resulting deep red solution was added 2.09 g (9 mmol) of the aldehyde 24 in 20 mL of Me$_2$SO. The mixture was stirred at room temperature for 16 h and heated to 55–60 °C for 1 h. The reaction mixture was diluted with 30 mL of H$_2$O and extracted with pentane (5 × 50 mL). The pentane extract was washed wth 1:1 H$_2$O/Me$_2$SO (2 × 100 mL) and brine (3 × 100 mL), dried (Na$_2$SO$_4$), and concentrated. The crude product was filtered through 10 g of silica gel with 200 mL of 3:1 pentane/EtOAc and concentrated to give 3.3 g of a pale yellow oil (93% crude yield).

LC (Radialpak B, 1% Et$_2$O/hexane, 2.0 mL/min, 285 nm) revealed two major products, t_R 10.2 (60%, isomer 26) and 10.9 min (40%, isomer 25). The crude product (3.3 g) was subjected to preparative LC (0.3% Et$_2$O/hexane) using the recycle technique. At each cycle, fractions containing 26 of greater than 90% isomeric purity were collected and the rest recycled. With three successive recycles, it was possible to obtain 0.9 g of 26 (90% isomeric purity), 0.87 g of 25 (85% isomeric purity), and 0.59 g of an approximately 1:1 mixture of both isomers. A second injection with 1.4 g of another batch of similarly prepared crude product gave 0.66 g of 25 (>90% isomeric purity) and 0.6 g of 26 (>90% isomeric purity). A 1.53-g portion of the mixture containing 90% isomer 25 was subjected to LC with three recycles, to give 0.8 g of 25 (98% isomeric purity) as a pale yellow oil. This oil was repurified by LC (0.4% Et$_2$O/hexane, three recycles) to give 0.56 g of 25 (99.9% isomeric purity by analytical LC). A final purification of this product on LC (2% EtOAc/hexane) gave 0.52 g of isomerically pure 25, followed by about 3% of some oxidation products by LC: analytical LC (Radialpak B, 1% Et$_2$O/hexane, 2.0 mL/min, 285 nm) t_R 10.9 min; IR (film) 2950, 1600, 1490, 1450, 1360, 1235, 1205, 1120, 1038, 960, 920, 870, 750 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.05 (s, 6, C$_R$-16, C$_R$-17 CH$_3$), 1.4–1.8 and 1.85–2.2 [2 m, 12, C$_R$-2, C$_R$-3, C$_R$-4 CH$_2$ and (CH$_2$)$_3$CHO], 1.74 (s, 3, C-18 CH$_3$), 2.04 (s, 3, C$_R$-19 CH$_3$), 3.4–4.1 (m, 2, CH$_2$O), 5.45 (br s, 1, OCHO), 6.21 (s, 2, C$_R$-7, C$_R$-8 HC=CH), 6.27 (d, J = 11 Hz, 1, C$_R$-10 C=CH), 6.8–7.6 (m, 6, C$_R$-11, C$_R$-12, HC=CH, and ArH). MS calcd for C$_{27}$H$_{36}$O$_2$, 392.2715; found, 392.2720.

A 0.52-g sample of isomer 26 (99.9% isomerically pure) was obtained as a pale yellow oil from repeated runs on LC (0.3% Et$_2$O/hexane): analytical LC (Radialpak B, 1% Et$_2$O/hexane, 2.0 mL/min, 285 nm) t_R 10.2 min; IR (film) 2940, 1610, 1495, 1465, 1380, 1365, 1240, 1205, 1180, 1120, 1040, 965, 920, 875, 745 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.07 (s, 6, C$_R$-16, C$_R$-17 CH$_3$), 1.4–1.7 and 1.8–2.1 [2 m, 12, C$_R$-2, C$_R$-3, C$_R$-4 CH$_2$ and (CH$_2$)$_3$CHO], 1.77 (s, 3, C$_R$-18 CH$_3$), 2.02 (s, 3, C$_R$-19 CH$_3$), 3.4–4.1 (m, 2, CH$_2$O), 5.48 (br s, 1, OCHO), 6.18 (d, J = 11 Hz, 1, C$_R$-10 C=CH), 6.21 (d, J = 16 Hz, 1, C$_R$-7 HC=CH), 6.68–7.53 (m, 7, C$_R$-8, C$_R$-11, C$_R$-12 HC=CH and ArH).

(*E*)-1-(2-Hydroxyphenyl)-4-methyl-6-(2,6,6-trimethyl-1-

cyclohexen-1-yl)-1,3,5-hexatriene (27). To 0.42 g (1.07 mmol) of 25 dissolved in 5 mL of EtOAc was added 20 mL of a 0.03 M solution of p-TsOH·H$_2$O in MeOH. The solution was stirred under argon for 1 h when TLC (1:1 hexane/ether) indicated that reaction was complete. The product was diluted with 10 mL of EtOAc and washed with 50 mL of H$_2$O, 40 mL of saturated NaHCO$_3$, and 40 mL of brine (twice), filtered through 10 g of Na$_2$SO$_4$, and concentrated to give 0.32 g of an oil (97%) which gradually crystallized to give a yellow solid: mp 121–123 °C dec; the product showed only one peak on both normal phase (Radialpak B, 4% Et$_2$O/hexane, 2 mL/min, 285 nm, t_R 13.4 min) and reverse phase (μBondapak/C$_{18}$, 10% H$_2$O/MeOH, 2 mL/min, 285 nm, t_R 6.9 min) LC; IR (mull) 3550 (OH), 1610, 1330, 1260, 1150, 1085, 980, 750 cm^{-1}; UV (EtOH) λ$_{max}$ 349 nm (ε 4.05 × 10^4), 240 (8.3 × 10^3). MS calcd for C$_{22}$H$_{28}$O, 308.2140; found, 308.2168.

(1*E*,3*Z*,5*E*)-1-(2-Hydroxyphenyl)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5-hexatriene (28). The 3(*Z*) isomer 28 was obtained as a light yellow solid from 0.42 g of the pure tetrahydropyranyl derivative 26 in 94% yield by the procedure used to convert 25 to 27: compound 28 showed one peak on both normal phase (Radialpak B, 4% Et$_2$O/hexane, 2 mL/min, 285 nm, t_R 13.4 min) and reverse phase (μBondapak/C$_{18}$, 15% H$_2$O/MeOH, 2 mL/min, t_R 8.0 min) LC; IR (mull) 3250 (OH), 1600, 1350, 1230, 1205, 1190, 1160, 1090, 970, 750 cm^{-1}; UV (EtOH) λ$_{max}$ 344 nm (ε 3.00 × 10^4), 250 (1.10 × 10^4). MS calcd for C$_{22}$H$_{28}$O, 308.2140; found, 308.2152.

ODC Assay. The ODC assay was performed as previously described[2b] using procedures reported by Raineri et al.[27] and O'Brien et al.[28]

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Synthesis and Anxiolytic Activity of 6-(Substituted-phenyl)-1,2,4-triazolo[4,3-b]pyridazines

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Received February 19, 1980

The synthesis of a series of 6-(substituted-phenyl)-1,2,4-triazolo[4,3-b]pyridazines (VIII) is reported. Some of these derivatives show activity in tests predictive of anxiolytic activity [(a) protection against pentylenetetrazole-induced convulsions; (b) thirsty rat conflict procedure]. They also represent a new class of compound which inhibits [^{3}H]diazepam binding. Structure–activity correlations, as well as the ability of structures VIII to inhibit [^{3}H]diazepam binding (in vitro), are discussed.

Intensive research in the benzodiazepine field has continued since the discovery and marketing of chlordiazepoxide and diazepam,[1] and this research has led to the discovery of many new derivatives with potent anxiolytic activity. However, very few new structures which are

unrelated to benzodiazepines but which display potent anxiolytic activity have been reported. Many derivatives of 5-phenyl-1,4-benzodiazepine with a ring fused at the 1,2 positions have been investigated.[2,3] 1,2,4-Triazolo[4,3-a][1,4]benzodiazepines,[2-4] 1,2,4-triazolo[5,1-a][2,4]benzo-

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diazepines,[5] imidazo[1,5-a][1,4]benzodiazepines,[6-8] imidazo[1,2-a][1,4]benzodiazepines,[9] 1,2,4-triazolo- and 1,2,5-triazino[4,3-d][1,4]benzodiazepinones,[10] 1,2,4-triazino-[4,3-a][1,4]benzodiazepines,[11] and pyrrolo[1,2-a][1,4]-benzodiazepines[12] have been synthesized in a search for superior drugs.

