were randomly divided into groups of 6–10 animals. To determine estrogenic activity, compounds were dissolved or suspended in olive oil (100 μL/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. 24 h after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid/40% formaldehyde/glacial acetic acid 15:5:1 by vol) for 2 h. Uteri were freed from connective tissue, washed with a saturated alcoholic solution of LiCl, dried at 100 °C for 24 h, and weighed. The relative uterine weight was calculated with the following formula: uterine dry weight (mg)/body weight (g), multiplied by 100. Agonistic activity (%) was estimated by the following formula: $(W_T - W_V)/(W_S - W_V) \times 100$ (W_S = relative uterine weight of animals treated with a standard dose of estrone (0.4 μg); W_T = relative uterine weight of animals treated with test compound; W_V = relative uterine weight of control animals).

To determine antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of test compound. Antagonism (%) was calculated with the following formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = relative uterine weight of animals treated with estrone + test compound). Experiments with a constant dose of the antagonist and varying doses of estrogen were performed in an analogous manner with diethylstilbestrol (DES) as agonist.

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Supplementary Material Available: ^{1}H NMR data of 5-methoxy-2-(4-methoxyphenyl)indoles (**3a–i, 6a–c**) and 1-(aminoalkyl)-5-hydroxy-2-(4-hydroxyphenyl)indoles (**5a–l**) (4 pages). Ordering information is given on any current masthead page.

Synthesis and Evaluation of a Series of Aryl[*e*]fused Pyrazolo[4,3-*c*]pyridines with Potential Anxiolytic Activity

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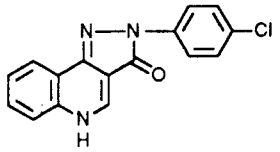
A series of pyrazolo[4,3-*c*]pyridines has been synthesized and evaluated as potential anxiolytic agents. Selected compounds from this series show a pharmacological profile of action different from that of diazepam. A number of the compounds possess higher affinity for central benzodiazepine receptors than diazepam, yet show less anticonvulsant activity and are less sedative. The structure–activity relationships of these potential anxiolytic agents are discussed.

Since the discovery of chlordiazepoxide and diazepam, the 1,4-benzodiazepines (BZs) have been a fruitful source of research.[1] However, anxioselective compounds,[2] which do not possess the undesirable side effects of BZs are needed. Some β-carboline[3] and 2-thienylpyrazoloquinoline[4] derivatives, related to the 2-(4-chlorophenyl)pyrazolo-[4,3-*c*]quinolin-3(5*H*)-one (CGS 9896) (**12**), but with different substituents on the parent skeleton, have a wide spectrum of biological activity. One compound from the



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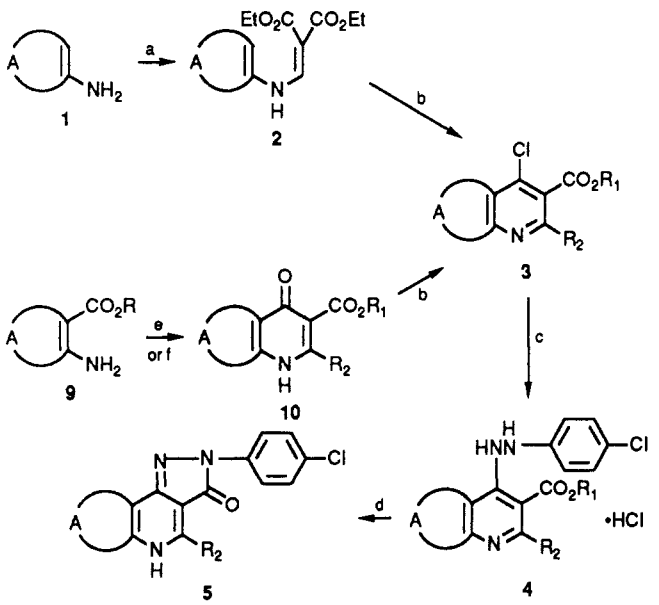
former series, 4-(methoxymethyl)-6-(phenylmethoxy)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid, ethyl ester (ZK 93423), represents a relatively selective anxiolytic,[5] whereas another, 4-(methoxymethyl)-5-(phenylmethoxy)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid, ethyl ester (ZK 91296), is a remarkable anticonvulsant.[6] The selective, non-BZ, pyrazoloquinoline **12**, which is a partial agonist at BZ receptors, is an important structural lead.[7] As part

Scheme I[a]



[a] (a) Diethyl ethoxymethylenemalonate/toluene/reflux. (b) $POCl_3$/reflux. (c) 4-$ClC_6H_4NHNH_2$/EtOH/reflux. (d) Xylene/reflux or K_2CO_3/*sec*-butyl alcohol/reflux. (e) $R_2C(OR_1)$=$CHCO_2R_1$/toluene/reflux followed by $NaOR_1$/ROH/toluene/reflux. (f) Procedure as in ref 13 using dimethyl acetylenedicarboxylate (DMAD).

of a program designed to identify novel compounds with potential anxiolytic activity, molecular modeling of the potential energy surfaces of known anxiolytic agents which act at central BZ receptors was carried out. These studies, which agree with the literature,[8] suggested that the BZ

