N-Ethyl-N-n-propyl-2-(4-fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (27). Compound **27** was prepared from **19**: yield 92%; uncrystallizable; NMR (DMSO-d_6) δ 9.52 (bs, 1 H, OH), 7.08 (dd, 1 H, H-5), 6.88 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 4.0 (bs, 1 H, NH^+), 3.20, 3.08, and 2.92 (three m, 8 H, 4 CH_2), 1.67 (m, 2 H, CCH_2C), 1.25 (t, 3 H, $NCCH_3$), 0.90 (t, 3 H, CH_3). Anal. ($C_{13}H_{21}BrFNO$) C, H, N.

N-Ethyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (28). Compound **28** was prepared from **20**: yield 89%; uncrystallizable; NMR (DMSO-d_6) δ 9.90 (bs, 1 H, OH), 7.32 and 7.25 (two m, 5 H, Harom), 7.05 (dd, 1 H, H-5), 6.92 (dd, 1 H, H-2), 6.73 (m, 1 H, H-6), 4.42 (bs, 1 H, NH^+), 3.32 (m, 6 H, 3 NCH_2), 3.05 and 2.96 (two t, 4 H, $ArCH_2$), 1.28 (t, 3 H, CH_3). Anal. ($C_{18}H_{23}BrFNO$) C, H, N.

N,N-Di-n-propyl-2-(4-fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (29). Compound **29** was prepared from **21**: yield 75%; mp 123-4 °C; NMR (DMSO-d_6) δ 9.85 (s, 1 H, OH), 9.22 (bs, 1 H, NH^+), 7.10 (dd, 1 H, H-5), 6.88 (dd, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.22 and 3.05 (two m, 6 H, 3 NCH_2), 2.90 (m, 2 H, $ArCH_2$), 1.50 (m, 4 H, 2 CCH_2C), 0.90 (t, 6 H, 2 CH_3). Anal. ($C_{14}H_{23}BrFNO$) C, H, N.

N-n-Propyl-N-(2-phenylethyl)-2-(4-fluoro-3-hydroxyphenyl)ethylamine Hydrobromide (30). Compound **30** was prepared from **22**: yield 83%; mp 138-40 °C; NMR (DMSO-d_6) δ 9.85 (s, 1 H, OH), 9.52 (bs, 1 H, NH^+), 7.28 (m, 5 H, Harom), 7.10 (dd, 1 H, H-5), 6.90 (m, 1 H, H-2), 6.72 (m, 1 H, H-6), 3.52, 3.31, and 3.26 (three m, 6 H, 3 NCH_2), 2.99 and 2.90 (two t, 4 H, 2 $ArCH_2$), 1.70 (m, 2 H, 2 CCH_2C), 0.90 (t, 3 H, CH_3). Anal. ($C_{19}H_{25}BrFNO$) C, H, N.

N-n-Propyl-N-(2-phenylethyl)-2-(3-fluoro-4-hydroxyphenyl)ethylamine Hydrobromide (31). Compound **31** was prepared from **25**: yield 86%; mp 154-55 °C; NMR (DMSO-d_6) δ 9.78 (s, 1 H, OH), 9.65 (bs, 1 H, NH^+), 7.30 (m, 5 H, Harom), 7.16 (dd, 1 H, H-2), 6.92 (m, 2 H, H-5,6), 3.45 and 3.17 (two m, 6 H, 3 NCH_2), 3.05 and 2.95 (two t, 4 H, 2 $ArCH_2$), 1.72 (m, 2 H, CCH_2C), 0.90 (t, 3 H, CH_3). Anal. ($C_{19}H_{25}BrFNO$) C, H, N.

Pharmacology. Binding Studies. Adult Sprague-Dawley rats were obtained from Charles River (Calco, Italy). [^{3}H]SCH 23390 (specific activity 77.7 Ci/mmol) and [^{3}H]spiperone (specific activity 24 Ci/mmol) were purchased from New England Nuclear. Unlabeled SCH 23390 was a generous gift of Dr. Ongini (Essex, Italy). The following substances were obtained commercially: apomorphine hydrochloride, dopamine hydrochloride (Sigma Chemical Co., St. Louis, MO).

