at −78 °C for 30 min and then the cooling bath was removed and the mixture allowed to stir at room temperature for 2 h. The reaction mixture was poured into water (100 mL) and ether (50 mL) and the resulting mixture saturated with NaCl. The organic layer was removed and the aqueous layer reextracted with ether (50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to a pale yellow oil (0.560 g, 94%). The crude product was taken up in methanol (50 mL) and the MOM ether removed by the addition of concentrated HCl (0.5 mL). The mixture was allowed to stir at 55 °C for 2 h. After cooling to room temperature, the reaction mixture was worked up by addition of 5% $NaHCO_3$ (3 mL) and concentrated in vacuo. After most of the methanol had been removed, the residue was partitioned between water and ether and the aqueous fraction reextracted with ether. The combined organic extracts were dried concentrated and chromatographed (10% ethyl acetate in hexane as eluant) to afford **64** (0.270 g, 56%) as a white crystalline solid and **3a** (0.050 g). Recrystallization of **64** from hexane afforded a sample identical with that prepared by hydrogenation of **63**.

2,3-Dihydro-4-*tert*-butyl-6-propen-3-yl-5-benzofuranol (69). To a suspension of sodium hydride (60% dispersion in mineral oil, 0.160 g, 3.99 mmol) in dry dimethylformamide (4.5 mL) was added a solution of **68**[22] (0.511 g, 2.66 mmol) in dimethylformamide (4 mL). After hydrogen evolution had ceased, a solution of allyl bromide (0.483 g, 3.99 mmol) in dimethylformamide (4 mL) was added and the mixture heated to 50 °C under nitrogen. After 1 h the reaction mixture was poured into 20% NaCl (125 mL) and extracted with ether (3 × 40 mL). The combined extracts were dried and concentrated to give a crude allyl ether (0.656 g). This material was taken up in 1,3-dichlorobenzene (6 mL) and heated to reflux under nitrogen for 16 h. Purification by flash chromatography using 2% ethyl acetate in hexane as eluant gave **69** (0.327 g, 53%) as a pale yellow solid: mp 92–95 °C; ^{1}H NMR δ 6.53 (s, 1 H), 6.06 (m, 1 H), 5.28 (m, 1 H), 5.22 (m, 1 H), 4.80 (s, 1 H), 4.46 (t, 2 H, J = 8 Hz), 3.48 (t, 2 H, J = 8 Hz), 3.38 (d, 2 H, J = 6 Hz), 1.50 (s, 9 H).

2,3-Dihydro-4-*tert*-butyl-6-propyl-5-benzofuranol (70). To a solution of **69** (0.230 g, 0.99 mmol) in absolute ethanol (12 mL) was added 10% palladium on carbon (0.020 g) and the solution hydrogenated at 3 atm of pressure. After 30 min the catalyst was removed by filtration through Celite and the filter cake washed with a small portion of ethanol. The combined filtrate and washings were concentrated to afford a crude product (0.210 g). Recrystallization from aqueous ethanol gave **70** (0.170 g, 73%) as a white solid: ^{1}H NMR δ 6.54 (s, 1 H), 4.46 (br s, 1 H), 4.44 (t, 2 H, J = 8 Hz), 3.47 (t, 2 H, J = 8 Hz), 2.52 (t, 2 H, J = 8 Hz), 1.66 (quintet, 2 H, J = 8 Hz), 1.51 (s, 9 H), 1.04 (t, 3 H, J = 8 Hz).

Human PMN LTB_4 Assay. A. Preparation of Human PMN. Human blood was obtained by antecubital venepuncture from consenting volunteers who denied having taken medication within the previous 7 days. The blood was immediately added to 10% (v/v) trisodium citrate (0.13 M) or 5% (v/v) sodium heparin (1000 IU/mL). PMNs were isolated from anticoagulated blood by dextran sedimentation and centrifugation through Ficoll-Hypaque (specific gravity 1.077), essentially as described.[33] Contaminating erythrocytes were removed by lysis following exposure to ammonium chloride (0.16 M) in Tris buffer (pH 7.65), and the PMNs resuspended at 5 × 10^5 cells/mL in HEPES (15 mM) buffered Hanks balanced salt solution containing Ca^{2+} (1.4 mM) and Mg^{2+} (0.7 mM), pH 7.4. Viability was assessed by Trypan blue exclusion and was typically greater than 98%.