(1) Sternbach, L. H. *The Benzodiazepines*; Garattini, S., Mussini, E., Randall, L. O., Eds.; Raven Press: New York, 1973; pp 1–25.
(2) Williams, M. *J. Med. Chem.* **1983**, *26*, 619.
(3) Braestrup, C.; Honoré, T.; Nielsen, M.; Petersen, E. N.; Jensen, H. *Biochem. Pharmacol.* **1984**, *33*, 859.
(4) Takada, S.; Shindo, H.; Sasatani, T.; Chomei, N.; Matsushita, A.; Eigyo, M.; Kawasaki, K.; Murata, S.; Takahara, Y.; Shintaku, H. *J. Med. Chem.* **1988**, *31*, 1738.
(5) Stephens, D. N.; Kehr, W.; Wachtel, H.; Schmiechen, R. *Pharmacopsychiatry* **1985**, *18*, 167.
(6) Petersen, E. N.; Jensen, L. H.; Honoré, T.; Braestrup, C.; Kehr, W.; Stephens, D. N.; Wachtel, H.; Seidelman, D.; Schmiechen, R. *Psychopharmacology* **1984**, *83*, 240.
(7) Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.

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Table I. Pharmacological Activities of Arylpyrazolopyridines

compound	[^{3}H]flunitrazepam binding assay: K_i, nM	rat Vogel test (n = 12) dose, mg/kg (ip)	rat Vogel test (n = 12) no. shocks taken[a]	percent change in seizure threshold (pentylenetetrazole) mouse; dose, mg/kg (po)[c]	percent of mice not grasping wire; dose, mg/kg (ip)[c]
5a	>10^5	20	6.7 ± 1.8	+18% at 30	NT
5b	1 × 10^3 ± 420	20	6.1 ± 1.2	+57% at 100	NT
5c	0.46 ± 0.21	20	14.0 ± 1.7	Ia at 100	Ia at 30
5d	1.8 ± 0.40	20	14.4 ± 3.4	Ia at 30	Ia at 30
5e	0.32 ± 0.20	20	17.0 ± 3.0	+47% at 30	Ia at 30
5f	810 ± 220	20	14.1 ± 1.8	Ia at 30	Ia at 30
5g	1.1 × 10^4 ± 600	20	Ia[b]	Ia at 100	NT
5h	99 ± 34	20	11.9 ± 2.6	+48% at 100	Ia at 30
5i	insol	20	10.0 ± 2.2	NT	+25% at 30
5j	12 ± 3.00	20	13.0 ± 2.3	Ia at 30	Ia at 30
5k	2.4 ± 0.50	20	13.0 ± 2.6	+37% at 30	Ia at 30
5l	0.29 ± 0.18	20	7.0 ± 1.4	NT	Ia at 30
5m	14 ± 3.40	20	9.0 ± 1.7	Ia at 30	NT
5n	0.46 ± 0.18	20	5.7 ± 1.1	Ia at 30	Ia at 30
5o	1.8 ± 0.42	20	12.0 ± 2.4	NT	Ia at 30
6	insol	20	16.8 ± 2.5	Ia at 30	Ia at 30
7a	2.6 × 10^3 ± 640	20	8.6 ± 3.5	Ia at 30	NT
8a	insol	20	13.7 ± 2.9	Ia at 30	NT
8b	insol	20	13.4 ± 1.5	Ia at 30	NT
12	0.35 ± 0.15	10	12.7 ± 2.7	ED_{50} 3.5 ± 1.2	+30% at 300
diazepam	14 ± 0.37	2.5	21.2 ± 3.6	ED_{50} 1.5 ± 0.4	ED_{50} 3.1 (3.8 po) ± 1.5

[a] Control no. of shocks (7.5 ± 0.2) taken by animals (n = 132) dosed with 1% methyl cellulose in 0.5% saline. [b] n = 6 animals tested. [c] Student's t test $p < 0.05$; NT = not tested; Ia = inactive.

receptor complex could tolerate a wide variety of π-aromatic rings. Therefore, a series of aromatic isosteric replacements of **12** was investigated in order to determine the effect of such changes on receptor-binding activity. We have already reported[9] that the pyrazolothienopyridines possess potential anxiolytic activity. Also, in an attempt to refine and optimize our model of the BZ receptor complex, chemical manipulation of the pyridine ring system has revealed some interesting structural and steric requirements. Here we describe the detailed synthesis and structure–activity relationships of the pyrazolopyridines **5**.

Chemistry

Scheme I outlines our synthetic routes to [e]-fused derivatives **5** of 2,5-dihydro-3*H*-pyrazolo[4,3-c]pyridines via the intermediates **3**. The appropriate 4-chloropyridine intermediates **3** were prepared either via the pyridones **10** (method C for R_2 = Me,Ph and method D for R_2 = H) or directly from the corresponding [(arylamino)methylene]-malonates **2** by a modified Gould–Jacobs cyclization,[10] with use of phosphorus oxychloride at reflux (method E). The desired pyrazolopyridines **5** were obtained by treatment of **3** with an ethanolic solution of (4-chlorophenyl)-hydrazine at reflux (method F), followed by base-promoted cyclization of the resulting 1,2-disubstituted hydrazines **4** with potassium carbonate (method B). However, in some cases, for example with **11**, the 1,2-disubstituted hydrazine intermediate could not be isolated and cyclized spontaneously to give the pyrazolone directly, thus avoiding a further step (method A).

The 2,5-dihydro-3*H*-pyrazolo[4,3-c]pyridin-3-one nucleus can exist in three possible tautomeric forms, e.g. in the thiophene-fused series **5c**, **7b**, and **8c**. An attempt was made to "freeze out" all three forms by appropriate methylation and this is illustrated in Scheme II. Treatment of the pyrazolopyridine **5c** with phosphorus oxychloride at reflux led to the chloro derivative **8a**. Displacement of the chloride in **8a** with methoxide gave the corresponding methoxy compound **8b**.