Radioreceptor binding studies were performed by using rat striatal membrane preparations. The tissue was homogenized in 100 volumes of Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 20.000g for 10 min. The resultant pellet was rehomogenized in buffer and centrifuged again. The final pellet was resuspended in 50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ to a final concentration of 3.5 mg wet weight/mL. Aliquots of 400 μL of membrane suspension were added to ice cold tubes containing 50 μL of [^{3}H]spiperone (final concentration 0.1 nM) or [^{3}H]SCH 23390 (final concentration 1 nM), 50 μL of displacer (6-7 different concentrations) in a final volume of 0.5 mL. Specific binding was determined in the presence of (+)-butaclamol (1 μM) for [^{3}H]-spiperone, and unlabeled SCH 23390 (10 μM) for [^{3}H]SCH 23390 binding. The experiments were performed in triplicate, and replicated three times on different days. Displacement analysis and IC_{50} determinations were carried out by using a computerized log-probit plot program. The new compounds were tested as hydrobromides.

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Registry No. **3**, 452-84-6; **4**, 63762-79-8; **5**, 63762-78-7; **6**, 82846-18-2; **7**, 128495-45-4; **8**, 128495-46-5; **9**, 128495-47-6; **10**, 128495-48-7; **11**, 128495-49-8; **12**, 128495-50-1; **13**, 128495-51-2; **14**, 128495-52-3; **15**, 128495-53-4; **16**, 128495-54-5; **17**, 128495-55-6; **18**, 128495-56-7; **19**, 128495-57-8; **20**, 128495-58-9; **21**, 128495-59-0; **22**, 128495-60-3; **23**, 128495-61-4; **24**, 128495-62-5; **25**, 128495-63-6; **26**, 128495-64-7; **27**, 128495-65-8; **28**, 128495-66-9; **29**, 128495-67-0; **30**, 128495-68-1; **31**, 128495-69-2; 2-(3-fluoro-4-methoxyphenyl)-ethylamine, 458-40-2.

Synthesis of Substituted 7,12-Dihydropyrido[3,2-*b*:5,4-*b*']diindoles: Rigid Planar Benzodiazepine Receptor Ligands with Inverse Agonist/Antagonist Properties

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A series of 1-, 2-, 3-, 4-, 5-, 6-, 7-, 10-, and 12-substituted pyridodiindoles were synthesized and screened in vitro against [^{3}H]diazepam for activity at the benzodiazepine receptor (BzR). In vitro, the 2-substituted pyridodiindoles were found to be the most potent (IC_{50} < 10 nM) of this new class of BzR ligands. In vivo, 2-methoxypyridodiindole **19a** (IC_{50} = 8 nM) was found to be the most potent partial inverse agonist (proconvulsant) of the series. The parent compound **2** (IC_{50} = 4 nM) was only slightly less potent. In addition, 2-hydroxypyridodiindole **21a** (IC_{50} = 6 nM) was found to exhibit potent proconvulsant activity when administered as a prodrug derivative, pivaloyl ester **22**. 2-Chloropyridodiindole **16a** (IC_{50} = 10 nM) was devoid of proconvulsant activity; however, **16a** was found to be the most potent antagonist of the anticonvulsant effects of diazepam in this class of BzR ligands. From the in vivo data available, substitution on ring E of **2** with electron-withdrawing groups results in antagonists at BzR, while replacement of hydrogen at C-2 with electron-releasing groups provides enhanced inverse agonist activity. The pyridodiindoles were used as "templates" for the formulation of a model of the inverse agonist/antagonist active site of the BzR. The proposed model consists of a hydrogen bond acceptor site (A^1) and a hydrogen bond donor site (D^2) disposed 6.0-8.5 Å from each other on the receptor protein. The hydrogen-bonding sites are believed to be located at the base of a narrow cleft. A large lipophilic pocket at the mouth of the narrow cleft serves to direct molecules into the binding site, while the presence of a small lipophilic pocket permits substitution only at position 2 of the pyridodiindole nucleus for maximum binding potency.