In laboratory animals, benzodiazepines produce a variety of activities such as sedative, muscle relaxant, anticonvulsant, and antiaggressive activity. Although different methods have been used to select compounds for clinical evaluation, the antagonism of convulsions induced by pentylenetetrazole in mice and rats has been used as a sensitive measure of anxiolytic activity[13] and correlates with the clinical activity of benzodiazepines. In 1977, Squires and Braestrup[14a] and Mohler and Okada[14b] reported on the stereospecific, high-affinity binding sites for benzodiazepines in brain tissues of rats. The presence of saturable, stereospecific, high-affinity diazepam-binding sites in the central nervous system has been demonstrated in vivo in rats[15] and in vitro in human brain tissue.[16] Excellent correlations between the IC_{50} values of various benzodiazepines for inhibiting [^{3}H]diazepam binding and the dose needed to antagonize pentylenetetrazole-induced convulsions have been reported.[16a,17] Studies to correlate specific pharmacological effects of anxiolytic drugs with specific receptor sites, as well as studies to determine whether an endogenous ligand[18] for the diazepam receptors is involved in mediating anxiety, should aid in finding drugs which are more selective in their pharmacological actions. We report on the pharmacological properties of

Scheme I
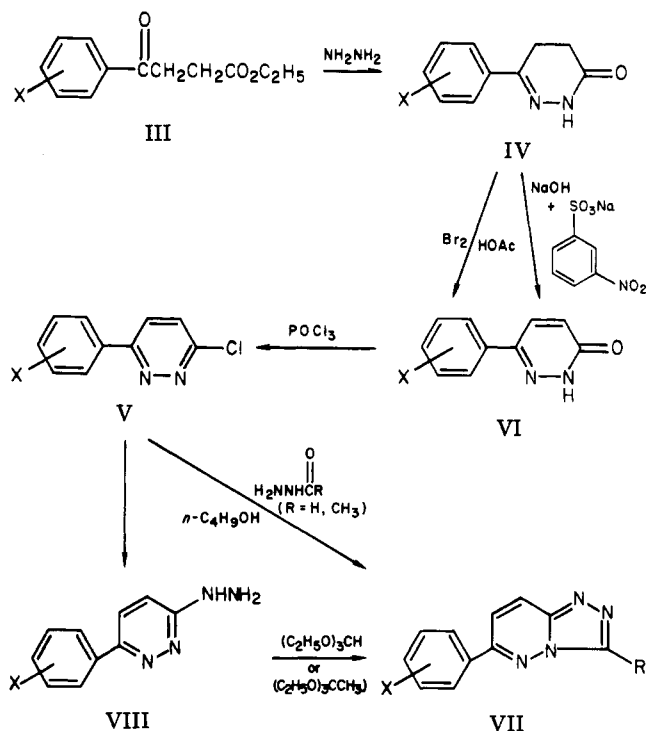
6-(substituted-phenyl)-1,2,4-triazolo[4,3-b]pyridazines, which represent a new class of compounds with activity in tests predictive of anxiolytic activity.[19] Some of these derivatives also exhibited hypotensive activity when tested in spontaneously hypertensive rats.

Chemistry. Interest in 6-aryl-1,2,4-triazolo[4,3-b]-pyridazines was stimulated by reports[20] that I is a me-
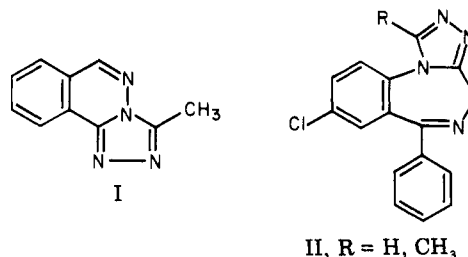
II, R = H, CH$_3$

tabolite of the hypotensive drug hydralazine, as well as reports of CNS activity in benzodiazepines II containing a fused 1,2,4-triazole ring.[2,3] A program directed toward the synthesis of 6-(substituted-phenyl)-1,2,4-triazolo[4,3-b]pyridazines as potential hypotensive and CNS agents was initiated in 1974. Our first objective was the development of a versatile synthetic method for the preparation of diverse ethyl 3-(substituted-benzoyl)propionates (III), which are key starting materials for the desired 1,2,4-triazolo[4,3-b]pyridazines (Scheme I). We have reported on the 1,4 addition of α-aryl-4-morpholineacetonitriles (derived from aryl aldehydes) to ethyl acrylate to synthesize ethyl 3-aroylpropionates III.[21] The reaction of 3-benzoylpropionates (III) with hydrazine to give 4,5-di-

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hydro-6-phenyl-3(2*H*)-pyridazinones (IV) and the preparation of diverse 6-(substituted-phenyl)-3-chloropyridazines has been reported.[21a]

In cases where oxidation of the 4,5-dihydropyridazinone with bromine in acetic acid gave bromination of the ring (such as IV; X = CH_3O), oxidation with sodium *m*-nitrobenzenesulfonate in the presence of sodium hydroxide gave pyridazinones VI in satisfactory yields. This oxidative method[22] has been used for the dehydrogenation of a variety of 6-aryl- and 6-heteroaryl-4,5-dihydro-3(2*H*)-pyridazinones.[23] Two methods were used to introduce the triazole ring. Reaction of 3-chloropyridazines V with acylhydrazines in refluxing 1-butanol gave triazolopyridazines VIII. Alternatively, reaction of V with hydrazine gave 3-hydrazino derivatives VII, which on reaction with ethyl orthoformate or ethyl orthoacetate gave the triazolopyridazines VIII. Through the sequence of reactions in Scheme I, over 100 derivatives of structure VIII were prepared. Representative compounds are listed in Table I.

Antihypertensive Activity. The derivatives in Table I were tested by a combined screening method for determining antihypertensive and diuretic activity.[24] Compounds which lowered the blood pressure of spontaneously hypertensive rats by at least 20 mmHg are listed in Table II. Except for the unsubstituted phenyl derivative 4, all the compounds showing antihypertensive activity have electron-withdrawing groups (Cl, F, CF_3, and CN) in the phenyl ring. In general, a methyl at the 3 position decreased or destroyed hypotensive activity. Derivatives in which a methyl was substituted at the C-7 or C-8 position (47, 48, 50, and 52) retained their activity.

One of the most active derivatives was the *p*-fluoro compound 10, which was tested in dogs (25 mg/kg) and showed reflex tachycardia and properties indicative of a peripheral vasodilator. None of the derivatives was as active as hydralazine, a potent peripheral vasodilator.

Anxiolytic Activity. Of special interest was the finding that a number of the 6-(substituted-phenyl)-1,2,4-triazolo[4,3-*b*]pyridazines showed potent anxiolytic activity and that hypotensive and anxiolytic activities did not parallel each other. Activities of these compounds in protection of rats against convulsions induced by pentylenetetrazole[13] (Table III) and in thirsty rat conflict procedure[25] were determined. The competitive inhibition of [³H]diazepam binding (in vitro) by 6-phenyl- and 6-(substituted phenyl)-1,2,4-triazolo[4,3-*b*]pyridazines is also listed (Table III). Of the compounds in Table III, approximately one-third exhibited ED_{50} values (antipentylenetetrazole) in the range of 1.5–10 mg/kg. The most active derivatives were the unsubstituted phenyl compound 2, *p*-fluoro (10 and 13), *m*-fluoro (14), and *m*-(trifluoromethyl) (24 and 25) analogues. In comparison of the activity of pairs of derivatives with a C-3 methyl group or a C-3 hydrogen atom and the same 6-aryl substituent, the C-3 methyl analogue was more active in antipentylenetetrazole and [³H]diazepam binding tests. Decreased activity or no activity results from introduction of ethyl, propyl, phenyl, benzyl, chloromethyl, or tri-
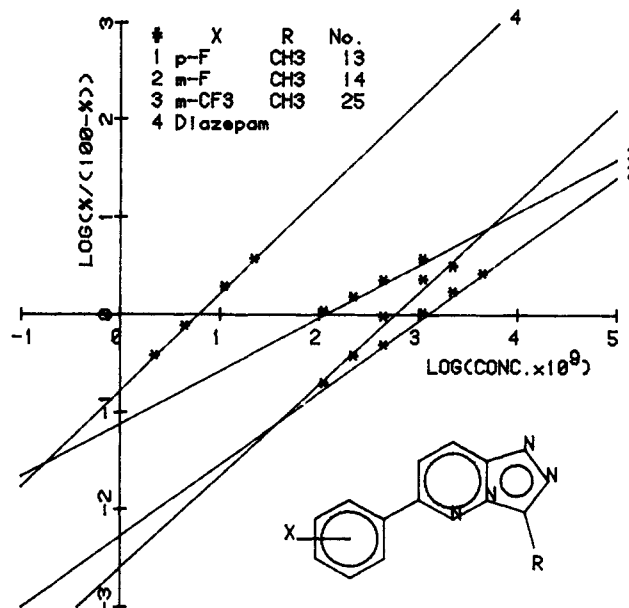