Methylation of **5c** using either sodium hydride and iodomethane or dimethyl sulfate gave the 5-methyl **6** and 1-methyl **7a** derivatives, respectively.

Pharmacological Methods

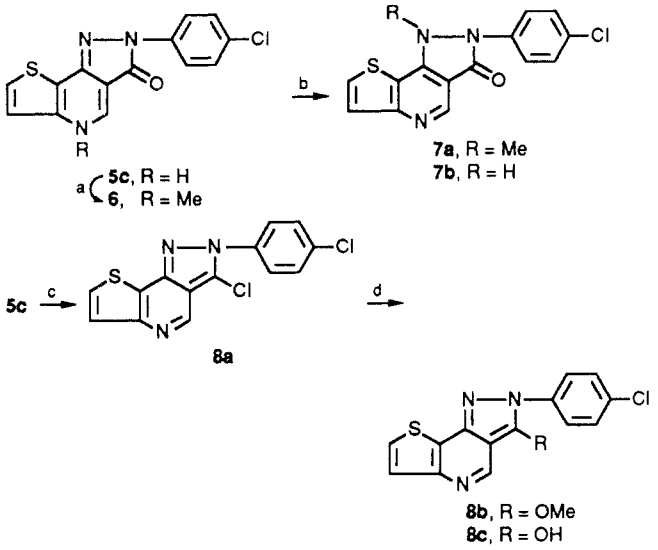
The pyrazolopyridines were tested in the following screens: [^{3}H]flunitrazepam binding assay,[11] anti-pentylenetetrazole test,[12] shock-induced suppression of drinking test[13] (Vogel test), and horizontal wire test.[14] The anticonvulsant properties of the compounds under investigation were evaluated by using the pentylenetetrazole (PTZ) infusion method in mice. The shock-induced suppression of drinking test was adapted from Vogel et al.,[13] and is considered to be a reliable and specific method for identifying potential anxiolytic activity. The details of these tests are given in the Experimental Section.

Results and Discussion

The structures and pharmacological data for the pyrazolo[4,3-c]pyridines are shown in Table I. Many of the compounds displace [^{3}H]flunitrazepam from CNS binding sites with a K_i comparable to that of diazepam (K_i 14 nM). However, the compounds **5f–h**, closely related to **12**, were only weakly active. In the thienopyridine series, substitution at the 4-position by phenyl or methyl (compounds **5a** and **5b**, respectively) caused a dramatic reduction in the in vitro affinity for BZ receptors (K_i >10^3 nM). This was also reflected by reduced anticonflict activity in the rat Vogel test. Comparison of the three isomeric thienopyridines **5c–e** reveals that the compounds have similar receptor affinities. In an attempt to provide information

(8) Fryer, R. I. *The Benzodiazepines from Molecular Biology to Clinical Practice*; Costa, E., Ed.; Raven Press: New York, 1983; pp 7–20.
(9) Forbes, I. T.; Thompson, M. Eur. Pat. EP 126,970; *Chem. Abstr.* **1985**, *102*, 220869.
(10) Khan, M. A.; Guarconi, A. E. *J. Heterocycl. Chem.* **1977**, *14*, 807.
(11) Martin, I. L.; Candy, J. M. *Neuropharmacology* **1978**, *17*, 993.
(12) (a) Everett, G. M.; Richards, R. K. *J. Pharmacol. Exp. Ther.* **1944**, *81*, 402. (b) Rommelspacher, H.; Nanz, C.; Borbe, H. O.; Fehske, K. J.; Muller, W. E.; Wollert, U. *Eur. J. Pharmacol.* **1981**, *70*, 409.
(13) Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacologia (Berlin)* **1971**, *21*, 1.
(14) Courvoisier, S. *J. Clin. Exp. Psychopathol.* **1956**, *17*, 25.

Scheme II[a]



[a] (a) NaH/THF/MeI/room temperature. (b) Me_2SO_4/110 °C. (c) $POCl_3$/reflux. (d) KOBu^t/MeOH/reflux.

for BZ receptor modeling studies, the various possible tautomeric forms of the pyrazolopyridine moiety were prepared by methylation at the 1, 3, or 5-position.

Methylation of **5c** gave two *N*-methyl derivatives **6** or **7a** depending upon the reaction conditions[7] (Scheme II). Location of the *N*-methyl group in **6** (5-Me) and **7a** (1-Me) was assigned from their 4-H chemical shifts (δ 8.78 and 9.04, respectively) when compared with the 4-H signal of **5c** which appears at δ 8.77. In addition, an NOE effect of 25% was observed at the 4-H and 6-H signals on irradiation of the 5-NMe group of **6**, no such effects were observed with compound **7a**. Comparison of the UV spectra of **5c** with those of **6** and **7a** further proved the structure. At neutral pH, the UV spectrum of **5c** in EtOH (λ_{max} 288 nm) resembles that of **6** (λ_{max} 291 nm) rather than that of **7a** (λ_{max} 275 and 241 nm).

Anticonflict activity was observed with the 3- and 5-methyl derivatives **8b** and **6**, but not with the 1-methyl isomer **7a**. At this stage, however, we cannot rule out the possibility that **8b** undergoes hydrolysis in vivo to **5c** which is responsible for the observed activity in the Vogel test. Replacement of the phenyl ring of **12** by an electron deficient ring as in naphthyridine **5g** or isothiazolopyridine **5h** was detrimental to in vitro potency (K_i >100 nM) but, interestingly, **5h** maintained in vivo activity. Reduced activity was observed with the linear tetracyclic compounds, benzothienopyridine **5f** and pyrazoloquinoline **5m** on in vitro BZ receptor binding, with the carboline **5i** inactive on in vivo parameters.