B. Generation and Radioimmunoassay of LTB_4. PMNs (0.5 mL; 2.5 × 10^5 cells) were placed in plastic tubes and incubated (37 °C, 2 min) with test compounds at the desired concentration or vehicle control (DMSO, final concentration 0.2%). The synthesis of LTB_4 was initiated by the addition of calcium ionophore A23187 (final concentration 10 μM) or vehicle in control samples and allowed to proceed for 5 min at 37 °C. The reactions were then terminated by the addition of cold methanol (0.25 mL) and samples of the entire PMN reaction mixture were removed for radioimmunoassay of LTB_4.

Samples (50 μL) of authentic LTB_4 of known concentration in radioimmunoassay buffer (RIA) buffer (potassium phosphate 1 mM; disodium EDTA 0.1 mM; Thimerosal 0.025 mM; gelatin 0.1%, pH 7.3) or PMN reaction mixture diluted 1:1 with RIA buffer were added to reaction tubes. Thereafter [^{3}H]LTB_4 (10 nCu in 100 μL of RIA buffer) and LTB_4 antiserum (100 μL of a 1:3000 dilution in RIA buffer) were added and the tubes vortexed. Reactants were allowed to equilibrate by incubation overnight at 4 °C. To separate antibody-bound from free LTB_4, aliquots (50 μL) of activated charcoal (3% activated charcoal in RIA buffer containing 0.25% Dextran T-70) were added and the tubes vortexed and allowed to stand at room temperature for 10 min prior to centrifugation (1500*g*, 10 min, 4 °C). The supernatants containing antibody-bound LTB_4 were decanted into vials, and Aquasol 2 (4 mL) was added. Radioactivity was quantified by liquid scintillation spectrometry. Preliminary studies established that the amount of methanol carried into the radioimmunoassay did not influence the results. The specificity of the antiserum and the sensitivity of the procedure have been described in detail elsewhere.[34] The amount of LTB_4 produced in test and control (ca. 20 ng/10^6 cells) samples were calculated. Inhibitory dose–response curves were constructed from five point titrations using a four-parameter algorithm and from these the IC_{50} values were determined. Unless otherwise noted, all compounds were tested in at least duplicate and the reported IC_{50} values reflect the mean value of all the IC_{50}s obtained.

Acknowledgment. We thank Dr. Stanley Wright of Dev Labs, Hoddeston, England, for a generous gift of 2,3-dihydro-5-benzofuranol (1) and for helpful procedures including the Lewis acid catalyzed Claisen rearrangement of 1 and the preparation of **4a** and **6a**.

Benzodiazepine Receptor Binding Activity of 6,9-Disubstituted Purines

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A series of 6,9-disubstituted purines were tested for their ability to bind to the benzodiazepine receptor in rat brain tissue. One of the most active compounds was 9-(3-aminobenzyl)-6-(dimethylamino)-9*H*-purine (**44**) with an IC_{50} = 0.9 μM, which was only 4.5-fold higher than the IC_{50} for chlordiazepoxide. Substitution of a 3-aminobenzyl or 3-hydroxybenzyl group at the 9-position of 6-(dimethylamino)purine led to over a 50-fold increase in receptor affinity. Compound **44** did not exhibit significant anxiolytic activity, nor did anticonvulsant activity correlate with relative receptor binding affinity.

The benzodiazepines (BZs) are a class of centrally acting drugs with broad therapeutic application as anxiolytics, hypnotics, muscle relaxants, and anticonvulsants.[1] High-affinity binding sites or receptors for BZs have been

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(1) Hollister, L. E. *Pharmacology of Benzodiazepines*; Usdin, E., et al., Eds.; Verlag Chemie: Weinheim, 1983; pp 29–35.

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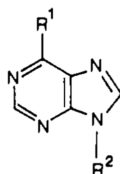
Table I. Physical Properties of 6,9-Disubstituted Purines



