC_6H_5	H	0.36	-7±9
12	OH (oxo)	H	0.31	
13	oxo ($1\text{-}CH_3$)	H	1.0	
14	SCH_3	H	0.075	-17±28
15	$SCH_2C_6H_5$	H	0.17	+25±18
16	SC_6H_5	H	0.056	-24±12
17	Cl	H	0.18	
18	N_3	H	0.22	
19	H	H	0.081	
chlordiazepoxide			0.2	+67±10 [d]
diaepam			0.006±0.001	

[a] The IC50s were determined by the method described in reference 7. The IC50 is the concentration of compound that decreased specific binding of 1.5 n*M* [^{3}H]diazepam to rat brain receptors by 50 percent. [b] The compounds were tested in Long-Evens rats as described in reference 7 on a modified Geller-Seifter Conflict schedule. Compounds were administered by oral gavage in a 0.5% methyl cellulose suspension at 25 mg/kg. [c] Data from reference 8. [d] Chlordiazepoxide was administered at 20 mg/kg.

EXPERIMENTAL

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 FT-80A, a Varian T-60, or a Hitachi Perkin-Elmer R-24 spectrometer and referenced to tetramethylsilane as an internal standard. The uv absorption spectra were measured on a Unicam SP 800 or Cary 118 UV-vis spectrophotometer. The thin layer chromatographs (tlc) were developed on Whatman 200 μ MK6F plates of silica gel with fluorescent indicator. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on tlc. Preparative flash chromatograph [16] was performed on a Silica Gel 60 (40-63 μm, E. Merck No. 9385). Elemental analyses were performed by Atlantic Microlab, Inc.

Method A. 6-Benzylamino-9-(3-formamidobenzyl)-9*H*-purine (**7**).

A solution of **17** [8] (0.50 g, 1.74 mmoles), benzylamine (0.5 ml, 0.49 g, 4.58 mmoles), and ethanol (5 ml) was stirred in a stoppered flask at ambient temperature for 44 hours. The insoluble product was collected by filtration and washed with water and ethanol. Recrystallization from ethanol-water gave 0.728 g (69%) of **7**, mp 185°. The analytical sample was obtained by recrystallization from water, mp 191-192.5°; uv (*p*H 1 + 9.5% ethanol): λ max 265 nm (ε 24300); uv (*p*H 7 + 9.5% ethanol): λ max 270 nm (ε 23500); uv (*p*H 13 + 9.5% ethanol): λ max 269 nm (ε 25500); ^{1}H nmr (60 MHz, DMSO-d_6): δ 10.1 (br m, 1H, NH), 8.67 (d, 0.5H, (E)-NHC*H*O, J = 11 Hz), 8.1-8.4 (m, 2.5H, purine H + (Z)-NHC*H*O), 6.9-7.7 (m, 4H, ArH), 5.34 (s, 2H, CH_2), 4.77 (br d, 2H, NH_2CH_2, J = 7 Hz).

Anal. Calcd. for $C_{20}H_{18}N_6O$: C, 67.02; H, 5.06; N, 23.45. Found: C, 67.08; H, 5.10; N, 23.42.

Method B. 6-Methylthio-9-(3-formamidobenzyl)-9*H*-purine (**14**).

A mixture of 6-methylthio-9*H*-purine (**24**) (1.07 g, 6.84 mmoles), 3-formamidobenzyl bromide [8] (1.6 g, 7.47 mmoles), potassium carbonate (1.5 g, 10.9 mmoles, powdered), and dimethylformamide (3.5 ml) was stirred for 18 hours. The reaction mixture was spin-evaporated *in vacuo*, and the residue was partitioned between dichloromethane and water. The combined dichloromethane layers were spin-evaporated *in vacuo*. The residual solids were purified by flash chromatography (silica gel, 3 cm x 25 cm column, 5% 2-propanol in dichloromethane). The fractions containing the higher R_f component were combined and spin-evaporated *in vacuo*. Recrystallization of the residual solids gave 0.717 g (37%) of **14**, mp 206-207°; ^{1}H nmr (80 MHz, DMSO-d_6): δ 8.75 (d, 0.15H, (E)-NHC*H*O, J = 11 Hz), 8.73 (s, 1H, purine H), 8.58 (s, 1H, purine H), 8.32 (d, 0.85H, (Z)-NHC*H*O, J = 0.8 Hz), 6.9-7.6 (m, 4H, ArH), 5.47 (s, 2H, CH_2), 2.65 (s, 3H, CH_3S).