The linear tetracyclic compounds **5f**, **5i**, and **5m** molecules are possibly too large to fit into the BZ receptor binding site thus suggesting a size constraint on the π-binding site. However, the angular dipyrazoloquinolines **5j–l,n** and the imidazoquinoline **5o** showed good in vitro potency but poor in vivo activity in the Vogel behavioral screen, possibly reflecting poor oral bioavailability of these compounds. On a qualitative basis, more than one-half of the compounds were similar to **12** in the rat Vogel test but substantially less active than CGS 9896 and diazepam in the anti-pentylenetetrazole test. Generally BZ agonists possess both anxiolytic and anticonvulsant properties, as observed with **12**. Only **5e**, **5h**, and **5k** have this profile. The lack of myorelaxant/sedative properties reported[15] for **12** has been confirmed in the wire test. The compounds, like **12**, were also less active than diazepam (ED_{50} 3.1 mg/kg ip) in the mouse wire test. This may suggest that the pyrazolopyridines, as a class of compounds, have weaker muscle-relaxant properties when compared to diazepam.

The antianxiety effects of **5e** and **5k** were confirmed by the Geller–Seifter test.[16] The degree of drug-induced enhancement of performance during an FR_5 schedule in Olac Hooded Lister male rats at 20 mg/kg ip was identical with that of **12** and diazepam at 10 and 2.5 mg/kg ip, respectively.

Modifications have been made to **12** which generate more information about drugs which interact with the BZ receptor. From these biological data, it is apparent that BZ receptor interacting drugs do have the potential to provide novel anxioselective agents for the treatment of anxiety.

Experimental Section

Chemistry. Melting points were determined by using a Kofler hot stage apparatus and are uncorrected. The elemental analyses indicated are within ±0.4% of the theoretical values. ^{1}H NMR spectra were obtained with a JEOL GX 270 or Varian CFT 20 spectrometer using tetramethylsilane as internal reference standard. Mass spectra were recorded on a JEOL JMS DX 303/DA 5000 system operating at 70 eV. All evaporations of solvents were carried out under reduced pressure, and organic solvents were dried over anhydrous Na_2SO_4. For column chromatography the silica gel used was Merck Kieselgel 60. Petrol refers to the light petroleum fraction boiling between 60 and 80 °C. Water determinations of the final products were made by the Karl Fischer method.

Synthetic Methods. Specific examples presented below illustrate general synthetic methods A–F for the preparations of 3 to 8 in Schemes I and II.

Ethyl 9-Chloro-1-methyl-1*H*-pyrazolo[3,4-*f*]quinoline-8-carboxylate (11b) (Method E). The compound was prepared from 6-amino-1-methylindazole and diethyl (ethoxymethylene)-malonate by the general procedure described by Khan et al.[10]: mp 85–86 °C; NMR ($CDCl_3$) δ 1.48 (3 H, t, J = 7 Hz), 4.41 (3 H, s), 4.52 (2 H, q, J = 7 Hz), 7.76 (1 H, d, J = 9 Hz), 8.02 (1 H, d, J = 9 Hz), 8.22 (1 H, s), 9.20 (1 H, s). Anal. ($C_{14}H_{12}N_3O_2Cl$) C, H, N.

The other compounds **3c**, **3e–i**, and **11c–e** in Tables II and III were prepared in a similar manner.

Methyl 4-Chlorothieno[3,4-*b*]pyridine-3-carboxylate (3d) (Method D). Methyl 3-aminothiophene-4-carboxylate[17] was converted into dimethyl 1,4-dihydro-4-oxothieno[3,4-*b*]-pyridine-2,3-dicarboxylate by using a procedure similar to that in ref 18, except that *tert*-butyl alcohol was used as solvent for the Michael addition to DMAD. Transformation into **3d** was carried out with use of a procedure similar to that described in ref 18.

The compounds **3c** and **3j** in Table II were prepared in a similar manner.

Ethyl 9-Chloro-2-methyl-2*H*-pyrazolo[4,3-*f*]quinoline-8-carboxylate (11a). 5-Aminoindazole (13.3 g, 100 mmol) and diethyl (ethoxymethylene)malonate (21.6 g, 100 mmol) in toluene (200 mL) were heated under reflux for 3 h. Ethanol was then removed by distillation, the mixture cooled to 5 °C, and the resultant crystalline product filtered off and washed with petrol (yield 20.4 g, 67%, mp 163–164 °C). A portion (19 g, 62.6 mmol) was treated with sodium ethoxide (5.46 g, 80.4 mmol) in dry dimethylformamide (150 mL). After 15 min, iodomethane (4.86 mL, 80.4 mmol) was added dropwise at 0 °C and the mixture stirred at room temperature for 18 h. Evaporation gave a solid

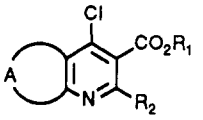
(15) Bennett, D. A.; Petrack, B. *Drug Dev. Res.* **1984**, *4*, 75.

(16) Geller, I.; Seifter, J. *Psychopharmacol.* **1960**, *1*, 482.

(17) (a) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1953**, *18*, 138. (b) Hromatka, O.; Binder, D.; Eichinger, K. *Monatsh. Chem.* **1973**, *104*, 1520.