no.	R^1	R^2	method[a]	yield, %	mp, °C	formula[b]
6	$N(CH_3)_2$	$(CH_2)_3C_6H_5$	A[c]	83[d]	70–72	$C_{16}H_{19}N_5$
9	$N(CH_3)_2$	$CH(C_6H_5)_2$	A,[e] B	43[f]	139–141	$C_{20}H_{19}N_5$
10	$N(CH_3)_2$	$CH(CH_2)_2C_6H_4$[g]	A, B	61[h]	122–124	$C_{16}H_{17}N_5$
11	$N(CH_3)_2$	$CH(CH_2)_3C_6H_4$[i]	A,[e] B	24[j]	82–84	$C_{17}H_{19}N_5$
15	$NHCH_2CH_3$	$CH_2C_6H_5$	A	89[k]	111–113	$C_{14}H_{15}N_5$
16	$N(CH_2CH_3)_2$	$CH_2C_6H_5$	A	63[l]	60–62[m]	$C_{16}H_{19}N_5$
18	$N(CH_2)_4$	$CH_2C_6H_5$	A	79[k]	129–130.5	$C_{16}H_{17}N_5$
43	$N(CH_3)_2$	$CH_2C_6H_4CH_2OH$-3	A[n]	75[f]	144–145	$C_{15}H_{17}N_5O$

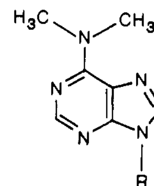
[a] A: see method E in reference 13. B: see method D in reference 13. [b] Analyzed for C, H, N. [c] For the starting 6-chloropurine, see: Schaeffer, H. J.; Odin, E.; Bittner, S. *J. Pharm. Sci.* 1971, *60*, 1184. [d] Recrystallized from toluene–petroleum ether (30–60 °C). [e] The crude product was purified by flash chromatography as in method B of reference 13. [f] Recrystallized from toluene. [g] 1-Indanyl substitutent. [h] Recrystallized from EtOH. [i] 1-(1,2,3,4-Tetrahydronaphthyl) substituent. [j] Recrystallized from pentane. [k] Recrystallized from heptane. [l] Recrystallized from petroleum ether (30–60 °C). [m] mp 63–64 °C, reported by: Itaya, T.; Ogawa, K. *Heterocycles* 1977, *6*, 965. [n] For the starting 6-chloropurine, see: Schaeffer, H. J.; Johnson, R. N.; Odin, E.; Hansch, C. *J. Med. Chem.* 1970, *13*, 452.

identified in the central nervous system.[2,3] There is a high correlation between the affinity of various BZs for this receptor and their pharmacological potencies.[3,4] 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] Various compounds have been suggested as possible endogenous ligands for the BZR.[7] The purines inosine and hypoxanthine were proposed as possible endogenous ligands,[8,9] although their affinity for the receptor is very weak. Several papers describe structure–activity studies on the interaction of purine compounds with the BZR.[10–12] As part of a program to discover novel compounds with specific binding activity for the BZR, we tested several purines for their ability to displace [^{3}H]-diazepam from high-affinity binding sites in rat brain tissue. The most active compounds were 9-benzylpurines; the structure–activity relationships for binding to the BZR of a series of 6,9-disubstituted purines are described here.

Chemistry

Most of the compounds in Table I were prepared by previously reported general methods. The 6-chloropurine precursors to **9–11** were available in high yield from 5-amino-4,6-dichloropyrimidine and the appropriate amine. The phenolic hydroxyl in **45**[13] was acylated with the aid of 4-(dimethylamino)pyridine to give **46** and **47**. The acetamide **55** was prepared from **56**[13] and acetic anhydride.

Table II. Benzodiazepine Receptor Binding Activity of 9-Substituted 6-(Dimethylamino)-9*H*-purines



no.	R	IC_{50}, μM[a]
1[b]	$CH_2C_6H_5$	13
2[b]	CH_3	(0)
3[c]	H	(57)
4[b]	C_6H_5	(0)
5[b]	$CH_2CH_2C_6H_5$	(0)
6	$CH_2CH_2CH_2C_6H_5$	(0)
7[b]	$CH(CH_3)C_6H_5$-(*R*)	(56)
8[b]	$CH(CH_3)C_6H_5$-(*S*)	2.1
9	$CH(C_6H_5)_2$	17
10	$CH(CH_2)_2C_6H_4$[d]	(48)
11	$CH(CH_2)_3C_6H_4$[e]	22

[a] Concentration of compound that decreased specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors by 50%. Values in parentheses are percent inhibition of [^{3}H]diazepam binding by 100 μM compound. [b] Synthesis: see reference 13. [c] Synthesis: Elion, G. B.; Burgi, E.; Hitchings, G. H. *J. Am. Chem. Soc.* 1952, *74*, 411. [d] 1-Indanyl substituent. [e] 1-(1,2,3,4-Tetrahydronaphthyl) substituent.