Since the discovery of benzodiazepine receptors (BzR) in 1977[1,2] more than a half-dozen structurally unique classes of ligands have been identified.[3-13] These ligands exhibit actions along a pharmacological continuum ranging from complete mimicry of the 1,4-benzodiazepines (full

† NIH.

(1) Squires, R. F.; Braestrup, C. *Nature* **1977**, *266*, 732.
(2) Mohler, H.; Okada, T. *Life Sci.* **1977**, *20*, 2101.

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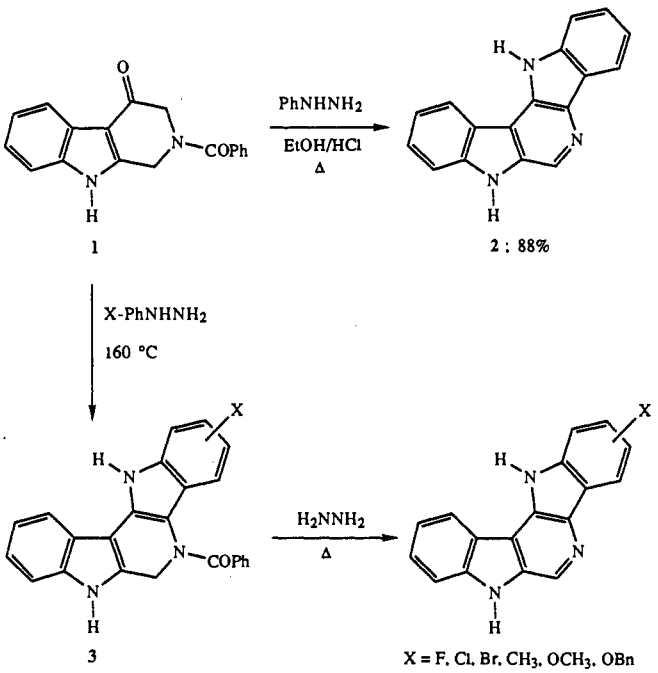
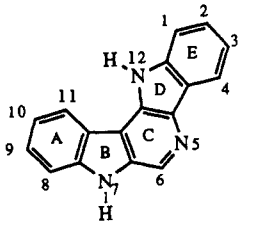
Scheme I



agonists)[3-13] to substances that produce convulsions,[14] reduce sleep,[15] and produce a syndrome resembling fear or anxiety (inverse agonists).[16]

The BzR ligands are thought to elicit their pharmacological actions by modulating the activity of GABA-gated chloride channels, constituted by at least three homologous but distinct proteins.[17] At present, the nature of the binding sites accommodating inverse agonist/antagonist and agonist ligands has not been resolved.[18-20] Structure–activity relationship (SAR) studies of various BzR ligands have led to the formulation of several models of the binding site of the BzR. Some of these models employ an active-analogue approach using both agonist and inverse agonist ligands in combined basis sets.[21-26] Several lines of evidence suggest that the inverse agonist/antagonist and agonist binding sites may be treated as separate entities in which these ligands bind to specific domains of the BzR.[18] With this strategy, specific ligand basis sets of similar efficacy have been employed to formulate active-site models (i.e., inverse agonist/antagonist and agonist