(18) Barker, J. M.; Huddleston, P. R.; Jones, A. W. *J. Chem. Res.* **1978**, 4701.

Table II. Physical Properties of 3 Prepared by Methods C, D, E

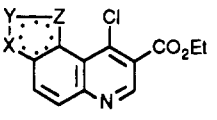


3a–j.

compound	A	R_1	R_2	method	% yield	mp, °C	formula
3a	[3,2-*b*]thieno	Et	Ph	C	75	73–75	$C_{16}H_{12}NO_2SCl$
3b	[3,2-*b*]thieno	Et	Me	C	55	115–118	$C_{11}H_{10}NO_2SCl$
3c	[3,2-*b*]thieno[a]	Me	H	D,E	52	95–97	$C_9H_6NO_2SCl$
3d	2-Et-[2,3-*b*]thieno	Et	H	E	30	37–41	$C_{12}H_{12}NO_2SCl$[b,e]
3e	[3,4-*b*]thieno	Me	H	D	63	89–90	$C_9H_6NO_2SCl$
3f	benzo[*b*][3,2-*b*]thieno	Et	H	E	63	88–89	*e*
3g	2-Me-[2,3-*b*]pyrido	Me	H	E	58	124–127	$C_{11}H_9N_2O_2Cl$
3h	3-Me-[5,4-*b*]isothiazolo	Et	H	E	35	77–78	$C_{10}H_9N_2O_2SCl$
3i	1-Me-[3,2-*b*]indolo	Me	H	D	44	105–108	$C_{14}H_{11}N_2O_2Cl$[d,e]
3j	1-Me-[6,5-*b*]indazolo	Et	H	E	5[c]	185–188	$C_{14}H_{12}N_3O_2Cl$

[a] The compound R_1 = Et, mp 79–81 °C was also prepared but not fully characterized. [b] MS *m/e* 269.0280 (M^+). [c] Minor product from the synthesis of **11c**. [d] MS *m/e* 274.0512 (M^+). [e] Satisfactory 270-MHz ^1H NMR obtained.

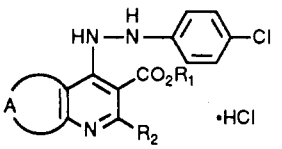
Table III. Physical Properties of Compound 11 Prepared by Method E



11a–e.

compound	nucleus	X	Y	Z	overall % yield	mp, °C	formula
11a	pyrazolo[4,3-*f*]	N	MeN	CH	56	–	–
11b	pyrazolo[3,4-*f*]	CH	N	MeN	73	85–86	$C_{14}H_{12}N_3O_2Cl$
11c	pyrazolo[4,3-*f*]	MeN	N	CH	54	135–137	$C_{14}H_{12}N_3O_2Cl$
11d	pyrazolo[3,4-*f*]	CH	MeN	N	30	151–152	$C_{14}H_{12}N_3O_2Cl$
11e	imidazo[4,5-*f*]	MeN	CH	N	50	170–173	$C_{14}H_{12}N_3O_2Cl$

Table IV. Physical Properties of 4 Prepared by Method F



4a–f.

compound	A	R_1	R_2	% yield	mp, °C	formula
4a	[3,2-*b*]thieno	Et	Ph	77	218–220	$C_{22}H_{19}N_3O_2SCl_2$
4b	[3,2-*b*]thieno	Et	Me	40	215–217	$C_{17}H_{17}N_3O_2SCl_2$
4c	[3,2-*b*]thieno	Me	H	40	165–168	$C_{15}H_{12}N_3O_2SCl_2$
4d	2-Et-[2,3-*b*]thieno	Et	H	25	188–189	$C_{18}H_{19}N_3O_2SCl_2$
4e	[3,4-*b*]thieno	Me	H	87	177–180	$C_{15}H_{13}N_3O_2SCl_2$
4f	benzo[*b*][3,2-*b*]thieno	Et	H	68	203–205	$C_{20}H_{17}N_3O_2SCl_2$

which was partitioned between dichloromethane and water. The organic phase was dried and evaporated to dryness. The residue was purified by column chromatography on silica, by eluting with 20% petrol/chloroform. Recrystallization from petrol gave diethyl [[*N*-(2-methyl-5-indazolyl)amino]methylene]malonate (7.3 g, 37%). The corresponding 1-methyl product (6.74 g, 34%) was also isolated. Treatment of a portion of the former (7.32 g, 23.1 mmol) with phosphorus oxychloride (100 mL) according to the general procedure in ref 10 gave **11a** (5.56 g, 83%) which was used without further purification: NMR ($CDCl_3$) δ 1.48 (3 H, t, J = 7.5 Hz), 4.30 (3 H, s), 4.50 (2 H, q, J = 7.5 Hz), 7.85 (1 H, d, J = 9 Hz), 7.98 (1 H, d, J = 9 Hz), 8.85 (1 H, s), 9.13 (1 H, s).