Figure 1. Dose–response curves. [³H]Diazepam inhibition (%) vs. concentration (μM). The slope for chlordiazepoxide hydrochloride is 0.94. Other compounds not plotted had the following slopes: **2** (0.70), X = H, R = CH_3; **20** (0.74), X = *m*-Cl, R = CH_3; **24** (0.53), X = *m*-CF_3, R = H; **32** (0.53), X = *m*-CF_3, R = CH_2Cl; **42** (0.78), X = *m*-NH_2, R = CH_3; **44** (0.84), X = *m*-$NHCOCH_3$, R = CH_3. The mean standard deviation of the slope is ±0.05.

fluoromethyl groups in the C-3 position. The compounds 8 and 33 with a 3-(methoxymethyl) group are active in antipentylenetetrazole tests, although both are poor in [³H]diazepam binding. Introduction of methyl groups at the C-7 and C-8 positions decreased or eliminated activity. Only the *m*-CF_3 analogues 46 and 47 with a methyl group at C-7 exhibited antipentylenetetrazole activity and very weak [³H]diazepam binding activity. [³H]Diazepam binding affinities (IC_{50} values) of the triazolopyridazines listed in Table III range from about 0.1 to 10 μM.

From the data in Table III it can be seen that the ED_{50} for protection of rats against seizures induced by pentylenetetrazole does not correlate consistently with potency in displacing [³H]diazepam from high-affinity receptors isolated from rat brain. Compounds 1, 8, 16, 17, and 33 are approximately equipotent in protection against seizures but are poor (<30%) as inhibitors of [³H]diazepam binding. Although compounds 10 and 33 are slightly more active than analogues 20 and 43 (antipentylenetetrazole), they are poor inhibitors (ca. 20%) of [³H]diazepam binding, while 20 and 43 inhibit binding by ca. 60%. A number of analogues (6, 9, 31, 32, and 44) are inactive (antipentylenetetrazole) but show greater than 50% inhibition of [³H]diazepam binding (in vitro). This latter result may reflect either lack of oral absorption or poor lipophilicity resulting in low or minimal concentrations in the brain.

In order to analyze the [³H]diazepam binding data of the various analogues and also to make a comparison with chlordiazepoxide hydrochloride, plots of dose–responses were generated by computer techniques. The first graph (Figure 1) plots logistically transformed diazepam binding data (inhibition vs. concentration) with straight lines obtained by weighted least squares for a representative selection of triazolopyridazines. These straight-line plots illustrate the variations in the slope of the dose–response. If no serious deviation from linearity appears, it is evident that a cross-over with diazepam could occur with compound 25 at low values of the inhibition. Apparent from these plots is the difference in slope of the dose–response

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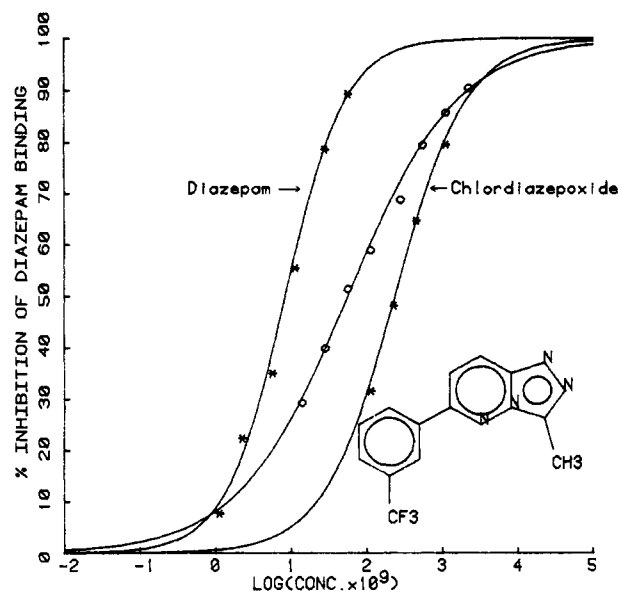(25) Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacologia* **1971**, *21*, 1.

Figure 2. [3H]Diazepam inhibition (%) vs. concentration (μM). Comparison of Diazepam and Chlordiazepoxide with compound **25**.

Figure 3. Correlation of oral antipentylenetetrazole activity with diazepam binding IC_{50} values.

Figure 4. Correlation of oral antipentylenetetrazole activity with diazepam binding activity (IC_2).

curves of **2, 13, 14, 20, 24, 25, 32, 42,** and **44**. Within this series of nine 6-phenyl-1,2,4-triazolo[4,3-b]pyridazines, the slope of the dose–response curve in [3H]diazepam binding varies from 1 to 0.53. Figure 1 illustrates that potencies based on comparison of IC_{50} values and IC_{25} values are equivalent only if the slopes of the dose–response curves are the same. The second graph (Figure 2) shows untransformed dose–response data for diazepam, chlordiazepoxide, and compound **25** and illustrates the greater potency of **25** compared with chlordiazepoxide. The graph (Figure 2) shows that, despite a much higher IC_{50} value (116 vs. 6 nm) at low concentrations (IC_{25}; 15 vs. 2 nm), compound **25** approaches the potency of diazepam in binding benzodiazepine receptors.

It has been demonstrated[26] that inhibition of pentylenetetrazole seizures of benzodiazepines is achieved at doses which displace only a small amount (5–30%) of [3H]flunitrazepam from its binding sites. Thus, only a small proportion of receptors need be occupied to block seizure activity (in vivo experiments[26a]). That only a small number of benzodiazepine receptors (ca. 15–20%) need be affected to obtain a significant anticonflict response also has been reported.[27]