Table I. Potencies of Substituted Pyridodiindoles



agent[a]	X	% yield (procedure)	IC_{50},[b] nM	analyses[c]
2	H	88 (A)	4[d]	$C_{17}H_{11}N_3$
4	3-F	69 (C)	6[d]	$C_{17}H_{10}N_3F$
5	3-Cl	68 (C)	2150[d]	$C_{17}H_{10}N_3Cl$[e]
6	3-Br	40 (C)	750	$C_{17}H_{10}N_3Br \cdot HCl \cdot {}^1/_2H_2O$
7	3-CH_3	58 (C)	224	$C_{18}H_{13}N_3 \cdot HCl \cdot H_2O$
8	3-OCH_3	90 (A)	890[d]	$C_{18}H_{13}N_3O \cdot HCl \cdot {}^1/_4H_2O$[e]
9	3-OBn	58 (C)	1600	$C_{24}H_{17}N_3O$
10	3-OH	81 (D)	114	$C_{17}H_{11}N_3O$
11	1-F	51 (B)	12[d]	$C_{17}H_{10}N_3F$
12	1-Cl	51 (B)	80[d]	$C_{17}H_{10}N_3Cl$
13	1-Br	25 (B)	28	$C_{17}H_{10}N_3Br \cdot HCl$
14	1-CH_3	71 (B)	83	$C_{18}H_{13}N_3 \cdot HCl \cdot {}^4/_5H_2O$
15	2-F	50	7[d]	$C_{17}H_{10}N_3F$
16a	2-Cl	64	10[d]	$C_{17}H_{10}N_3Cl$
17a	2-Br	33	19	$C_{17}H_{10}N_3Br$
18a	2-CH_3	36	8	$C_{18}H_{13}N_3 \cdot HCl$
19a	2-OCH_3	66	8[d]	$C_{18}H_{13}N_3O \cdot HCl \cdot {}^3/_4H_2O$
20a	2-OBn	26	>4000	$C_{24}H_{17}N_3O$
21a	2-OH	90 (D)	6	$C_{17}H_{11}N_3O$
22	2-Piv	79	200[f]	$C_{21}H_{19}N_3O_2$
16b	4-Cl	11	715[d]	$C_{17}H_{10}N_3Cl$
18b	4-CH_3	4	>5000	$C_{18}H_{13}N_3 \cdot HCl \cdot {}^3/_4H_2O$
19b	4-OCH_3	12	250	$C_{18}H_{13}N_3O$
20b	4-OBn	6	>4000	$C_{24}H_{17}N_3O$
21b	4-OH	57 (D)	578	$C_{17}H_{11}N_3O$
33	6-C_2H_5	35	250	$C_{19}H_{15}N_3 \cdot HCl \cdot {}^1/_4H_2O$
34	7-CH_3	77[g]	1136	$C_{18}H_{13}N_3 \cdot HCl$[g]
35	12-CH_3	32[g]	157	$C_{18}H_{13}N_3 \cdot HCl \cdot {}^1/_4H_2O$
36	7,12-CH_3	36[g]	1970	$C_{19}H_{15}N_3 \cdot HCl$[g]

[a] All pyridodiindoles were tested as the hydrochloride salt. [b] Measured against [^{3}H]diazepam, and flunitrazepam (10 μM) was used to define nonspecific binding. [c] Analyzed for C, H, and N. [d] Previously cited in ref 31. [e] See ref 33 for experimental details. [f] Measured against [^{3}H]flunitrazepam, and Ro 15-1788 (10 μM) was used to define nonspecific binding. [g] See ref 36 for experimental details.