Ethyl 7-Chloro-5-phenylthieno[3,2-*b*]pyridine-6-carboxylate (3a) (Method C). A mixture[19] (1:1) of ethyl 3-ethoxycinnamate and ethyl 3,3-diethoxy-3-phenylpropionate (4.18 g, 17.8 mmol) was added to methyl 3-aminothiophene-2-carboxylate (2.67 g, 17 mmol) in xylene (80 mL), containing toluene-4-sulphonic acid (10 mg), and refluxed vigorously for 50 min, with removal of ethanol by distillation. After cooling, the solution was added dropwise to 0.4 M ethanolic sodium ethoxide (45 mL, 18 mmol) and the mixture heated under reflux for 2 h. The reaction mixture was concentrated, the residue was diluted with water and extracted with ether. The pH of the aqueous layer was adjusted to 4, the mixture chilled, and filtered to afford ethyl 4,7-dihydro-7-oxo-5-phenylthieno[3,2-*b*]pyridine-6-carboxylate (4.1 g, 81%), mp 239–242 °C. Treatment of the foregoing pyridone (3.8 g, 12.7 mmol) with phosphorus oxychloride (40 mL) with use of the method as described in ref 10 gave **3a** (3.75 g, 93%) as pale yellow crystals: mp 73–75 °C; NMR ($CDCl_3$) δ 1.10 (3 H, t, J = 7 Hz), 4.24 (2 H, q, J = 7 Hz), 7.30–7.80 (6 H, m), 7.93 (1 H, d, J = 6 Hz). Anal. ($C_{16}H_{12}NO_2SCl$) C, H, N.

The compound **3b** in Table II was prepared in a similar manner.

Ethyl 7-(2-(4-Chlorophenyl)hydrazino)-5-phenylthieno-[3,2-*b*]pyridine-6-carboxylate, Monohydrochloride (4a) (Method F). A solution of **3a** (3.05 g, 9.6 mmol) and (4-

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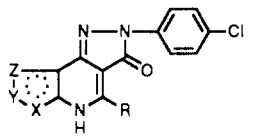
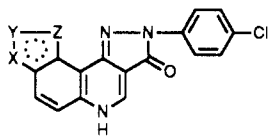
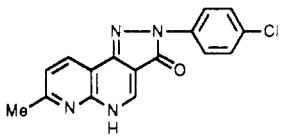
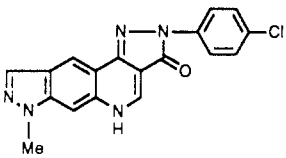
Table V. Physical Properties of **5** Prepared by Methods A and B

compound	X	Y	Z	R	method	% yield	mp, °C	formula
								
5a	CH	CH	S	Ph	B	60	285–290	$C_{20}H_{12}N_3OSCl\cdot0.75H_2O$
5b	CH	CH	S	Me	A	89	321–325	$C_{15}H_{10}N_3OSCl$
5c	CH	CH	S	H	A	30	336–340	$C_{14}H_8N_3OSCl\cdot H_2O$
5d	S	CEt	CH	H	A	37	295–300	$C_{16}H_{12}N_3OSCl\cdot H_2O$
5e	CH	S	CH	H	B	88	>300 dec	$C_{14}H_8N_3OSCl\cdot0.5H_2O$
5f	benzo fused		S	H	B	75	>330	$C_{18}H_{10}N_3OSCl\cdot0.5H_2O$
5h	S	N	CMe	H	A	68	>340	$C_{14}H_9N_4OSCl$[a]
5i	benzo fused		NMe	H	B	61	>320	$C_{19}H_{13}N_4OCl\cdot H_2O$
								
5j	CH	N	MeN		A	40	>300	$C_{18}H_{12}N_5OCl\cdot1.5H_2O$
5k	MeN	N	CH		A	69	>300	$C_{18}H_{12}N_5OCl\cdot0.5H_2O$
5l	CH	MeN	N		A	56	>300	$C_{18}H_{12}N_5OCl\cdot H_2O$
5n	N	MeN	CH		A	55	>300	$C_{18}H_{12}N_5OCl\cdot H_2O$
5o	MeN	CH	N		A	88	>300	$C_{18}H_{12}N_5OCl\cdot2H_2O$
								
5g					A	64	332–336	$C_{16}H_{11}N_4OCl\cdot H_2O$
								
5m					A	88	>300	$C_{18}H_{12}N_5OCl\cdot2H_2O$

[a] C, N: calcd, 53.08, 17.69; found, 52.40, 16.29. MS *m/e* 316.0183 (M^+).

chlorophenyl)hydrazine (1.38 g, 9.6 mmol) in dry ethanol (30 mL) was heated under reflux and under nitrogen, for 18 h. The solution was cooled to 5 °C and the resultant solid removed by filtration. Recrystallization from ethanol gave **4a** (3.4 g, 77%) as white crystals: mp 218–220 °C; NMR (DMSO-d_6) δ 0.87 (3 H, t, J = 7 Hz), 4.04 (2 H, q, J = 7 Hz), 6.92 (2 H, d, J = 9 Hz), 7.32 (2 H, d, J = 9 Hz), 7.62 (5 H, s), 7.65 (1 H, d, J = 6 Hz), 8.38 (1 H, d, J = 6 Hz), 9.18 (1 H, s, exchanges with D_2O). Anal. ($C_{22}H_{19}N_3O_2SCl_2$) C, H, N.

The other compounds **4b–f** in Table IV were prepared in a similar manner.

2-(4-Chlorophenyl)-7-methyl-3*H*-pyrazolo[4,3-*c*]-1,8-naphthyridin-3-one (5g) (Method A). A solution of **3g** (1.0 g, 4.24 mmol) and (4-chlorophenyl)hydrazine (0.75 g, 5.24 mmol) in sodium dry xylene (25 mL) was heated under reflux and under nitrogen, for 8 h. The resultant orange precipitate was removed by filtration and dissolved in 10% aqueous sodium hydroxide. The solution was washed with ethyl acetate and the pH of the aqueous layer adjusted to 8.5 with saturated ammonium chloride solution. The resultant precipitate was filtered, washed well with water, methanol, and ether, and dried in vacuo. Recrystallization from methanol/chloroform gave **5g** (0.84 g, 64%) as gold needles: mp 332–336 °C; NMR (DMSO-d_6) δ 2.64 (3 H, s), 7.35 (3 H, m), 8.25 (2 H, d, J = 8.5 Hz), 8.46 (1 H, d, J = 8 Hz), 8.53 (1 H, s); MS *m/e* 310.0635 (M^+). Anal. ($C_{16}H_{11}N_4OCl\cdot H_2O$) C, H, N.