Biological Results and Discussion

The compounds in Table II–IV were tested for activity in the BZR binding assay. The percent inhibition values in parentheses represent inhibition of specific binding of 1.5 nM [^{3}H]diazepam to rat brain receptors by 100 μM compound. An IC_{50} value was usually determined if the percent inhibition was greater than 75%. Increased inhibition of [^{3}H]diazepam binding was assumed to reflect increased affinity for the receptor by a compound.

Structure requirements for good binding by 9-substituted purines were highly specific (Table II). The parent 9-benzylpurine **1** binds to the BZR with an IC_{50} = 13 μM.

(2) Squires, R. F.; Braestrup, C. *Nature (London)* 1977, *266*, 732.
(3) Möhler, H.; Okada, T. *Science* 1977, *198*, 849.
(4) Braestrup, C.; Squires, R. F. *Eur. J. Pharmacol.* 1978, *48*, 263.
(5) Haefely, W.; Kyburz, E.; Gerecke, M.; Möhler, H. In *Advances in Drug Research*; Testa, B., Ed.; Academic Press: London, 1985; pp 165–299.
(6) Petrack, B.; Yokoyama, N. *Annu. Rep. Med. Chem.* 1985, *20*, 1.
(7) Marangos, P. J.; Paul, S. M.; Goodwin, F. K.; Skolnick, P. *Life Sci.* 1979, *25*, 1093.
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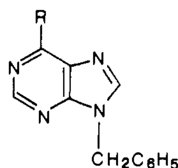
(10) Davies, L. P.; Cook, A. F.; Poonian, M.; Taylor, K. M. *Life Sci.* 1980, *26*, 1089.
(11) Grozinger, K.; Freter, K. R.; Farina, P.; Gladczuk, A. *Eur. J. Med. Chem.* 1983, *18*, 221.
(12) Sung, S.-C.; Saneyoshi, M. *Biochem. Pharmacol.* 1984, *33*, 1737.
(13) Kelley, J. L.; Krochmal, M. P.; Linn, J. A.; McLean, E. W.; Soroko, F. E. *J. Med. Chem.* 1988, *31*, 606.

Table III. Benzodiazepine Receptor Binding Activity of 6-Substituted 9-Benzyl-9*H*-purines



no.	R	IC_{50}, μM[a]
1[b]	$N(CH_3)_2$	13
12[b]	$NHCH_3$	24
13[b]	NH_2	37
14[b]	$N(CH_3)CH_2CH_3$	19
15	$NHCH_2CH_3$	19
16	$N(CH_2CH_3)_2$	(83)
17[b]	$N(CH_2)_3$	15
18	$N(CH_2)_4$	16
19[b]	$N(CH_3)$-cyclopentyl	14
20[b]	$NHCH_2CH_2OH$	(73)
21[b]	NH-cyclopropyl	(68)
22[b]	OCH_3	14
23[c]	oxo (1-CH_3)	15.5
24[b]	OH (oxo)	19
25[b]	SCH_3	3.3
26[b]	Cl	(40)
27[b]	H	(34)

[a] See Table II. [b] Synthesis: see reference 13. [c] Synthesis: Townsend, L. B.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 990.

Removal of the benzene ring, as in **2**, resulted in complete loss of receptor affinity at 100 μM. However, the 9-unsubstituted purine **3** had weak affinity, causing 57% inhibition at 100 μM. This level of activity of **3** is comparable to that reported by Davies et al.[10] for 6-(dimethylamino)purine (**3**). Substitution of a 9-phenyl group on **3** was poorly tolerated (see **4**), and replacement of the one-carbon bridge with a two- (**5**) or three- (**6**) carbon bridge resulted in compounds with no measurable affinity at 100 μM. The benzyl substituent gives optimum binding with simple 9-substituted 6-(dimethylamino)purines.

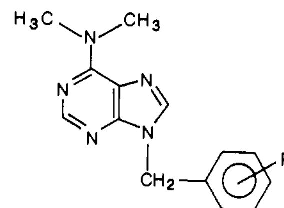
Substitution on the benzylic methylene of **1** resulted in a striking contrast in receptor binding. Introduction of a methyl in the *R* configuration (see **7**) led to a 7-fold loss in binding, whereas a methyl in the *S* configuration (see **8**) gave a 6-fold increase in receptor affinity. The BZR exhibits a high degree of stereochemical specificity for a methyl group on the benzylic methylene of **1**.