The correlation of in vivo antipentylenetetrazole ED_{50} values with diazepam binding IC_{50} values is shown in the third graph (Figure 3). A correlation of the in vivo antipentylenetetrazole ED_{50} (mol/kg) with the diazepam inhibition (IC_n) as a function of n was determined by regressing a group of ED_{50} values against each group of IC_n values for n = 1–99. Such IC_n values can be calculated from the regression lines described above. The inhibitions actually measured in these experiments generally range from ca. 25 to ca. 90% of the total inhibition. Consequently, IC_n values for $n > 25$ and $n < 90$ represent interpolation of actual data, while IC_n values for $n < 25$ or $n > 90$ are extrapolations. Extrapolated values assume that the slope of the dose–response does not change at
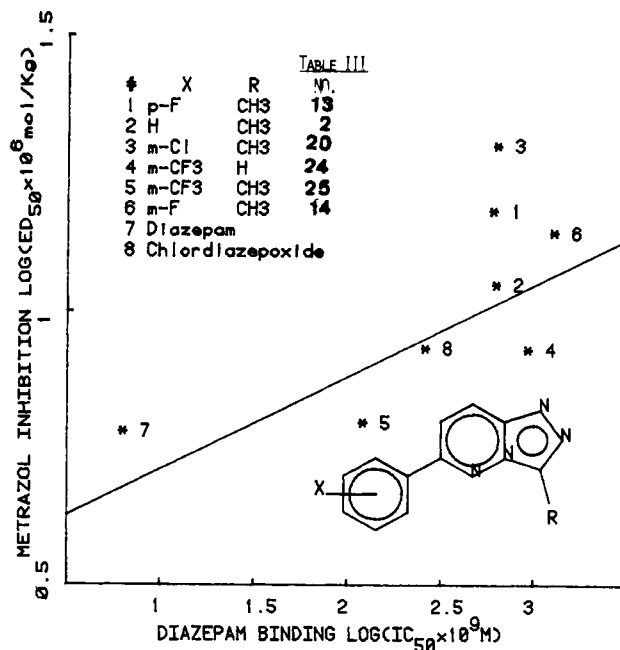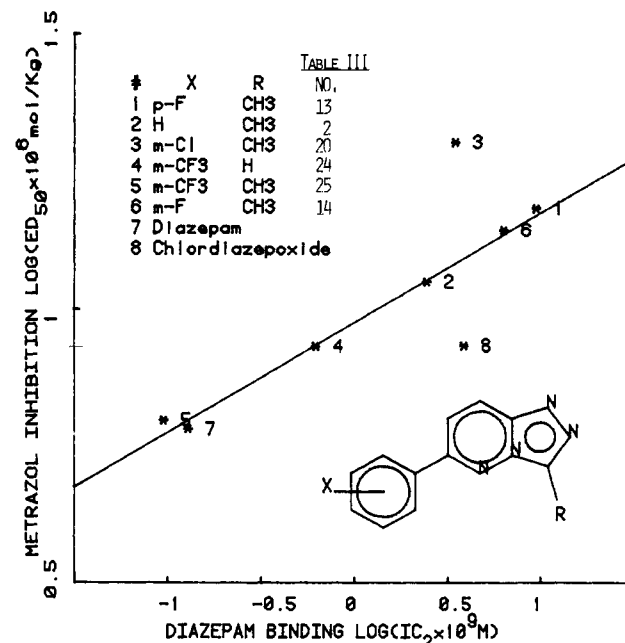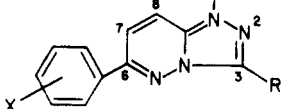
lower values of the inhibition. If one considers only the region of inhibitions actually measured, the best correlation, as determined by the correlation coefficient (r), of diazepam inhibitions and antipentylenetetrazole values is found using IC_{25} values. However, a better correlation occurs for the extrapolated IC_2 values (Figure 4) [r (IC_{50}) = 0.69; r (IC_{25}) = 0.79, r (IC_2) = 0.85]. These findings are consistent with the concept that only a small fraction (ca. 10–20%) of benzodiazepine receptors need be occupied for a pharmacological response.[26,27]

Selected compounds were screened in the thirsty rat conflict procedure, and compounds **1, 2, 8, 10, 13, 14, 16, 19, 20, 21, 24, 25, 28, 33,** and **42** showed activity when dosed at 25 to 200 mg/kg orally. Oral minimal effective doses were determined only for chlordiazepoxide (3.0 mg/kg), diazepam (1.5 mg/kg), and compound **25** (0.8 mg/kg). Overall the most active compound in all the tests was the

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Table I. 6-Aryl-1,2,4-triazolo[4,3-b]pyridazines

no.	X	R₁	yield, %	proce-dure	mp, °C	recrystn solvent	formula	anal.
1	H	H	83	A	138-139	C₂H₅OH	C₁₁H₈N₄	C, H, N
2	H	CH₃	76	A	191-194	C₂H₅OH	C₁₂H₁₀N₄	C, H, N
3	H	C₂H₅	61	A	133-135	C₂H₅OH	C₁₃H₁₂N₄	C, H, N
4	H	n-C₃H₇	70	A	123-125	C₂H₅OH	C₁₄H₁₄N₄	C, H, N
5	H	CF₃	40	B	183-184	C₂H₅OH	C₁₂H₇N₄F₃	C, H, N, F
6	H	C₆H₅	80	A	213-215	C₂H₅OH	C₁₇H₁₂N₄	C, H, N
7	H	CH₂C₆H₅	77	A	155-157	C₂H₅OH	C₁₈H₁₄N₄	C, H, N
8	H	CH₂OCH₃	90	C	133	CH₂Cl₂-hexane	C₁₃H₁₂N₄O	C, H, N
9	H	CH₂Cl	22	D	165-166	CH₂Cl₂-hexane	C₁₂H₉N₄Cl	C, H, N, Cl
10	p-F	H	31	A	197-198	CH₃OH	C₁₁H₇N₄F	C, H, N, F
11	m-F	H	22	A	159-161	CH₃OH	C₁₁H₇N₄F	C, H, N, F
12	o-F	H	21	A	161-163	CH₃OH	C₁₁H₇N₄F	C, H, N, F
13	p-F	CH₃	78	A	227-229	C₂H₅OH	C₁₂H₉N₄F	C, H, N, F
14	m-F	CH₃	61	A	184-186	CH₃OH	C₁₂H₉NF	C, H, N, F
15	o-F	CH₃	73	A	147-149	CH₂Cl₂-hexane	C₁₂H₉NF	C, H, N, F
16	p-Cl	H	61	A	216-218	C₂H₅OH	C₁₁H₇N₄Cl	C, H, N, Cl
17	m-Cl	H	50	A	175-177	C₂H₅OH	C₁₁H₇N₄Cl	C, H, N, Cl
18	o-Cl	H	60	A	156-158	CHCl₃-hexane	C₁₁H₇N₄Cl	C, H, N, Cl
19	p-Cl	CH₃	25	D	208-210	EtOH	C₁₂H₉N₄Cl	C, H, N
20	m-Cl	CH₃	56	A	171-172	CH₂Cl₂-hexane	C₁₂H₉N₄Cl	C, H, N, Cl
21	o-Cl	CH₃	60	A	146-148	CHCl₃-hexane	C₁₂H₉N₄Cl	C, H, N, Cl
22	p-Cl	C₂H₅	58	A	197-199	C₂H₅OH	C₁₃H₁₁N₄Cl	C, H, N, Cl
23	p-Cl	C₆H₅	72	A	254-256	C₂H₅OH	C₁₇H₁₁N₄Cl	C, H, N, Cl
24	m-CF₃	H	59	A	132-134	DMF-H₂O	C₁₂H₇N₄F₃	C, H, F; Nᵃ
25	m-CF₃	CH₃	78	A	193-194	CH₃OH	C₁₃H₉N₄F₃	C, H, N, F
26	m-CF₃	C₂H₅	54	A	183-185	CHCl₃-hexane	C₁₄H₁₁N₄F₃	C, H, N, F
27	m-CF₃	n-C₃H₉	44	A	117-118	DMF-H₂O	C₁₅H₁₃N₄F₃	C, H, N, F
28	p-CF₃	H	61	A	160-161	CH₂Cl₂-hexane	C₁₂H₇N₄F₃	C, H, N, F
29	p-CF₃	CH₃	58	A	199-201	CH₂Cl₂-hexane	C₁₃H₉N₄F₃	C, H, N, F
30	p-CF₃	C₂H₅	55	A	194-196	CHCl₃-hexane	C₁₄H₁₁N₄F₃	C, H, N, F
31	m-CF₃	CF₃	38	B	189	CHCl₃-hexane	C₁₃H₆N₄F₆	C, H, N, F
32	m-CF₃	CH₂Cl	26	D	180-182	CH₂Cl₂-hexane	C₁₃H₆N₄F₆	C, H, N, F
33	m-CF₃	CH₂OCH₃	88	C	149-151	CH₂Cl₂-hexane	C₁₄H₁₁N₄OF₃	C, H, N, F
34	m-OCH₃	H	92	A	124-125	CH₃OH	C₁₂H₁₀N₄O	C, H, N, F
35	m-OCH₃	CH₃	46	A	151-153	CH₃OH	C₁₃H₁₂N₄O	C, H, N
36	p-OCH₃	CH₃	51	A	147-148	CH₂Cl₂-hexane	C₁₃H₁₂N₄O	H, N; Cᵇ
37	m-CH₃	H	33	A	164-166	CHCl₃-hexane	C₁₂H₁₀N₄	H, N; Cᶜ
38	m-CH₃	CH₃	30	A	160-162	CH₂Cl₂-hexane	C₁₃H₁₂N₄	H, N; Cᵈ
39	m-CH₃	CF₃	5	B	176-178	CH₃OH	C₁₃H₉N₄F₃	C, H, N, F
40	m-NO₂	H	73	A	231-233	C₂H₅OH	C₁₁H₇N₅O₂	C, H, N
41	m-NO₂	CH₃	78	A	240-242	C₂H₅OH	C₁₂H₉N₅O₂	C, H, N
42	m-NH₂	H	92	E	226-228	C₂H₅OH	C₁₁H₉N₅	C, H, N
43	m-NH₂	CH₃	62	E	206-208	C₂H₅OH	C₁₂H₁₁N₅	C, H, N
44	m-NHCOCH₃	CH₃	75	F	245-247	C₂H₅OH-ether	C₁₄H₁₃N₅O	H, N; Cᵉ
45	m-CN	H (7-CH₃)	84	A	214-216	CH₃OH	C₁₃H₉N₅	C, H, N
46	m-CF₃	H (7-CH₃)	65	A	163-165	CH₂Cl₂-hexane	C₁₃H₉N₄F₃	C, H, N, F
47	m-CF₃	H (7-CH₃)	72	A	185-187	CH₂Cl₂-hexane	C₁₄H₁₁N₄F₃	C, H, N, F
48	m-CF₃	H (8-CH₃)	59	A	181-183	CH₂Cl₂-hexane	C₁₃H₉N₄F₃	C, H, N, F
49	m-CF₉	CH₃ (8-CH₃)	70	A	196-198	CH₂Cl₂-hexane	C₁₄H₁₁N₄F₃	C, H, N; Fᶠ
50	p-F	H (7-CH₃)	71	A	147-149	CH₃OH	C₁₂H₉N₄F	C, H, N, F
51	p-F	CH₃ (7-CH₃)	67	A	230-232	CH₃OH	C₁₃H₁₁N₄F	C, H, N, F
52	p-F	CH₃ (8-CH₃)	64	A	196-187	CH₃OH	C₁₃H₁₁N₄F	C, H, N, F
53	m-F	H (7-CH₃)	67	A	190-192	CH₃OH	C₁₂H₉N₄F	C, H, N, F
54	m-F	CH₃ (7-CH₃)	73	A	258-260	CH₃OH	C₁₃H₁₁N₄F	C, H, N, F
55	3,4-Cl₂	H	88	A	249-251	DMF-H₂O	C₁₁H₆N₄Cl₂	C, H, N, Cl
56	3,4-Cl₂	CH₃	50	A	267-269	DMF-H₂O	C₁₂H₈N₄Cl₂	C, H, N, Cl