(3) Skolnick, P.; Paul, S. *GABA and Benzodiazepine Receptors*; Squires, R., Ed.; CRC Press: Boca Raton, FL, 1988; Vol. II, pp 91–102.
(4) Greenblatt, D. J.; Shader, R. I. *Benzodiazepines in Clinical Practice*; John Wiley and Sons: New York, 1974.
(5) Tallman, J. F.; Paul, S. M.; Skolnick, P.; Gallagher, D. W. *Science* **1980**, *207*, 274.
(6) Haefely, W.; Piere, L.; Polc, P.; Schaffner, R. *Handbook of Experimental Pharmacology*, Hoffmeister, F.; Stille, G., Eds.; Springer-Verlag: Berlin, 1981; Part II, pp 55, 13.
(7) Schmidt, R. F.; Vogel, M. E.; Zimmermann, M. *Naunyn Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **1967**, *258*, 69.
(8) Gray, J. A. *Handbook of Psychopharmacology*, Iversen, L. L.; Iversen, S.; Snyder, S. H., Eds.; Plenum Press: New York, 1977; p 433.
(9) Braestrup, C.; Honore, T.; Nielsen, M.; Petersen, E. N.; Jensen, H. *Biochem. Pharmacol.* **1984**, *33*, 859.
(10) Stephens, D. N.; Shearman, G. T.; Kehr, W. *Psychopharmacology* **1984**, *83*, 233.
(11) Loscher, W.; Schneider, H.; Kehr, W. *Eur. J. Pharmacol.* **1985**, *114*, 261.
(12) Hagen, T. J.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1985**, *30*, 750.
(13) Wettstein, J. G.; Spealman, R. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 471.
(14) Schweri, M.; Cain, M.; Cook, J. M.; Paul, S. M.; Skolnick, P. *Pharmacol. Biochem. Behav.* **1982**, *17*, 457.
(15) Mendelson, W. B.; Cain, M.; Cook, J. M.; Paul, S. M.; Skolnick, P. *Science* **1983**, *219*, 414.
(16) Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. *Science* **1982**, *218*, 1332.
(17) Pritchett, D. B.; Sontheimer, M.; Shivers, B. D.; Ymer, S.; Kettenmann, H.; Schofield, P. R.; Seeburg, P. H. *Nature* **1989**, *338*, 582.
(18) (a) Skolnick, P.; Schweri, M.; Kutter, E.; Williams, E.; Paul, S. M. *J. Neurochem.* **1982**, 1142. (b) Fujimoto, M.; Hirae, F.; Okadayaski, T. *Life Sci.* **1982**, *30*, 51. (c) Mhatre, M.; Ticku, M. K. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 164.
(19) Hirsh, J. D.; Garrett, M.; Beer, B. *Pharmacol. Biochem. Behav.* **1985**, *23*, 681.
(20) Ehlert, F. J.; Roeske, R.; Gee, K. W.; Yamamura, H. *Biochem. Pharmacol.* **1983**, *32*, 274.
(21) Crippen, G. M. *Mol. Pharmacol.* **1982**, *22*, 11.
(22) Loew, G. H.; Nienow, J. R.; Paulson, M. *Mol. Pharmacol.* **1984**, *26*, 19.
(23) Codding, P. W. *Mol. Pharmacol.* **1985**, *28*, 178.
(24) Fryer, R. I. *Life Sci.* **1986**, *36*, 1947.
(25) Borea, P. A.; Gilli, G.; Bertolasi, V.; Ferretti, V. *Mol. Pharmacol.* **1986**, *31*, 334.
(26) Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 153.

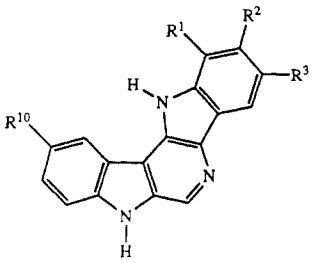
site models).[27,28] A comparison of all these models reveals that, in general, the only common feature required for high-affinity binding is that the ligand assume a planar or pseudoplanar topography.[29,30] However, each individual model differs in the manner in which agonist ligands interact with the active site of the receptor relative to inverse agonist ligands. In an attempt to unequivocally identify primary ligand–receptor interactions, the SAR of the rigid, planar 7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindoles has been investigated.[31] This is the only class of BzR ligands which possesses a planar topography and is completely rigid. The pyridodiindoles are termed "templates", since the rigid nature of these BzR inverse agonist/antagonist ligands permits formulation of a qualitative model of the inverse agonist/antagonist active site.