Introduction of a phenyl substituent (see **9**) on the benzylic methylene resulted in no significant loss in binding, which suggests the presence of an area of bulk tolerance. Restriction of the conformational freedom of the phenyl group, as in **10** and **11**, did not result in improved affinity for the BZR.

The effect of various 6-substituents on BZR binding affinity can be gleaned from Table III. There was little significant difference in IC_{50}'s for receptor binding among 6-amino substituents ranging in size from amino (**13**) to cyclopentylmethylamino (**19**). The 6-methoxy- (**22**) and 6-oxopurines **23** and **24** had binding affinity comparable to **1**. The 6-(methylthio) analogue **25** was more active with an IC_{50} = 3.3 μM; however, substitution of chloro (**26**) or hydrogen (**27**) for the 6-(dimethylamino) group of **1** gave compounds with IC_{50}'s >100 μM.

Analogues of **1** that contain substitutions in the phenyl moiety are listed in Table IV. Introduction of an ortho substituent resulted in little or no significant change in affinity for the receptor (see **28–33**). Substitution in the para position of the phenyl ring in **1** with 10 different substituents resulted in a significant loss in receptor binding (see **48–57**). There is little tolerance in the receptor site for substituents in the para position of **1**.

Table IV. Benzodiazepine Receptor Binding Activity of 6-(Dimethylamino)-9-benzyl-9*H*-purines Substituted in the Phenyl Moiety



compd	R	IC_{50}, μM[a]
1[b]	H	13
28[b]	2-F	5.4
29[b]	2-CH_3	(70)
30[b]	2-Cl	13
31[b]	2-OCH_3	20
32[b]	2-NO_2	(48)[c]
33[b]	2-NH_2	(19)[c]
34[b]	3-F	7.3
35[b]	3-CH_3	(62)
36[b]	3-Cl	(59)
37[b]	3-Br	(63)
38[b]	3-CF_3	(49)
39[b]	3-I	(64)
40[b]	3-OCH_3	(70)
41[b]	3-NO_2	(48)
42[b]	3-CN	(70)
43	3-CH_2OH	5
44[d]	3-NH_2	0.9
45[b]	3-OH	1.2
46	3-$OCOC(CH_3)_3$	1.6
47	3-$OCOCH_3$	0.44
48[b]	4-F	(23)
49[b]	4-CH_3	(22)
50[b]	4-Cl	(24)
51[b]	4-OCH_3	(0)
52[b]	4-$OCH_2C_6H_5$	(11)
53[b]	4-NO_2	(23)
54[b]	4-CN	(26)
55	4-$NHCOCH_3$	(22)
56[b]	4-NH_2	(67)
57[b]	4-OH	(15)[c]
chlordiazepoxide		0.2
diazepam		0.006

[a] See Table II. [b] Synthesis: see reference 13. [c] Percent inhibition at 10^{-5} M due to limited compound solubility. [d] Synthesis: see Kelley, J. L.; Miller, C. A.; Selway, J. W. T.; Schaeffer, H. J. *Eur. J. Med. Chem.* **1988**, *23*, 319.

A variety of substitutions in the meta position of **1** resulted in some loss in binding except with the small fluoro (**34**) and polar CH_2OH (**43**), NH_2 (**44**), and OH (**45**) substituents. The *m*-amino and *m*-hydroxy groups increase receptor binding affinity by 14- and 11-fold, respectively. The pivalate (**46**) and acetate (**47**) esters of **45** also had good affinity for the BZR.

Thus, substitution of a 3-aminobenzyl or 3-hydroxybenzyl group at the 9-position of 6-(dimethylamino)purine (**3**) led to over a 50-fold increase in BZR binding affinity. Compound **44** had an IC_{50} only 4.5-fold higher than chlordiazepoxide and thus represents a significant and novel structural lead for further BZR binding studies.

Fifteen of the most active BZR binding purines were tested for activity on a modified Geller–Seifter conflict schedule.[14,15] In a typical group of rats used for evaluating the effect of the purines, chlordiazepoxide (CDP) produced significant dose-related increases in responding during the

(14) Geller, I.; Seifter, J. *Psychopharmacologia* **1960**, *1*, 482.
(15) Pollard, G. T.; Howard, J. L. *Psychopharmacology* **1979**, *62*, 117.