Anal. Calcd. for $C_{14}H_{13}N_5OS$: C, 56.17; H, 4.38; N, 23.40. Found: C, 56.22; H, 4.28; N, 23.35.

Method C. 6-Anilino-9-(3-formamidobenzyl)-9*H*-purine (**8**).

A solution of **20** (0.117 g, 0.37 mmole) and ethyl formate (30 ml) was heated in a stainless steel bomb for 24 hours at 110°. The solvent was removed by spin-evaporation *in vacuo*, and the residual solid was recrystallized from ethyl acetate-hexanes to give 0.104 g (80%) of **8**, mp 156-158°; uv (*p*H 1 + 9.5% ethanol): λ max 274.5 nm (ε 21900); uv (*p*H 7 + 9.5% ethanol): λ max 287 nm (ε 23900); uv (*p*H 13 + 9.5% ethanol): λ max 287 nm (ε 24400); ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.15 (br m, 1H, NH), 8.78 (d, 0.3H, (E)-NHC*H*O, J = 11.2 Hz), 8.45 (s, 2H, purine H), 8.25 (d, 0.7H, (Z)-NHC*H*O, J = 1.6 Hz), 6.8-7.5 (m, 9H, ArH), 5.43 (s, 2H, CH_2).

Anal. Calcd. for $C_{19}H_{16}N_6O \cdot 1/2H_2O$: C, 64.58; H, 4.85; N, 23.78. Found: C, 64.45; H, 4.86; N, 23.76.

6-Amino-9-(3-formamidobenzyl)-9*H*-purine (**3**).

A mixture of **18** (0.270 g, 0.91 mmole), methanol (50 ml), and 5% palladium on carbon (50 mg) was shaken in the presence of hydrogen at 2-3 atmospheres for 18 hours. The reaction mixture was heated on a steam bath to dissolve the precipitated product, and the catalyst was removed by filtration. The filtrate was spin-evaporated *in vacuo*, and the residual solid was recrystallized from ethanol-water to give 0.226 g (72%) of **3**, mp 227-228°; uv (*p*H 1 + 9.5% ethanol): λ max 256.5 nm (ε 23300); uv (*p*H 7 + 9.5% ethanol): λ max 256 nm (ε 22000); uv (*p*H 13 + 9.5% ethanol): λ max 258 nm (ε 22300); ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.16 (br m, 1H, NH), 8.75 (d, 0.25H, (E)-NHC*H*O, J = 11.2 Hz), 8.24 (s, 1.75H, purine H + (Z)-NHC*H*O), 8.79 (s, 1H, purine H), 6.9-7.6 (m, 6H, ArH + NH_2), 5.37 (s, 2H, CH_2).

Anal. Calcd. for $C_{13}H_{12}N_6O$: C, 58.2; H, 4.51; N, 31.33. Found: C, 57.99; H, 4.55; N, 31.18.

6-Methoxy-9-(3-formamidobenzyl)-9*H*-purine (**9**).

A solution of **17** [8] (0.5 g, 1.74 mmoles), sodium methoxide (0.100 g, 1.85 mmoles), and methanol (10 ml) was refluxed on a steam bath for 10 minutes and then cooled to 0° for 2 hours. The precipitated product was collected by filtration to give 0.390 g (80%) of **9**, mp 174-175.5°. The analytical sample was prepared by recrystallization from methanol, mp 174.5-175.5°; uv (*p*H 1 + 9.5% ethanol): λ max 246.5 nm (ε 24000); uv (*p*H 7 + 9.5% ethanol): λ max 247 nm (ε 24400); uv (*p*H 13 + 9.5% ethanol): λ max 248 nm (ε 24400); ^{1}H nmr (60 Hz, DMSO-d_6): δ 10.2 (br m, 1H, NH), 8.6 (d, 0.28H, (E)-NHC*H*O, J = 12 Hz), 8.56 (s, 1H, purine H), 8.51 (s, 1H, purine H), 8.2 (d, 0.72H, (Z)-NHC*H*O, J = 1.6 Hz), 6.9-7.6 (m, 4H, ArH), 5.50 (s, 2H, CH_2), 4.13 (s, 3H, CH_3O).