Chemistry

The phenylhydrazone of 2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (**1**)[32] was generated in situ in a solution of ethanol. A mixture of ethanolic hydrogen chloride was added and the solution was heated to reflux for 1 h (procedure A). This afforded the 7,12-dihydropyridodiindole **2** as the sole product in 88% yield, which resulted from an acid-mediated Fischer indole cyclization (Scheme I).[33] The 1-substituted and 3-substituted pyridodiindoles were readily prepared by heating **1** in a 5–10-fold excess of the appropriately substituted phenylhydrazine for 4–6 h at 160 °C. This usually furnished the intermediate benzamide derivative **3** via a thermally mediated Fischer indole cyclization (Scheme I). Generally, **3** was not isolated, but rather a 25-fold excess of anhydrous hydrazine was added to the reaction mixture and the solution was heated to reflux for 12 h. This procedure gave the desired 1-substituted (procedure B) and 3-substituted (procedure C) pyridodiindoles, respectively, in yields which ranged from 50 to 90% (Table I). The 2-substituted pyridodiindoles were prepared in similar fashion by heating **1** in the corresponding 3-substituted phenylhydrazine as described above. However in this case, as expected, the Fischer indole cyclization gave two isomeric diindoles. In all cases, the 2-substituted isomer predominated over the corresponding 4-substituted analogue and the isomers could be separated and obtained in pure form by flash chromatography. The yields and synthetic procedures employed for the synthesis of the various ring E substituted analogues are summarized in Table I.

Hydroxypyridodiindoles **10**, **21a**, and **21b** were prepared from the corresponding benzyloxy analogues **9**, **20a**, and **20b** by hydrogenolysis (H_2, 45 psi) in dry ethanol over 10% palladium on carbon (procedure D). 2-Pivaloyl ester **22** was prepared from the corresponding 2-hydroxy derivative **21a**. Treatment of **21a** with pivaloyl chloride in trifluoroacetic acid at 50 °C furnished **22** in 79% yield.[34]

Table II. Potencies of the 10-Substituted 7,12-Dihydropyridodiindoles



agent[a,b]	R^1	R^2	R^3	R^{10}	IC_{50},[c] nM
2	H	H	H	H	4
23	H	H	H	NO_2	4
24	H	H	Cl	NO_2	1190
25	H	Cl	H	NO_2	126
26	H	H	H	NH_2	44
27	H	H	Cl	NH_2	>350
28	H	Cl	H	NH_2	98
29	H	H	H	Br	6
30	H	H	Br	Br	>320
31	Cl	H	H	Cl	310
32	H	H	$NaSO_3$	$NaSO_3$	5000

[a] All dihydropyridodiindoles were tested as the hydrochloride salt. [b] See ref 35 for synthetic details and analytical data. [c] Measured against [^{3}H]diazepam, and flunitrazepam (10 μM) was used to define nonspecific binding.

The 10-substituted pyridodiindoles (Table II) were prepared from the corresponding ring E substituted derivatives by electrophilic aromatic substitution reactions in acidic media.[35] Protonation of the pyridyl nitrogen atom N(5) effectively directed electrophilic substitution toward position 10 with a high degree of regioselectivity in excellent yield.[35]

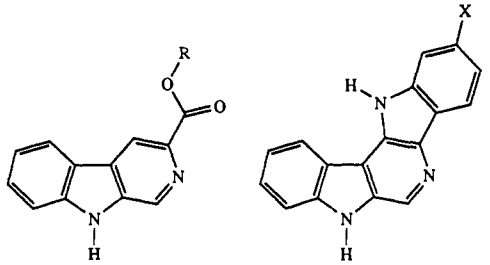
Biological Results and Discussion

The potencies of compounds **2–22** (Table I) strongly suggest that both steric and to a lesser degree electronic constraints are imposed upon the rigid pyridodiindole ligands by the receptor site.[31] The receptor site will tolerate the small fluoro substituent at any position in ring E of **2** (**4**, **11**, and **15**). However, larger substituents at position 3 (**5–10**) resulted in a dramatic decrease in the potency of these ligands. Substitution at position 1 with larger substituents (**12–14**) decreased ligand affinity by approximately 1 order of magnitude compared to that of the parent compound **2**. As previously reported, in contrast to the 1- and 3-substituted pyridodiindoles, the 2-substituted congeners exhibited the highest affinities for the BzR in this class of ligands.[31] As illustrated in Table I, the electronic nature of the substituent at position 2 did not affect the in vitro affinity of the ligand. However, large bulky substituents at position 2, such as benzyloxy (**20a**) or pivolyl (**22**), greatly reduced the potency of the ligand for BzR. Lastly, substitution at position-4 decreased the affinity of the pyridodiindoles for BzR by at least 2 orders of magnitude (Table I).