Table V. Effect of Selected Purines on Conflict Responding in Long-Evans Rats[a]

compd	% change in conflict responding[b]	compd	% change in conflict responding[b]
1	−17 ± 44	**25**	+13 ± 7
8	+5 ± 7	**28**	+17 ± 10
12	−3 ± 6	**43**	+4 ± 10
17	−7 ± 9	**44**	+4 ± 9
18	+10 ± 7	**45**	+2 ± 10
19	−10 ± 13	**46**	+6 ± 11
22	+26 ± 18	**47**	+2 ± 9
23	−16 ± 23	chlordiazepoxide[c]	+67 ± 10

[a] Compounds were tested as described under Experimental Section on a modified Geller–Seifter conflict schedule. [b] Compounds were administered by oral gavage in a 0.5% methylcellulose suspension at 25 mg/kg. [c] Chlordiazepoxide was administered at 20 mg/kg.

conflict portion of the operant schedule (5 mg/kg po, +24 ± 5; 10 mg/kg po, +46 ± 4; 20 mg/kg po, +67 ± 10). None of the purines tested at 25 mg/kg po produced any significant change in conflict responding (see Table V). No activity was seen over a wider dose range by the oral route (12.5–50 mg/kg) for compounds **1**, **12**, and **47** or by the intraperitoneal route up to 80 mg/kg for compounds **8** and **44**. Administration of compound **44** prior to administration of CDP did not alter the increase in conflict responding produced by CDP.

Most of these compounds were also tested for anticonvulsant activity against maximal electroshock-induced seizures (MES) in rats.[13] Although several compounds are potent anticonvulsant agents, there is no positive correlation between BZR affinity and anti-MES activity. For example, the ranked order of affinity for the BZR is **44** (3'-NH_2) = **45** (3'-OH) > **25** (6-SCH_3) > **28** (2'-F) > **1** (H) > **36** (3'-Cl). The order of ip anticonvulsant potency is **36** (3'-Cl) ≈ **1** (H) > **28** (2'-F) > **25** (6-SCH_3) > **44** (3'-NH_2) ≈ **45** (3'-OH).[13]

We have discovered several 9-benzylpurines that possess good affinity for the BZR. In vivo, however, these compounds did not produce increased responding in the Geller–Seifter conflict test, which is expected for BZ agonists.[5,6] In more limited testing, they did not produce the effects expected for BZ antagonists or inverse agonists, either.[16,17] The most plausible explanation for this lack of in vivo activity, considering their binding potency, is that these compounds do not reach central BZRs in sufficient concentration to produce a behavioral effect. Many factors—such as lack of absorption, rapid metabolism, or lack of penetration into the CNS—could limit concentrations at central BZRs. The lack of correlation between BZR affinity and in vivo anti-MES activity[13,18] could be accounted for by these same factors. Alternatively, it is possible that these compounds bind to a subtype of BZR[19] that is not involved in conflict behavior.

Since minor structural changes can alter the agonist, antagonist, and inverse agonist nature of BZR binding,[5,6,17,20] as well as the distribution and metabolism of compounds,[21] the study of analogoues of **44** and **45** may lead to agents with different binding properties and in vivo activity. These studies are ongoing, and results will be reported later.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and are uncorrected. NMR 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. UV 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. TLC's were developed on Whatman 200-μm MK6F plates of silica gel with fluorescent indicator. Preparative flash chromatography[22] was performed on silica gel 60 (40–63 μm, E. Merck No. 9385). The analytical samples gave combustion values for C, H, and N within 0.4% of theoretical. Elemental analyses were performed by Atlantic Microlab, Inc.

(A) Chemistry. 6-(Dimethylamino)-9-[3-(pivaloyloxy)-benzyl]-9*H*-purine (46). A mixture of **45**[13] (4.00 g, 14.9 mmol), pivalic anhydride (3.60 g, 19.4 mmol), 4-(dimethylamino)pyridine (2.13 g, 17.4 mmol), and dichloromethane (30 mL) was stirred under a nitrogen atmosphere for 18 h. The solution was diluted with dichloromethane (100 mL), washed with 5% aqueous sodium bicarbonate until neutral, dried ($MgSO_4$), and spin evaporated in vacuo. The residual oil was added to a column of silica gel 60, which was wetted with hexanes and eluted with a mixture of ethyl acetate and hexanes (1:1) by flash chromatography. The fractions containing **46** were combined and concentrated by spin evaporation in vacuo. Recrystallization from hexanes gave 4.80 g (87%) of **46**, mp 80–81 °C. Anal. ($C_{19}H_{23}N_5O_2$) C, H, N.