2-(4-Chlorophenyl)-5,10-dihydro-10-methyl-2*H*-dipyrazolo[4,3-*c*:3′,4′-*f*]quinolin-3-one (5j) was prepared from **11b** according to method A except that the product was reprecipitated with 5 M H_2SO_4 and the yellow solid washed with water, ethanol and dried in vacuo: mp >300 °C; NMR (DMSO-d_6) δ 4.90 (3 H, s), 7.40 (1 H, d, J = 9 Hz), 7.50 (2 H, d, J = 9 Hz), 7.95 (1 H, d, J = 9 Hz), 8.20 (1 H, s), 8.25 (2 H, d, J = 9 Hz), 8.75 (1 H, s); MS *m/e* 349.0722 (M^+). Anal. ($C_{18}H_{12}N_5OCl\cdot1.5H_2O$) C, H, N.

The other compounds **5b–d**, **5h**, and **5k–5o** in Table V were prepared in a similar manner.

2-(4-Chlorophenyl)-2,5-dihydro-3*H*-pyrazolo[3,4-*d*]-1-benzo[*b*]thieno[3,2-*b*]pyridin-3-one (5f) (Method B). A stirred solution of **4f** (1.14 g, 2.64 mmol) in *sec*-butyl alcohol (45 mL) containing 2 equiv of potassium carbonate (0.73 g) was heated under reflux and under nitrogen, for 20 h. Workup as in method A gave **5f** (0.74 g, 80%) as a yellow powder: mp 330 °C dec; NMR (DMSO-d_6) δ 7.25–8.60 (9 H, m), 8.85 (1 H, s); MS *m/e* 351.0222 (M^+). Anal. ($C_{18}H_{10}N_3OSCl\cdot0.5H_2O$) C, H, N.

The compounds **5a**, **5e**, and **5i** in Table V were prepared in the same manner.

2-(4-Chlorophenyl)-2,5-dihydro-5-methyl-3*H*-pyrazolo-[3,4-*d*]thieno[3,2-*b*]pyridin-3-one (6). Sodium hydride (80%; 0.12 g, 4.0 mmol) was added to a stirred suspension of **5c** (1.00 g, 3.3 mmol) in dry tetrahydrofuran (20 mL) at 25 °C, under nitrogen. Stirring was continued for 30 min and iodomethane (0.3 mL, 4.8 mmol) was added over a period of 1 h. After 18 h, the yellow precipitate was removed by filtration, washed with ether and recrystallization from tetrahydrofuran gave **6** (0.64 g, 61%): mp 300 °C dec; NMR (DMSO-d_6) δ 4.07 (3 H, s), 7.48 (2 H, d, J = 9 Hz), 7.66 (1 H, d, J = 6 Hz), 8.11 (1 H, d, J = 6 Hz), 8.24

(2 H, d, J = 9 Hz), 8.78 (1 H, s); MS m/e 315.0224 (M^+). Anal. ($C_{15}H_{10}N_3OSCl·0.5H_2O$) C, H, N.

2-(4-Chlorophenyl)-1,2-dihydro-1-methyl-3*H*-pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridin-3-one (7a). A mixture of **5c** (0.71 g, 2.36 mmol) and dimethyl sulfate (20 mL) was heated at 110 °C for 18 h and then evaporated to dryness. The residue was partitioned between 10% aqueous sodium hydroxide and dichloromethane. The organic phase was dried, and evaporation gave a green solid (0.50 g) which on recrystallization from charcoal–methanol afforded **7a** (0.28 g, 38%) as beige crystals: mp >300 °C; NMR (DMSO-d_6) δ 3.46 (3 H, s), 7.64 (4 H, s), 7.78 (1 H, d, J = 5 Hz), 8.41 (1 H, d, J = 5 Hz), 9.04 (1 H, s); MS m/e 315.0240 (M^+). Anal. ($C_{15}H_{10}N_3OSCl$) C, H, N.

3-Chloro-2-(4-chlorophenyl)-2*H*-pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridine (8a). A mixture of **5c** (0.70 g, 2.32 mmol) and phosphorus oxychloride (15 mL) was heated under reflux for 2 h. Evaporation gave a solid which was partitioned between dichloromethane and saturated aqueous sodium hydrogen carbonate. The organic phase was dried, and evaporation gave a white solid (0.70 g). Recrystallization from acetone gave **8a** (0.61 g, 82%) as white crystals: mp 131–132 °C; NMR ($CDCl_3$) δ 7.50–7.85 (6 H, m), 9.09 (1 H, s); MS m/e 318.9748 (M^+). Anal. ($C_{14}H_7N_3SCl_2$) C, H, N.