Anal. Calcd. for $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72. Found: C, 59.25; H, 4.65; N, 24.68.

6-Benzyloxy-9-(3-formamidobenzyl)-9*H*-purine (**10**).

A mixture of sodium hydroxide (0.070 g, 1.75 mmoles) and benzyl alcohol (15 ml) was heated on a steam bath until homogeneous. To this solution was added **17** [8] (0.5 g, 1.74 mmoles), water (25 ml), and dimethylformamide (25 ml), and the reaction was heated on a steam bath for 25 hours. The volatiles were removed by spin-evaporation *in vacuo* (0.02 Torr). The residue was recrystallized from ethanol-water to give 0.475 g (73%) of **10**, mp 151-152°; uv (*p*H 1 + 9.5% ethanol): λ max 249 nm (ε 25200); uv (*p*H 7 + 9.5% ethanol): λ max 248 nm (ε 25300); uv (*p*H 13 + 9.5% ethanol): λ max 250 nm (ε 25500); ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.15 (br m, 1H, NH), 8.73 (d, 0.16H, (E)-NHC*H*O, J = 12 Hz), 8.56 (s, 1H, purine H), 8.51 (s, 1H, purine H), 8.32 (d, 0.84H, (Z)-NHC*H*O, J = 1.2 Hz), 6.9-7.6 (m, 9H, ArH), 5.64 (s, 2H, CH_2O), 5.47 (s, 2H, CH_2N).

Anal. Calcd. for $C_{20}H_{17}N_5O_2$: C, 66.84; H, 4.77; N, 19.49. Found: C, 66.58; H, 4.84; N, 19.39.

6-Phenoxy-9-(3-formamidobenzyl)-9*H*-purine (**11**).

A solution of **17** [8] (0.5 g, 1.74 mmoles), phenol (0.325 g, 3.46 mmoles), potassium hydroxide (0.225 g, 3.46 mmoles), water (5 ml), and dimethylformamide (5 ml) was stirred at ambient tem-

perature for 18 hours. The reaction solution was diluted with water (50 ml) and extracted with ethyl acetate (4 x 75 ml). The extracts were combined and spin-evaporated *in vacuo*. The residual solid was purified by flash chromatography (silica gel, 3 cm x 20 cm column, ethyl acetate). Recrystallization from ethanol-water gave 0.382 g (64%) of **11**, mp 156-157°. The analytical sample was prepared by a second recrystallization, mp 156-156.5°; uv (*p*H 1 + 9.5% ethanol): λ max 249.5 nm (ε 23800); uv (*p*H 7 + 9.5% ethanol): λ max 248.5 nm (ε 24200); uv (*p*H 13 + 9.5% ethanol): λ max 250 nm (ε 24200); ^{1}H nmr (60 Hz, DMSO-d_6): δ 10.17 (br m, 1H, NH), 8.73 (d, 0.2H, (E)-NHC*H*O, J = 12 Hz), 8.6 (s, 1H, purine H), 8.4 (s, 1H, purine H), 8.20 (d, 0.8H, (Z)-NHC*H*O, J = 1.6 Hz), 6.9-7.7 (m, 9H, ArH), 5.50 (s, 2H, CH_2).

Anal. Calcd. for $C_{19}H_{15}N_5O_2$: C, 66.08; H, 4.38; N, 20.28. Found: C, 66.02; H, 4.36; N, 20.24.

9-(3-Formamidobenzyl)-1,9-dihydro-6*H*-purine-6-one (**12**).

A solution of **17** [8] (0.50 g, 1.74 mmoles) and 90% formic acid was heated at 80° for 1 hour. The reaction solution was spin-evaporated *in vacuo*, and the residual solid was recrystallized from ethanol-water to give 0.352 g (75%) of **12**, mp 255°. The analytical sample was prepared by recrystallization from ethanol, mp 259-262° eff; ^{1}H nmr (80 Hz, DMSO-d_6): δ 12.1 (br s, 1H, NH), 10.16 (br m, 1H, N*H*CHO), 8.72 (d, 0.21H, (E)-NHC*H*O, J = 11.2 Hz), 8.23 (d, 0.79H, (Z)-NHC*H*O, J = 1.8 Hz), 8.19 (s, 1H, purine H), 8.04 (s, 1H, purine H), 6.9-7.6 (m, 4H, ArH), 5.36 (s, 2H, CH_2).