ᵃ N; calcd, 21.21; found, 20.25. ᵇ C: calcd, 64.98; found, 64.34. ᶜ C: calcd, 68.55; found, 67.85. ᵈ C: calcd, 69.62; found, 68.60. ᵉ C: calcd, 62.91; found, 61.10. ᶠ F: calcd, 19.50; found, 20.08.

m-(trifluoromethyl)phenyl derivative **25** (CL 218 872),[28] which displays in vivo activity in the range of chlordiazepoxide and diazepam.

Compound **25** shows protection against seizures induced by pentylenetetrazole for more than 7 h (Table IV) and, thus, exhibits duration of activity. In tests to measure its ability to inhibit convulsions induced by bicuculline, isoniazid, and strychnine,[28] **25** is less potent than chloridaz-

epoxide and diazepam. These properties, along with lower potency than diazepam in tests to measure muscular incoordination, suggest that derivative **25** may be more se-

(28) For a discussion of the pharmacological properties of compound **25** (CL 218 872), see Lippa, A. S.; Coupet J.; Greenblatt, E. N.; Klepner, C. A.; Beer, B. *Pharmacol. Biochem. Behav.* **1979**, *11*, 99.

Table II. Hypotensive Activity[a]

compd	X	R	MABP[b] 100 mg/kg po	MABP[b] 25 mg/kg po
4	H	n-C$_3$H$_7$	110	145[d]
10	p-F	H	88	123[e] (−26)[h] (−31)
12	o-F	H	112	
16	p-Cl	H	110	136[d] (−15)[h] (−17)
18	o-Cl	H	127	
21	o-Cl	CH$_3$	123	
22	p-Cl	C$_2$H$_5$	141[d]	
24	m-CF$_3$	H	90	117[d]
27	m-CF$_3$	n-C$_3$H$_7$	112	165[d]
28	p-CF$_3$	H	85	136[f] (−15)[h] (−12)
29	p-CF$_3$	CH$_3$	123 (50 mg/kg)[d,g]	157
30	p-CF$_3$	C$_2$H$_5$	129[e]	157
45	m-CN	H (7-CH$_3$)	88	135[f]
47	m-CF$_3$	CH$_3$ (7-CH$_3$)	129[e]	
48	m-CF$_3$	H (8-CH$_3$)	102	
50	p-F	H (7-CH$_3$)	105	
52	p-F	H (8-CH$_3$)	126[e]	
53	m-F	H (7-CH$_3$)	142[e]	
controls[c]			169 ± 2	
clonidine hydrochloride			102 ± 2.8[i]	
hydralazine hydrochloride				82 ± 1.5[j]

[a] Only those compounds from Table I which lowered the mean arterial blood pressure (MABP) by at least 20 mm are listed. Compounds 7, 11, 13, 17, 20, 30, 40, 41, 43, and 44 (Table I) were not tested. [b] Mean arterial blood pressure (MABP) in saline-loaded spontaneously hypertensive rats (SHR) dosed (po) with compound at 0 and 24 h and blood pressure measured at 28 h. Except where noted, data are for one rat. [c] Averaged value for MABP of 30 saline-loaded spontaneously hypertensive rats. [d] Average (two rats). [e] Average (three rats). [f] Average (four rats). [g] Toxic at 100 mg/kg (death, two rats). [h] Average reduction in blood pressure (mm) over controls (four rats) at 2 and 4 h in SH rats (n = 4) when tested in dose–response procedure. [i] Average of seven rats; for data on other hypotensive drugs, see ref 24. [j] Data from ref 24.

lective than current therapeutic drugs as an antianxiety agent.[28] Compound 25 does not potentiate the effects of alcohol at therapeutic doses in rats and is presently under clinical evaluation as an anxiolytic agent.

Conclusion

In order to develop selective CNS agents which possess only anxiolytic activity without concomitant sedative, muscle-relaxant properties and other liabilities such as potentiation of the effects of alcohol, activities in animal models must be correlated with clinical effects. It is a reasonable expectation that a better understanding of receptor sites in the brain and their involvement with specific pharmacological properties[29] will aid in the discovery of drugs with selective CNS activity.

The triazolopyridazines represent one of the first nonbenzodiazepine classes of compound[31] to show *potent* binding affinities for specific benzodiazepine receptor sites in brain tissues of rats. They are also active in antipentylenetetrazole tests and in the thirsty rat conflict procedure. Other compounds, such as 1-methylisoguanosine and analogues and purine compounds, inhibit [^{3}H]diazepam binding but only at relatively high concentrations[32] (IC$_{50}$ ≈ 20–200 μM).

(29) (a) Paul, S. M.; Skolnick, P. *Science* 1978, *202*, 892. (b) Babbini, M.; Gaiardi, M.; Bartoletti, M. *Life Sci.* 1979, *25*, 15. (c) Squires, R. F.; Benson, D. I.; Braestrup, C.; Coupet J.; Kelpner, C. A.; Myers, V.; Beer, B. *Pharmacol. Biochem. Behav.* 1979, *10*, 825.

(30) Calculated by the arc-sine transformation of Finney: Finney, D. J. "Statistical Methods in Biological Assay", 2nd ed.; Haffner: New York, 1964.

(31) See Blanchard, J. C.; Boireau, A.; Garret, C.; Julou, L. *Life Sci.* 1979, *24*, 2417 for a nonbenzodiazepine compound with potent binding activity.

(32) Davies, L. P.; Cook, A. F.; Poonian, M.; Taylor, K. M. *Life Sci.* 1980, *26*, 1089.

Methyl and ethyl esters of β-carboline-3-carboxylic acid exhibit [^{3}H]diazepam binding activity at concentrations of 7–19 nM.[33] Since the latter compounds[32,33] are not reported to possess activity in other anxiolytic tests, in vitro binding activity for benzodiazepine receptors cannot be used as the only criterion for selecting potential anxiolytic agents.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are not corrected. Samples for analysis were dried in vacuo over anhydrous calcium chloride at 70 °C for 16–24 h. ^{1}H NMR spectra were determined with a Varian A-100 spectrometer, and chemical shifts (δ) relative to internal tetramethylsilane were as expected. Solvents were removed under reduced pressure by the use of a rotary evaporator.