Examination of the in vitro binding data of ring E substituted pyridodiindoles (Table I) indicates that, in general, electronic effects exhibit only a small influence on the affinity of the pyridodiindoles; both electron-withdrawing and electron-releasing substituents at position

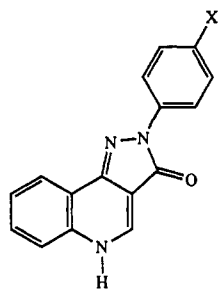
(27) (a) Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, P. W.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1988**, *31*, 1854. (b) Codding, P. W.; Roszak, A. W.; Szkaradzinska, M. B.; Cook, J. M.; Aha, L. I. *Trends Med. Chem.* **1988**, 109.
(28) Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1990**, *33*, 1062.
(29) Hagen, T. J.; Guzman, F.; Schultz, C.; Cook, J. M.; Skolnick, P.; Shannon, H. E. *Heterocycles* **1986**, *24*, 2845.
(30) Cain, M.; Weber, R.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J.; Paul, S. M.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081.
(31) Trudell, M. L.; Basile, A. S.; Shannon, H. E.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1987**, *30*, 456.
(32) Cain, M.; Mantei, R.; Cook, J. M. *J. Org. Chem.* **1982**, *47*, 4923.
(33) Trudell, M. L.; Fukada, N.; Cook, J. M. *J. Org. Chem.* **1987**, *52*, 4293.
(34) (a) Horn, A. S.; Griever-Kazemier, H.; Dijkstra, D. *J. Med. Chem.* **1982**, *25*, 993. (b) Bergman, R.; Baldessarini, R. J.; Wilson, K. G. *J. Med. Chem.* **1976**, *19*, 717.
(35) Trudell, M. L.; Lifer, S. L.; Tan, Y. C.; England, W. B.; Cook, J. M. *J. Org. Chem.* **1988**, *53*, 4185.

Scheme II



(BCCE) R = Et
(BCCt) R = t-Bu

2; X = H
18a; X = Cl
19a; X = OCH_3



37a; X = H
37b; X = Cl
37c; X = OCH_3

2 are well-tolerated by the binding site, while substituents at position 3 are not tolerated; moreover, binding decreases somewhat with groups attached to C-1. This suggests that steric constraints imposed by the receptor binding site upon the ligand dominate the SAR of the pyridodiindoles.

Substitution of ring A appears to have a less dramatic effect on the affinity of the pyridodiindoles for BzR (Table II). Electron-withdrawing substituents placed at position 10 [**23** (10-NO_2), **29** (10-Br)] did not affect the in vitro affinity of the monosubstituted ligands. However, introduction of an electron-releasing substituent at position 10 [**26** (10-NH_2)] resulted in an approximate order of magnitude reduction of potency. This indicates that the acidity of the indole N(7)–H, which is potentiated by substituents at position 10, may influence the binding of the pyridodiindoles and strongly implies that the indole N(7)–H moiety is involved in a hydrogen-bonding interaction at the active site.[28]

The 2,10-disubstituted pyridodiindoles and the 3,10-disubstituted pyridodiindoles were found to be less potent than the corresponding 2-monosubstituted pyridodiindoles (Table II). Moreover, the 3,10-disubstituted pyridodiindoles demonstrated low potencies, similar to those observed for the 3-monosubstituted congeners. From this data it appears that interaction of the bis-substituted pyridodiindoles at BzR is more sensitive to ring E substituents than substituents attached to the A ring.