Anal. Calcd. for $C_{13}H_{11}N_5O_2 \cdot 1/4H_2O$: C, 57.04; H, 4.25; N, 25.58. Found: C, 56.93; H, 4.13; N, 25.5.

9-(3-Formamidobenzyl)-1-methyl-1,9-dihydro-6*H*-purine-6-one (**13**).

To a stirred solution of **12** (0.40 g, 1.49 mmoles), 1 *N* sodium hydroxide (1.5 ml), and water (1.5 ml) was added dimethyl sulfate (0.15 ml, 0.189 g, 1.49 mmoles). The reaction was stirred for 1 hour, and then the volatiles were spin-evaporated *in vacuo*. The residual solid was purified by flash chromatography (silica gel, 1% methanol in dichloromethane). Recrystallization from dichloromethane-hexanes gave 0.104 g (41%) of **13**, mp 178.5-179°; uv (*p*H 1 + 9.5% ethanol): λ max 248 nm (ε 2100); uv (*p*H 7 + 9.5% ethanol): λ max 246.5 nm (ε 20800); uv (*p*H 13 + 9.5% ethanol): λ max 249.5 nm (ε 20700); ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.15 (br m, 1H, NH), 8.75 (d, 0.36H, (E)-NHC*H*O, J = 11 Hz), 8.37 (s, 1H, purine H), 8.20 (s, 1H, purine H), 8.24 (d, 0.64H, (Z)-NHC*H*O, J = 1.6 Hz), 6.9-7.6 (m, 4H, ArH), 5.35 (s, 2H, CH_2).

Anal. Calcd. for $C_{14}H_{13}N_5O_2$: C, 59.34; H, 4.36; N, 24.72. Found: C, 59.16; H, 4.68; N, 24.61.

9-(3-Formamidobenzyl)-6-phenylthio-9*H*-purine (**16**).

To a stirred solution of benzenethiol (0.165 g, 1.5 mmoles), sodium hydroxide (0.050 g, 1.25 mmoles), and ethanol (5 ml) was added **17** [8] (0.287 g, 1.0 mmole). After 1.5 hours the reaction solution was diluted with dichloromethane (200 ml) and washed successively with water (2 x 25 ml), 1 *N* sodium hydroxide (3 x 25 ml), and water (2 x 25 ml). The dichloromethane was spin-evaporated *in vacuo*, and the residual solid was dissolved in ethyl acetate, filtered through a pad of silica gel (2.5 cm x 2.5 cm), and washed with ethyl acetate. The filtrate was spin-evaporated *in vacuo*, and the residual solid was recrystallized from ethyl acetate-hexanes to give 0.295 g (82%) of **16**, mp 189-190°; uv (*p*H 1 + 9.5% ethanol): λ max 292.5 nm (ε 21100); λ sh 287 nm (ε 19400); uv (*p*H 7 + 9.5% ethanol): λ max 291.5 nm (ε 22300); λ sh 240 nm (ε 17700); uv (*p*H 13 + 9.5% ethanol): λ max 291.5 nm (ε 22800); λ sh 287.5 nm (ε 22300); ^{1}H nmr (60 MHz, DMSO-d_6): δ 10.1 (br m, 1H, NH), 8.6 (d, 0.1H, (E)-NHC*H*O, J = 11 Hz), 8.63 (s, 1H, purine H), 8.43 (s, 1H, purine H), 8.2 (d, 0.9H, (Z)-NHC*H*O, J = 1.6 Hz), 6.9-7.6 (m, 9H, ArH), 5.47 (s, 2H, CH_2).

Anal. Calcd. for $C_{19}H_{15}N_5OS$: C, 63.14; H, 4.18; N, 19.38. Found: C, 63.22; H, 4.23; N, 19.36.

6-Azido-9-(3-formamidobenzyl)-9*H*-purine (**18**).