3-Methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo-[4,3-b]pyridazine (25). General Procedure A. A solution of 6.0 g (23.2 mmol) of 3-chloro-6-[3-(trifluoromethyl)phenyl]-pyridazine and 3.4 g (46.4 mmol) of acetylhydrazine in 75 mL of 1-butanol was refluxed for 48 h. The solvent was removed and the residue treated with activated carbon in ethanol. Filtering and chilling the filtrate gave 5.0 g (78%) of orange crystals. Two recrystallizations from methyl alcohol afforded 3.7 g of crystals, mp 193–194 °C.

In the general procedure followed, a mixture of 0.1 mol of the 3-chloro-6-arylpyridazine and 0.21 mol of the acylhydrazine in 150 mL of 1-butanol was refluxed for 40–48 h. The solution was cooled and filtered, and the solid was washed with hexane and then recrystallized. If the product did not precipitate on cooling, the 1-butanol was removed in vacuo, the residue was triturated with water and filtered, and the solid was recrystallized.

6-Phenyl-3-(trifluoromethyl)-1,2,4-triazolo[4,3-b]-pyridazine (5). General Procedure B. To a mixture of 3.5 g of 3-hydrazino-6-phenylpyridazine in 35 mL of pyridine was added 6.3 g of trifluoroacetic anhydride. The mixture was refluxed

(33) Braestrup, C.; Nielsen, M.; Olsen, C. E. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 2288.

Table III. Anxiolytic Activity of 6-Phenyl-1,2,4-triazolo[4,3-*b*]pyridazines

compd	X	R_1	antiphenylenetetrazole ED_{50},[a-c] mg/kg	[3H]diazepam % inhibn (1 µM)[d]	[3H]diazepam binding IC_{50}, nM
1	H	H	9.0; 4.8	23	nt
2	H	CH_3	2.3; 2.9	55	633
3	H	C_2H_5	i	nt	nt
4	H	n-C_3H_7	i	38	nt
5	H	CF_3	15; 18	nt	nt
6	H	C_6H_5	i	74	nt
7	H	$CH_2C_6H_5$	i	nt	nt
8	H	CH_2OCH_3	9.5; 6.4	<10	nt
9	H	CH_2Cl	i	45; 47	nt
10	p-F	H	5.0; 2.2	16; 23; 15	nt
11	m-F	H	5.5	nt	nt
12	o-F	H	17	16	nt
13	p-F	CH_3	3.4; 2.0	65; 64	589
14	m-F	CH_3	3.1	47; 43	1200
15	o-F	CH_3	10.4	37	7450
16	p-Cl	H	5.3; 9.8	<10	nt
17	m-Cl	H	8.2	30	2250
18	o-Cl	H	18	nt	nt
19	p-Cl	CH_3	9.9; 10	28	nt
20	m-Cl	CH_3	8.0; 4.8	60; 61	709
21	o-Cl	CH_3	14	<10	nt
22	p-Cl	C_2H_5	i	nt	nt
23	p-Cl	C_6H_5	i	nt	nt
24	m-CF_3	H	4.5; 2.5	55	905
25	m-CF_3	CH_3	1.7; 1.1	77	116
26	m-CF_3	C_2H_5	24	73	280
27	m-CF_3	n-C_3H_9	i	nt	nt
28	p-CF_3	H	5.8	21	nt
29	p-CF_3	CH_3	A (15; ip)	17	nt
30	p-CF_3	C_2H_5	~50	nt	nt
31	m-CF_3	CF_3	i	60	nt
32	m-CF_3	CH_2Cl	i	67; 68	391
33	m-CF_3	CH_2OCH_3	6.4	23	nt
34	m-OCH_3	H	A (50, ip)	34; 29	nt
35	m-OCH_3	CH_3	A (50, ip)	74	309
36	p-OCH_3	CH_3	i	nt	nt
37	m-CH_3	H	~50	nt	nt
38	m-CH_3	CH_3	~50	68	nt
39	m-CH_3	CF_3	i	nt	nt
40	m-NO_2	H	22	nt	nt
41	m-NO_2	CH_3	11.6	53	nt
42	m-NH_2	H	15.0; 16.6	32	nt
43	m-NH_2	CH_3	12.0	60; 58	510
44	m-$NHCOCH_3$	CH_3	i	56	633
45	m-CN	H (7-CH_3)	34	nt	nt
46	m-CF_3	H (7-CH_3)	12.9	21	nt
47	m-CF_3	H (7-CH_3)	13.8	26	nt
48	m-CF_3	H (8-CH_3)	i	<10	nt
49	m-CF_3	CH_3 (8-CH_3)	i	<10	nt
50	p-F	H (7-CH_3)	i	nt	nt
51	p-F	CH_3 (7-CH_3)	i	<10	nt
52	p-F	CH_3 (8-CH_3)	i	<10	nt
53	m-F	H (7-CH_3)	i	nt	nt
54	m-F	CH_3 (7-CH_3)	i	<10	nt
55	3,4-Cl_2	H	i	nt	nt
56	3,4-Cl_2	CH_3	i	nt	nt
chlordiazepoxide hydrochloride			1.7	75	248
diazepam			1.8		6
meprobamate				1.8	nt

[a] Oral dose in rats (10 per group) except where noted by ip (intraperitoneal dosing); i indicates no activity at 50 mg/kg (administered either ip or po); ED_{50} (median effective dose), dose which is estimated to be effective in 50% of the animals. [b] Where more than one determination was carried out, results for repeat experiments are given. [c] Symbol A indicates activity observed but no ED_{50} determined. [d] nt indicates the compound was not tested.

for 6 h and concentrated in vacuo. The dark residue was dissolved in ethyl acetate and passed through a short column of hydrous magnesium silicate. The filtrate was concentrated in vacuo and the residue recrystallized (twice) from ethanol to give 2.0 g of crystals, mp 183–184 °C.

3-(Methoxymethyl)-6-phenyl-1,2,4-triazolo[4,3,*b*]-pyridazine (8). General Procedure C. A mixture of 3.4 g of 9 in 200 mL of methanol containing 0.89 g of sodium methoxide was refluxed for 18 h. The mixture was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 and passed through a

Table IV. Effect of Compound **25** on
Phenylenetetrazole Seizures at Various
Time Periods in Rats[a]

time, h	ED_{50},[b] mg/kg
0.5	0.5 (0.2-1.0)
1	0.6 (0.4-1.1)
2	0.7 (0.4-1.3)
3	1.0 (0.6-1.5)
4	1.8 (1.2-2.7)
5	1.7 (1.2-2.3)
6	2.0 (1.3-3.0)
7	2.5 (1.6-4.0)

[a] Three doses per study with six rats per dose (po).
[b] Median effective dose (95% confidence interval).[30]

short column of hydrous magnesium silicate. The eluent was concentrated and the residue triturated with hexane to give 3.0 g (90%) of white crystals, mp 130–133 °C. A sample was recrystallized from CH_2Cl_2-hexane to give white crystals, mp 133–134 °C.

3-(Chloromethyl)-6-phenyl-1,2,4-triazolo[4,3-*b*]pyridazine (9). General Procedure D. To a stirred suspension of 16.9 g of 3-hydrazino-6-phenylpyridazine in 250 mL of dry THF cooled in an ice bath was added portionwise 10.3 g (7.24 mL) of chloroacetyl chloride in 50 mL of THF over 30 min. The cooled mixture was stirred for 1 h, allowed to warm to room temperature, and stirred for 3 h. The mixture was concentrated in vacuo. The residue was stirred with saturated sodium carbonate solution and filtered, and the solid was washed with H_2O to give 23 g of crystals. Recrystallization from CH_2Cl_2-hexane gave 11.3 g (48%) of chloroacetic acid 2-(6-phenyl-3-pyridazinyl)hydrazide, mp 133–134 °C. Anal. $(C_{12}H_{11}ClN_4O)$ C, H, N, Cl.