2-(4-Chlorophenyl)-3-methoxy-2*H*-pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridine (8b). A stirred suspension of **8a** (1.0 g, 3.13 mmol) and potassium *tert*-butoxide (0.37 g, 3.30 mmol) in dry methanol (90 mL) was heated under reflux, under nitrogen, for 18 h. Evaporation gave a residue which was partitioned between chloroform and water. The organic phase was dried and evaporation, followed by recrystallization from methanol–ether, gave **8b** (0.82 g, 83%) as off-white crystals: mp 180 °C dec; NMR ($CDCl_3$) δ 4.50 (3 H, s), 7.48 (2 H, d, J = 10 Hz), 7.60 (2 H, s), 7.83 (2 H, d, J = 10 Hz), 9.16 (1 H, s). Anal. ($C_{15}H_{10}N_3OSCl$) C, H, N.

Shock-Induced Suppression of Drinking in the Rat. Naive rats (Hacking and Churchill CFY, male 200–250 g) were deprived of water overnight before being familiarized to the test apparatus on the day prior to the test. In the familiarization period, rats were allowed to drink freely for 3 min with no footshock. Water was then provided for a 4-h period before being withdrawn overnight. On the test day animals were allowed to drink freely for 30 s and then received a mild footshock (0.225 mA for 0.5 s) for every 5 s of drinking time accumulated in a 3 min session. Drugs were administered intraperitoneally to groups of 6–12 rats 30 min before testing. The number of shocks taken during a given test period was recorded and drug effects expressed as the percentage change from controls. Statistical comparisons were made using the Mann Whitney "U" test (two-tailed). Anxiolytic agents cause a significant release of behavior suppressed by punishment.

Horizontal Wire Test for Sedative/Muscle Relaxant Properties. Male CD-1 mice (19–25 g) were randomly allocated to groups of 10 and dosed intraperitoneally with drug or vehicle and tested 0.5, 1, 2, 4, and 6 h postdose for traction. Mice were lifted by the tail and allowed to grasp a horizontally strung wire (20-cm height and 1-mm diameter) with their forepaws and then released. The number of mice per treatment group that did not actively grasp the wire with at least one hind paw within 10 s was determined. In control animals this number was consistently zero. The ED_{50} was calculated graphically by the method of Litchfield and Wilcoxon[20] and is the dose which renders 50% of the animals incapable of grasping the wire with at least one hind paw.

Anticonvulsant Properties. Animals were lightly restrained and PTZ (8 mg/mL) infused at a constant rate of 0.5 mL/min into a tail vein. The time taken to induce tonic extension of the hindlimbs was recorded and the dose of PTZ required to produce the seizure calculated from the equation:

$$\text{dose (mg/kg) of PTZ} = \frac{\text{time to convulsion (s)}(0.5)(8)}{60} \frac{(1000)}{\text{body wt (g)}}$$

Drugs were administered orally to groups of 10 mice (Charles River CD-1, male 18–24 g) 60 min before testing. Drug effects are expressed as the percentage change in seizure threshold relative to control values and statistical comparisons made by using a Student's t test (two-tailed). Anticonvulsant agents increase the dose of PTZ required to induce the tonic seizure.

Radioligand Binding Studies. [^{3}H]Flunitrazepam selectively labels BZ receptors and displacement of this specific binding in vitro by novel compounds in well-washed, frozen rat whole brain membranes was measured essentially as described by Martin and Candy.[11] At the fixed concentration of 0.5 nM used, specific binding of ^{3}H ligand represents 80–90% of the total radioactivity bound. Nonspecific binding was defined by 10 μM clonazepam. IC_{50} values were calculated from log [concn] against percentage inhibition curves; K_i values were determined by using the Cheng–Prusoff equation.

All determinations were performed in triplicate.

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Registry No. **3a**, 96516-59-5; **3b**, 96516-55-1; **3c**, 96516-52-8; **3d**, 96516-60-8; **3e**, 96516-63-1; **3f**, 128056-39-3; **3g**, 128056-40-6; **3h**, 128056-41-7; **3i**, 128056-42-8; **4a**, 96516-79-9; **4b**, 96516-77-7; **4c**, 128056-43-9; **4d**, 128056-44-0; **4e**, 96516-83-5; **4f**, 128056-45-1; **5a**, 96516-15-3; **5b**, 96516-85-7; **5c**, 96516-86-8; **5d**, 96516-09-5; **5e**, 96539-76-3; **5f**, 128056-46-2; **5g**, 92972-72-0; **5h**, 128056-47-3; **5i**, 128056-48-4; **5j**, 128056-49-5; **5k**, 128056-50-8; **5l**, 128056-51-9; **5m**, 128056-52-0; **5n**, 128056-53-1; **5o**, 128056-54-2; **6**, 96516-13-1; **7a**, 96516-14-2; **8a**, 128056-55-3; **8b**, 128056-56-4; **11a**, 128056-57-5; **11b**, 128056-58-6; **11c**, 128056-59-7; **11d**, 128056-60-0; **11e**, 128056-61-1; 5-aminoindazole, 19335-11-6; diethyl (ethoxymethylene)malonate, 87-13-8; diethyl [[*N*-(2-methyl-5-indazolyl)amino]methylene]malonate, 128083-62-5; diethyl [[*N*-(1-methyl-5-indazolyl)amino]methylene]malonate, 128056-62-2; ethyl 3-ethoxycinnamate, 57293-23-9; ethyl 3,3-diethoxy-3-phenylpropionate, 96516-58-4; methyl 3-aminothiophene-2-carboxylate, 22288-78-4; ethyl 4,7-dihydro-7-oxo-5-phenylthieno[3,2-*b*]pyridine-6-carboxylate, 96516-57-3; (4-chlorophenyl)hydrazine, 1073-69-4; ethyl 8-chloro-5,8-dihydro-3-methylpyrazolo[4,3-*g*]quinoline-7-carboxylate, 128056-63-3.

(20) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.