9-(3-Acetoxybenzyl)-6-(dimethylamino)-9*H*-purine (47). A mixture of **45**[13] (43.7 g, 13.8 mmol), acetic anhydride (1.8 g, 18.0 mmol), 4-(dimethylamino)pyridine (2.02 g, 16.6 mmol), and dichloromethane (30 mL) was stirred under a nitrogen atmosphere for 18 h. The solution was diluted with dichloromethane (100 mL), washed with 5% aqueous sodium bicarbonate until neutral, dried ($MgSO_4$), and spin evaporated in vacuo. The residue was recrystallized from toluene–hexanes to give 3.6 g (84%) of **47**, mp 126–127 °C. Anal. ($C_{16}H_{17}N_5O_2$) C, H, N.

9-(4-Acetamidobenzyl)-6-(dimethylamino)-9*H*-purine (55). A solution of **56**[13] (5.00 g, 18.6 mmol), acetic acid (50 mL), and acetic anhydride (50 mL) was stirred at ambient temperature for 20 h. The volatiles were removed by spin evaporation. The white residue was dissolved in hot ethanol (800 mL), filtered, and cooled to give 4.40 g (76%) of white crystals. Concentration of the filtrates gave a second crop. The solids were combined and recrystallized from ethanol (neutral norite) to give 4.64 g (80%) of **55**, mp 259–261 °C. Anal. ($C_{16}H_{18}N_6O$) C, H, N.

5-Amino-4-(benzhydrylamino)-6-chloropyrimidine (58). This compound was prepared from 5-amino-4,6-dichloropyrimidine and aminodiphenylmethane on a 60-mmol scale by method A of reference 13. The crude product was purified by flash chromatography as in method B of reference 13 to give 18.1 g (95%) of **58** that was a single spot on TLC. Recrystallization of a sample from toluene gave the analytical sample, mp 157–159 °C. Anal. ($C_{17}H_{15}ClN_4$) C, H, N.

5-Amino-4-chloro-6-(1-indanyl)pyrimidine (59). This compound was prepared from 5-amino-4,6-dichloropyrimidine and 1-aminoindane by method A of reference 13 with the following modification. After evaporation of the solvent, the crude product was dissolved in dichloromethane and added to silica gel 60 (10 g) and spin evaporated in vacuo. The residual solids were introduced onto a column of silica gel 60 that had been wetted with hexanes. The column was eluted with hexanes (1 L), ethyl acetate–hexanes (1:2) (1 L), ethyl acetate–hexanes (2:1) (1 L), and finally with ethyl acetate (1 L) by flash chromatography. The fractions containing **59** were combined and spin evaporated in vacuo. The residue was recrystallized from ethanol to give 3.3 g (35%) of **59**, mp 187–188 °C. Anal. ($C_{13}H_{12}N_3Cl$) C, H, N.

5-Amino-4-chloro-6-(1,2,3,4-tetrahydro-1-naphthyl)pyrimidine (60). This compound was prepared from 5-amino-4,6-

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dichloropyrimidine and 1,2,3,4-tetrahydro-1-naphthylamine hydrochloride by the method of preparation of **59** with ethyl acetate-hexanes (1:1) as the flash chromatography elution solvent to give 1.46 g (20%) of **60**, mp 221-222 °C. Anal. ($C_{14}H_{14}N_3Cl$) C, H, N.

(B) **Benzodiazepine Binding Assay.** Male Sprague-Dawley rats weighing between 110 and 220 g were decapitated, and the brains were rapidly removed and chilled in ice-cold 0.9% NaCl. The cerebellum and pons medulla were removed, and the rest of the brain was homogenized in 20 volumes of 0.32 M sucrose in a Potter-Elvehjem homogenizer fitted with a Teflon pestle with a clearance of 0.25 mm. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was decanted and centrifuged at 30000*g* for 15 min. The pellet was frozen at a -60 °C for later use.