A mixture of the preceding compound (24.6 g) and 300 mL of HOAc was refluxed for 1 h. The mixture was treated with activated carbon and filtered, and the filtrate was concentrated. The residual oil was triturated with ether to give a solid, which was filtered and washed with ether (22 g). The solid was dissolved in $CHCl_3$, and the solution was passed through a column of hydrous magnesium silicate. The eluent was concentrated to give 10.8 g of crystals, mp 162–164 °C. Recrystallization of a sample from CH_2Cl_2-hexane gave white crystals, mp 165–166 °C.

6-(3-Aminophenyl)-1,2,4-triazolo[4,3-*b*]pyridazine (42). General Procedure E. A cooled mixture of 0.07 mol of **40**, 200 mL of trifluoroacetic acid, and 1.0 g of 10% palladium on carbon catalyst was shaken in a Parr hydrogenator under about 45 lb of initial hydrogen pressure until the theoretical amount of hydrogen was absorbed (1–1.5 h). The catalyst was filtered off and the mother liquor was concentrated to remove most of the acid. The residue was dissolved in 200 mL of H_2O and the pH was adjusted to about pH 5 with 5 N NaOH. The precipitate was filtered off, washed well with water, a little ethanol, and then ether, and dried in a vacuum oven. The solid was recrystallized from ethanol.

3-Methyl-6-(3-acetamidophenyl)-1,2,4-triazolo[4,3-*b*]-pyridazine (44). General Procedure F. A mixture of 0.03 mol of amine **43** and 25 mL of acetic anhydride was allowed to stand at room temperature for 4 h and was diluted with ether. The mixture was filtered and the solid recrystallized from ethanol-ether to give crystals, mp 245–247 °C.

General Procedure for the Preparation of Intermediate 3-Chloropyridazines. Intermediates for compounds in Table I which are not described have been reported.[21]

(a) 3-Chloro-6-(4-fluorophenyl)pyridazine. 4,5-Dihydro-6-(4-fluorophenyl)-3(2*H*)-pyridazinone[22] (87 g, 0.41 mol) in 800 mL of HOAc was stirred and heated on a steam bath. A 15-mL portion of a solution of 24.1 mL (0.44 mol) of bromine in 100 mL of HOAc was added dropwise. When the color had lightened, the remainder of the bromine solution was added over 30 min. The mixture was heated for 1 h, poured onto crushed ice, and filtered. The solid was washed with H_2O to give 82 g (95%) of 6-(4-fluorophenyl)-3(2*H*)-pyridazinone as off-white crystals, mp 265–268 °C.

A mixture of 82 g of pyridazinone in 500 mL of phosphorus oxychloride was heated on a steam bath for 5 h. The solvent was removed and 1 L of ice–water was added with stirring. The

mixture was filtered and the solid washed with H_2O. The solid was dissolved in 2 L of $CHCl_3$ and treated with activated carbon, and the mixture was filtered. The filtrate was concentrated and filtered to give 64 g (71%) of crystals, mp 161–163 °C. Anal. $(C_{10}H_7ClFN_2)$ C, H, N, Cl, F.

(b) 3-Chloro-6-(3-nitrophenyl)pyridazine. 4,5-Dihydro-6-(3-nitrophenyl)-3(2*H*)-pyridazinone[22] (77 g) was reacted with 18.5 mL of bromine in HOAc (680 mL) to give 76 g (100%) of 6-(3-nitrophenyl)-3(2*H*)-pyridazinone, mp 275–278 °C. A 7-g sample was heated with 400 mL of ethanol and filtered (6.0 g, mp 275–278 °C). Chilling the filtrate and filtering gave 0.7 g of crystals, mp 272–275 °C. Anal. $(C_{10}H_7N_3O_3)$ H, N; C: calcd, 55.3; found, 55.9. A 60-g sample of the above compound was heated with 350 mL of phosphorus oxychloride to give 67 g of solid. The solid was heated with 1 L of $CHCl_3$ and the mixture was filtered. Concentration of the filtrate to 150 mL and filtering gave 29.6 g (45%) of yellow crystals, mp 209–211 °C. Anal. $(C_{10}H_6ClN_3O_2)$ C, H, N, Cl.

(c) 3-Chloro-6-(3-methoxyphenyl)pyridazine. A mixture of 4,5-dihydro-6-(3-methoxyphenyl)-3(2*H*)-pyridazinone[22] (85 g, 0.42 mol), 123.8 g (0.55 mol) of sodium 3-nitrobenzenesulfonate, and 84.0 g (2.1 mol) of NaOH in 1.5 L of H_2O was heated on a steam bath for 3 h. Activated carbon was added and the mixture was filtered. The filtrate was chilled, acidified with concentrated HCl, and filtered. The solid was washed with H_2O to give 72 g of yellow crystals, mp 161–171 °C. Two recrystallizations from CH_3OH gave 31 g (36%) of cream-colored crystals, mp 183–185 °C. Anal. $(C_{11}H_{10}N_2O_3)$ C, H, N.

Reaction of the above compound (31 g, 0.15 mol) with 150 mL of phosphorus oxychloride gave 19 g (57%) of off-white crystals, mp 98–99 °C. Anal. $(C_{11}H_9ClN_2O)$ H, N; C: calcd, 59.9; found, 60.4; Cl: calcd, 16.1; found, 16.6.

(d) 3-Chloro-6-(3-cyanophenyl)-7-methylpyridazine. 4,5-Dihydro-6-(3-cyanophenyl)-5-methyl-3(2*H*)-pyridazinone[22] (1.0 g, 4.7 mmol) was reacted with 0.78 g (4.9 mmol) of bromine in 5 mL of HOAc to give 0.90 g (91%) of yellow crystals, mp 285–290 °C. Recrystallization of a sample from ethanol gave crystals, mp 289–294 °C. A mixture of 10 g (0.0474 mol) of 6-(3-cyanophenyl)-5-methyl-3(2*H*)-pyridazinone and 100 mL of phosphorus oxychloride was heated on a steam bath for 3 h to give 8.2 g (75%) of cream-colored crystals, mp 155–158 °C. Recrystallization of a sample from ethyl acetate gave off-white crystals, mp 159–162 °C. Anal. $(C_{12}H_8ClN_3)$ C, H, N, Cl.

(e) 3-Chloro-6-(3,4-dichlorophenyl)pyridazine. To a solution of α-(3,4-dichlorophenyl)-4-morpholineacetonitrile[21a] (136.9 g, 0.505 mol) in 600 mL of THF was added dropwise 10 mL of ethanol containing 30% KOH. A 25-mL portion of ethyl acrylate was added dropwise over 10 min (exotherm). After the solution was stirred for 2 h, an additional 10 mL of ethanol containing 30% KOH was added and 25 mL of ethyl acrylate added dropwise over 10 min. The mixture was stirred for 24 h, and the solvent was removed in vacuo. The residual oil was diluted with ether and filtered. The solvent was removed to give 132.6 g (0.358 mol) of yellow oil. To the oil in 500 mL of ethanol was added 36 g (0.72 mol) of hydrazine hydrate, and the mixture was refluxed overnight. The mixture was chilled and filtered to give 63.5 g of crude 4,5-dihydro-6-(3,4-dichlorophenyl)-3(2*H*)-pyridazinone as yellow crystals, mp 168–182 °C. From a similar run, a sample was recrystallized from ethanol to give yellow crystals, mp 171–173 °C. Anal. $(C_{10}H_8Cl_2N_2O)$ C, H, N, Cl.

A mixture of 63.5 g (0.26 mol) of the preceding compound, 67.5 g (0.30 mol) of sodium 3-nitrobenzenesulfonate, and 52 g (1.30 mol) of NaOH in 2 L of H_2O was heated on a steam bath for 20 h. The mixture was filtered and the filtrate treated with activated carbon. The filtrate was acidified with concentrated HCl and filtered, and the solid was washed with H_2O to give 26.4 g (42%) of tan crystals, mp 215–218 °C. A 10-g sample was heated with 100 mL of phosphorus oxychloride to give 8.9 g of solid. The solid was chromatographed on silica gel with $CHCl_3$–CH_3OH (95:5) as eluent to give 5.5 g of crystals, mp 180 °C. Recrystallization of a sample from ethyl acetate gave off-white crystals, mp 188–189 °C. Anal. $(C_{10}H_5Cl_3N_2)$ C, H, N, Cl.