A mixture of **17** [8] (0.50 g, 1.74 mmoles), sodium azide (0.130 g, 2.0 mmoles), and dimethylformamide (4 ml) was stirred at ambient temperature for 18 hours. The reaction solution was poured into water (200 ml) and extracted with ethyl acetate (5 x 50 ml). The extracts were combined, washed with water (50 ml), dried (magnesium sulfate), and spin-evaporated *in vacuo*. The residual solid was recrystallized from ethyl acetate to give 0.372 g (73%) of **18**, mp 193-195°; ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.15 (br m, 1H, NH), 10.09 (s, 1H, purine 8-H), 8.79 (s, 1H, purine 2-H), 8.75 (d, 0.25H, (E)-NHC*H*O, J = 11.2 Hz), 8.23 (d, 0.75H, (Z)-NHC*H*O, J = 1.2 Hz), 6.9-7.6 (m, 4H, ArH), 5.65 (s, 2H, CH_2).

Anal. Calcd. for $C_{13}H_{10}N_8O$: C, 53.06; H, 3.43; N, 38.08. Found: C, 53.02; H, 3.47; N, 38.07.

9-(3-Formamidobenzyl)-9*H*-purine (**19**).

A mixture of **17** [8] (0.50 g, 1.74 mmoles), sodium acetate (0.164 g), methanol (50 ml), and 20% palladium hydroxide on carbon (50 mg) was shaken in the presence of hydrogen at 2-3 atmospheres for 3 hours. The catalyst was removed by filtration, and the filtrate was spin-evaporated *in vacuo*. The residual solid was recrystallized from ethanol-water to give 0.316 g (72%) of **19**, mp 150-155°. The analytical sample was prepared by recrystallization from ethyl acetate, mp 155-156.5°; uv (*p*H 1 + 9.5% ethanol): λ max 245 nm (ε 13700); uv (*p*H 7 + 9.5% ethanol): λ max 246 nm (ε 14200); uv (*p*H 13 + 9.5% ethanol): λ max 149.5 nm (ε 14200); ^{1}H nmr (80 MHz, DMSO-d_6): δ 10.05 (br m, 1H, NH), 9.18 (s, 1H, purine H), 8.94 (s, 1H, purine H), 8.73 (s, 1H, purine H), 8.75 (d, 0.18H, (E)-NHC*H*O, J = 12 Hz), 8.21 (d, 0.72H, (Z)-NHC*H*O, J = 1.6 Hz), 6.9-7.6 (m, 4H, ArH), 5.51 (s, 2H, CH_2).

Anal. Calcd. for $C_{13}H_{11}N_5O$: C, 61.65; H, 4.38; N, 27.65. Found: C, 61.57; H, 4.41; N, 27.62.

6-Anilino-9-(3-aminobenzyl)-9*H*-purine (**20**).

A solution of **17** [8] (0.287 g, 1.0 mmole), aniline (1.0 ml, 0.978 g, 10.5 mmoles), and ethanol (5 ml) was stirred at ambient temperature for 18 hours, and then heated to 80° for 1 hour. The reaction solution was spin-evaporated *in vacuo*, and the residual solid was recrystallized from ethyl acetate to give 0.291 g (68%) of **20**, mp 202-205°. Recrystallization from ethyl acetate gave the analytical sample, mp 204-205°; uv (*p*H 1 + 9.5% ethanol): λ max 274.5 nm (ε 21100); uv (*p*H 7 + 9.5% ethanol): λ max 288.5 nm (ε 25400); uv (*p*H 13 + 9.5% ethanol): λmax 288.5 nm (ε 25000); ^{1}H nmr (60 MHz, DMSO-d_6): δ 9.9 (br m, 1H, NH), 8.40 (s, 1H, purine H), 8.36 (s, 1H, purine H), 8.1-7.8 (m, 2H, ArH), 8.5-6.8 (m, 4H, ArH), 6.6-6.3 (m, 2H, ArH), 5.30 (s, 2H, CH_2), 5.10 (s, 2H, NH_2).

Anal. Calcd. for $C_{18}H_{16}N_6$: C, 68.34; H, 5.1; N, 26.65. Found: C, 68.34; H, 5.17; N, 26.56.

6-Cyclopropylamino-9-(3-aminobenzyl)-9*H*-purine (**21**).