The frozen pellet was resuspended at the time of use in 20 volumes of Tris-HCl buffer, pH 7.5. A 0.5-mL aliquot of this membrane suspension was incubated with 1.5×10^{-9} M [^{3}H]-diazepam and enough Tris-HCl buffer (pH 7.5) to give a final volume of 2.0 mL for 30 min at 4 °C. Ten milliliters of ice-cold buffer was added to each tube, and the samples were filtered through Whatman GF/C glass filters. The filters were washed with an additional 10 mL of the ice-cold buffer, removed, placed in scintillation vials together with 10.0 mL of Bray's solution, and counted in a Packard Tri-Carb liquid scintillation counter, Model 3320.

Nonspecific [^{3}H]diazepam binding was determined from parallel samples containing 3×10^{-6} M cold diazepam. Specific binding is defined as the difference between total and nonspecific binding and was ~90% of the total binding. This procedure is a modification of the techniques of Braestrup and Squires.[2]

(C) **Pharmacology.** Ovariectomized Long-Evans (Charles River) rats, deprived of food 22 h/day, worked for food pellets in daily 1-h sessions on a modified Geller-Seifter conflict schedule.[14,15] The operant chambers (Coulbourn Instruments) were located in sound-attenuating enclosures and were equipped with a lever manipulandum, a cue light above the lever, a house light, a pellet dispenser delivering 45-mg pellets (BioServ, Inc.) to a lighted feeder bin, and a water spout. Response-contingent footshock was delivered to a grid floor from a Coulbourn shock generator during the conflict portion of the schedule. Environmental control and data acquisition were performed by a Data General Nova 3/12 minicomputer via an Interact interface (BRS/LVE).

Food reinforcement was delivered on a mult VI 2-min/CRF (food + shock) schedule consisting of four 12-min periods of variable interval reinforcement during which a lever press produced a food pellet every 2 min on the average, alternating with four 3-min periods of continuous reinforcement, signaled by a cue light, during which every lever press produced a pellet and a footshock. Shock level was 0.00 mA for the first response in each conflict period and was increased by 0.05 mA for each successive response in the period.

In general, compounds were administered by oral gavage (po) in a 0.5% methylcellulose suspension 60 min prior to the operant session. Several compounds were also tested following intraperitoneal administration. Results on days of drug administration are expressed as a percent change in conflict responses from the preceding day and represent the mean and SEM of at least four rats.

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Synthesis and Structure-Activity Relationship of Substituted Tetrahydro- and Hexahydro-1,2-benzisothiazol-3-one 1,1-Dioxides and Thiadiazinones: Potential Anxiolytic Agents

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Several novel substituted tetrahydro- and hexahydro-1,2-benzisothiazol-3-one 1,1-dioxides and thiadiazinones were prepared and examined in a series of in vitro and in vivo tests to determine their pharmacological profile. Most compounds were orally active in blocking the conditioned avoidance response (CAR) but did not antagonize apomorphine-induced stereotyped behavior. Several compounds demonstrated moderate to high affinity for the 5-HT_{1A} receptor binding site, with compounds **37** and **38** containing 2-pyrimidinylpiperazinyl and [3-(trifluoromethyl)phenyl]piperazinyl moieties and compound **47** containing the 2-pyrazinylpiperazinyl moiety displaying the highest affinity (K_i values of 10, 4, and 9 nM, respectively). Compound **37**, 3-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]hexahydro-4,7-etheno-1*H*-cyclobut[*f*]-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, buspirone, and ipsapirone showed similarities in their neurochemical and behavioral profiles. They were similar in potency in blocking CAR with AB_{50} values of 39, 32, and 42 mg/kg, respectively. They also demonstrated high affinity and selectivity for the 5-HT_{1A} receptor site (K_i = 10 nM) and exhibited partial agonist/antagonist activity in the serotonin syndrome test. In addition, compound **37** inhibited apomorphine-induced climbing behavior much more potently (ED_{50} of 3.4 mg/kg) than stereotyped behavior (ED_{50} of 32.2 mg/kg) and will be evaluated further. Structure-activity relationships within this series of compounds are discussed.

The discovery of buspirone as a non-benzodiazepine anxiolytic agent revolutionized the drug therapy of anxiety and has led to the synthesis of several compounds possessing high 5-HT_{1A} affinity, many of which are under development as anxiolytic agents.[1,2] In addition to treatment of anxiety, 5-HT_{1A} partial agonists such as gepirone (**1**) are now being examined for their mixed activity as anxiolytic-antidepressant agents.[3] The therapeutic potential of the 5-HT_{1A} agonists in the treatment of multi-CNS disorders was recently extended to the development of compounds that may have antipsychotic and

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