(f) 3-Hydrazino-6-[3-(trifluoromethyl)phenyl]pyridazine. A mixture of 20.0 g (0.077 mol) of 3-chloro-6-[3-(trifluoromethyl)phenyl]pyridazine, 11.5 g (0.23 mol) of hydrazine hydrate, and 250 mL of 1-butanol was refluxed for 24 h. The mixture was

chilled and filtered. The solid was washed with petroleum ether (bp 30–60 °C) and with CH_2Cl_2 to give 17.5 g (89%) of cream-colored solid, mp 160–164 °C. The crude product was used in further reactions.

Ability to Protect against Pentylenetetrazole Convulsions. Groups of 10 rats deprived of food for 24 h were treated orally with graded doses (4–6) of compound, followed 60 min later by PTZ (23 mg/kg, intravenously into the caudal vein). This dose of PTZ was estimated to cause seizures in 99% of the rats. Animals were observed for at least 5 s of uninterrupted clonic seizures, which occurred in untreated animals within several seconds following PTZ administration. The number of animals protected from convulsions were recorded and median effective doses calculated.[19]

Thirsty Rat Conflict Procedure.[25] Groups of six naive, Wistar strain rats, weighing 200–240 g each, were deprived of water for 48 h and food for 24 h prior to testing. The test compounds were administered in single or graded, oral or intraperitoneal doses, suspended in a 2% starch vehicle containing 0.5%, v/v, polyethylene glycol and one drop of polysorbate 80. Control animals received the vehicle alone. At 30 or 60 min each rat was placed in an individual black Plexiglas chamber (4.25 in. wide by 6.5 in. deep). A 10% dextrose solution was available ad libitum from a tap located in the rear of the chamber. A 0.3-mA constant current, 60-Hz pulsed DC shocking current was established between the stainless-steel grid floor and the tap. Each rat was allowed 20 s of nonshocked drinking, after which time alternating cycles of 5-s "shock off" and 5-s "shock on" (where each lick on the tap was accompanied by shock) began and continued for a total of 5 min. The number of shocks taken by each rat during the 5-min interval was recorded and compared to a control group. The test compounds were considered active if the shocks received by the test group were significantly different from the control group by the Mann–Whitney U test. (Wilcoxon rank sum test) ($p < 0.05$).[34]

Diazepam Binding Assay. A modification of the diazepam binding assay described by Squires[14a] and Mohler et al.[14b] was used. The frontal cortex of rats was homogenized gently in 20 vol of ice-cold 0.32 M sucrose, centrifuged twice at 1000g for 10 min, and recentrifuged at 30000g for 20 min to produce a crude P_2-synaptosomal fraction. The P_2 fraction was resuspended, twice the original volume, in hypotonic 50 mM Tris-HCl (pH 7.4). The binding assay consisted of 300 μL of the P_2-fraction suspension ($\sim$0.350 mg), 100 μL of test drug, and 100 μL of [^{3}H]diazepam (1.5 nM), which was added to 1.5 mL of 50 mM Tris-HCl (pH 7.4). Nonspecific binding controls and total binding controls received 100 μL of diazepam (3 μM) and 100 μL of deionized water, respectively, in place of test drug. Incubation for 20 min proceeded in ice and was terminated by filtration, under vacuum, through Whatman GF/C glass-fiber filters. The filters were washed twice with 5 mL of ice-cold 50 mM Tris-HCl (pH 7.4) and placed in scintillation vials. After drying at 50–60 °C for 30 min, 10 mL of Beckman Ready-Solve HP was added and radioactivity determined in a Beckman scintillation counter. Calculation of percent inhibitions were carried out as follows:

$$CPM_{specific} = CMP_{total} - CPM_{nonspecific}$$

$$pmol\ bound = CMP_{specific}\left(\frac{3\ pmol}{CPM_{[^3H]diazepam} - CPM_{background}}\right)$$

$$\%\ inhibn = \left(\frac{pmol_{total} - pmol_{drug}}{pmol_{total}}\right)100$$

Compounds were further evaluated in the diazepam binding assay to determine the drug concentration required to cause a 50% displacement of diazepam from its receptors (IC$_{50}$). Appropriate concentrations of each drug were tested in the assay, and the percent inhibition vs. drug concentration was plotted on log paper. The IC$_{50}$ was read directly from the graph. Data for Figures 1 and 2 are average values from triplicate runs.

Procedures for Antihypertensive Screen in Spontaneously Hypertensive Rats.[24] Male spontaneously hypertensive rats of Okamoto strain of 8 weeks old were purchased from Taconic Farms, Inc. (Germantown, NY). These rats were kept on Purina laboratory chow and tap water ad libitum for 8 weeks in our animal facilities before use. The present method, using sequential analysis techniques, required from one to three rats per compound. One male adult rat weighing about 300 g was dosed by gavage with each test compound at 100 mg/kg of body weight with 0.9% sodium chloride loading at 25 mL/kg at 0 h. The test compound was suspended in 2% boiled starch at 50 mg/mL. The rat was put in a metabolism cage. The 0–5 h urine was collected, and urinary Na$^+$, K$^+$, and Cl$^-$ were determined by the Technicon Auto-Analyzer, method no. N-20 for Na$^+$ and K$^+$ and method no. N-5b for Cl$^-$. At the end of 5 h, the rat was returned to a regular animal cage and was provided with water ad libitum. After 24 h, a second identical dose of the test compound was given by gavage without 0.9% sodium chloride loading. Four hours after the second dose, the rat was restrained in a supine position with elastic tapes (Elastikon, Johnson & Johnson). The femoral area was locally anesthetized by subcutaneous infiltration of 2% lidocaine. The iliac artery was isolated and punctured with a 26-gauge, thin-wall needle which was connected to a Statham P23Db pressure transducer–Beckman dynograph recorder system for monitoring blood pressure. Usually the blood pressure was recorded for 15 min or until it was stabilized. The heart rate was determined by counting the pulse waves per unit of time. Then the needle was removed and the puncture site of the artery was sealed with an extremely fast setting cyanoacrylate ester adhesive (Super Bonder, Part No. 49450, Loctite Corp., Newington, CT) The skin of the rat was closed by means of wound clips and treated with an antiseptic, thimerosal tincture (1:1000). The rat was put back in the cage for recovery. The rats employed in many studies were used only once. However, in routine testing, the rats can be reused after a rest of at least 7 days. The blood flow through the hindlimb after sealing of the puncture hole in the artery using the liquid adhesive was relatively unimpaired. The untouched contralateral iliac artery was used for monitoring blood pressure when the rat was reused.

Evaluation of Antihypertensive Compounds in Conscious Spontaneously Hypertensive Rats (Dose-Response Procedure). Male spontaneously hypertensive rats (Taconic Farms, Germantown, NY) of the Okomoto strain of 300–350 g of body weight were restrained in a supine with elastic tape. The area at the base of the tail was locally anesthetized by subcutaneous infiltration with 2% procaine. The ventral caudal artery was isolated, and a cannula made of PE (polyethylene) 10 and PE 20 tubing was passed into the lower abdominal aorta. The cannula was secured, heparinized (100 IU/cm^3), and sealed, and the wound was closed. The animals were returned to their cages for a short period and then placed in plastic restraining cages in an upright position. The cannula was attached to a Statham P23Db blood pressure transducer, and blood pressure (pulsatile) was recorded for 10–15 min. Heart rate was calculated from the pulse wave/unit of time. The cannula was then reheparinized and sealed. Each animal was dosed po with either vehicle (2% boiled starch) at 1 mL/100 g or compound to be tested at 1, 5, 10, and 25 mg/kg. Twenty hours later the animals were connected via the cannula to the Statham transducer, and a record of blood pressure was obtained. Immediately following, each animal received a second po dose, and blood pressure was monitored continuously for 4 h. Heart rate was taken at 0, 15, 30, 60, 120, 180, and 240 min postdosing. Systolic, diastolic, and mean blood pressure were tabulated at these same time periods. All animals had food and water ad libitum except during the recording periods.

(34) Swinscow, T. D. W. "Statistics at Square One"; Dawson and Goodall Ltd, The Mendip Press: Bath, England, 1978; p 58.