A mixture of **22** (13.2 g, 43.2 mmoles), platinum oxide (200 mg), and acetic acid (200 ml) was shaken in the presence of hydrogen at 2-3 atmospheres for 35 minutes. The catalyst was removed by

filtration. The filtrates were acidified with concentrated hydrochloric acid (5 ml) and spin-evaporated *in vacuo*. The resulting dark oil was dissolved in 1*N* hydrochloric acid and the solution was stirred with decolorizing carbon (Norite, 1.5 g) for 10 minutes, filtered through Celite (Preiser Scientific, Inc.), and spin-evaporated *in vacuo*. The residual solids were recrystallized from 2-propanol/water to give 12.3 g (82%) of **21**, mp 210° dec. The analytical sample was prepared by a second recrystallization from the same solvent, mp 210° dec; uv (*p*H 7 + 10% ethanol): λ max 270 nm (ε 20300); ^{1}H nmr (100 MHz, DMSO-d_6): δ 10.17 (br m, 3H, NH + 2HCl), 8.70 (s, 1H, purine H), 8.52 (s, 1H, purine H), 7.6-7.2 (m, 4H, ArH), 5.58 (s, 2H, CH_2), 3.01 (m, 1H, C*H*(CH_2)$_2$), 0.80-1.04 (m, 4H, CH(C*H*$_2$)$_2$).

Anal. Calcd. for $C_{15}H_{16}N_6 \cdot 2HCl \cdot H_2O$: C, 48.53; H, 5.43; N, 22.64. Found: C, 48.45; H, 5.44; N, 22.52.

6-Cyclopropylamino-9-(3-nitrobenzyl)-9*H*-purine (**22**).

A solution of **23** [15] (20 g, 69.2 mmoles), cyclopropylamine (14.2 g, 250 mmoles), and ethanol (100 ml) was stirred for 0.5 hour at ambient temperature and then heated (60° oil bath) for 3 hours. The reaction solution was spin-evaporated *in vacuo*, and the residual damp paste was slurried with water (150 ml). The resulting solids were collected by filtration and washed well with water and recrystallized from ethanol to give 20.1 g (93%) of **22**, mp 165-170°. The analytical sample was prepared by recrystallization from ethanol, mp 168-168.5°; uv (*p*H 1 + 9.5% ethanol): λ max 271 nm (ε 30100); uv (*p*H 7 + 9.5% ethanol): λ max 269 nm (ε 32900); uv (*p*H 13 + 9.5% ethanol): λ max 271 nm (ε 30100); ^{1}H nmr (80 MHz, DMSO-d_6): δ 8.32 (s, 1H, purine H), 8.25 (s, 1H, purine H), 8.25-8.07 (m, 1H, ArH), 7.93 (br d, 1H, J = 4.0 Hz, NH), 7.50-7.85 (m, 3H, ArH), 5.54 (s, 2H, CH_2), 3.1 (m, 1H, C*H*(CH_2)$_2$), 0.55-0.80 (m, 4H, CH(C*H*$_2$)$_2$).

Anal. Calcd. for $C_{15}H_{14}N_6O_2$: C, 58.06; H, 4.55; N, 27.08. Found: C, 57.99; H, 4.55; N, 26.96.

6-Methylthio-7-(3-formamidobenzyl)-7*H*-purine (**26**).

After elution of the 9-isomer **14**, the 7-isomer was isolated by further elution with 10% 2-propanol in dichloromethane. Fractions containing the lower R_f 7-isomer were collected, combined, and spin-evaporated *in vacuo* to give 0.222 g (12%) of **24**, mp 225-225.5°; uv (*p*H 1 + 9.5% ethanol): λ max 304.5 nm (ε 13400); uv (*p*H 7 + 9.5% ethanol): λ max 295 nm (ε 15200); uv (*p*H 13 + 9.5% ethanol): λ max 295 nm (ε 15500); ^{1}H nmr (60 MHz, DMSO-d_6): δ 10.13 (br m, 1H, NH), 8.9-8.5 (m, 2.25H, purine H_2 + (E)-NHC*H*O), 8.3 (br d, 0.75H, (Z)-NHC*H*O), 7.8-6.7 (m, 4H, ArH), 5.76 (s, 2H, CH_2), 2.7 (s, 3H, CH_3).

Anal. Calcd. for $C_{14}H_{13}N_5OS$: C, 56.17; H, 4.38; N, 23.4. Found: C, 56.05; H, 4.43; N, 23.31.

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