Since the pyridodiindoles (3,4-indolo-β-carbolines) are similar in structure to the β-carbolines (Scheme II), 6-ethylpyridodiindole **33** and methylated indole derivatives **34**–**36**[36] (Table I) were prepared for comparison to the SAR of the β-carbolines.[30] Similar to trends in the SAR of β-carbolines, an ethyl group at position 6 (**33**) resulted in a net reduction of ligand potency. Moreover, 7-methylpyridodiindole **34** was found to be significantly less potent than dihydro analogue **2**. Conversely, 12-methylpyridodiindole **35** maintained moderate potency, similar to that

Table III. In Vivo Activity of 7,12-Dihydropyridodiindoles

agent[a]		ED_{50}, mg/kg: proconvulsant actions[b,c]	ED_{50}, mg/kg: diazepam antagonism[b,d]	log P^e (oct/H_2O)
2		15.1[f]	21.3[f]	3.87
15	(2-F)	>30[f,g]	>30[f,h]	4.01
16a	(2-Cl)	>30[f,g]	2.5[f]	4.58
17a	(2-Br)	>40[g]	>40[h]	4.73
18a	(2-CH_3)	>40[g]	>40[h]	4.53
19a	(2-OCH_3)	7.0[f]	3.9[f]	4.04
21a	(2-OH)	>40[g]	>40[h]	0.95[i]
4	(3-F)	>30[f,g]	24.0[f]	4.01
10	(3-OH)	>40[g]	>40[h]	
22	(2-Piv)	17.5		

[a] All dihydropyridodiindoles were tested as the hydrochloride salt. [b] See ref 30 and text for biological protocols. [c] Dose necessary to induce convulsions in 50% of the mice that had been previously given a subconvulsant dose of PTZ (40 mg/kg). [d] Dose necessary to antagonize the anticonvulant effects of diazepam (2.5 mg/kg) in mice given a convulsant dose of PTZ (80 mg/kg). [e] Calculated from fragmentation constants (ref 43). [f] Previously cited in ref 31. [g] No proconvulsant action or anticonvulsant action observed at the highest dose tested. [h] Did not antagonize the anticonvulsant effects of diazepam at highest dose tested. [i] Determined experimentally (refs 43 and 44). The highest dose tested under *g* and *h* was 30 mg or 40 mg/kg, as illustrated.

of the 1-substituted congeners **12** (1-Cl) and **14** (1-CH_3). As expected the 7,12-dimethyl analogue **36** exhibited low potency. These data suggest that the pyridodiindoles may interact with the active site of the BzR in a similar fashion to that of the β-carbolines. It appears that the indole N(7)–H functionality (H-bond acceptor) is a necessary structural feature for high-affinity binding of the pyridodiindoles to BzR. These results correlate well with similar trends observed for the SAR of the β-carbolines [N(9)–H][30] and 2-arylpyrazoloquinolinones **37** [N(5)–H].[37,38]

In Vivo Data

The structural similarities between the pyridodiindoles and pyrazoloquinolinones (Scheme II) suggested that **2**, **16a**, and **19a** would exhibit pharmacologic profiles similar to those of the 2-aryl-2,5-dihydro-5*H*-pyrazolo[4,3-*c*]-quinolin-3-ones, CGS-8216 (**37a**, antagonist/partial inverse agonist), CGS-9896 (**37b**, partial agonist), and CGS-9895 (**37c**, partial agonist/antagonist), respectively.[37,39] Likewise, efficacy similar to those of the structurally related β-carboline-3-carboxylic acid esters (BCCE, partial inverse agonist,[16] and/or BCCt, antagonist[40]) was also anticipated.

Since the pharmacological classification of the CGS compounds **37a**, **37b**, and **37c** was based in part on their abilities to affect pentalenetetrazole (PTZ) induced convulsions,[39,41] the pyridodiindoles were examined in similar paradigms. The diindoles were tested in mice for anticonvulsant properties with the convulsant PTZ (80 mg/kg). Under these conditions, none of the compounds tested at

(36) Trudell, M. L.; Tan, Y. C.; Cook, J. M. *J. Org. Chem.* **1988**, *53*, 4873.

(37) Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.

(38) (a) Takada, S.; Shindo, H.; Sasatini, T.; Chomei, H.; Matushita, A.; Eigyo, M.; Kawaski, K. K.; Murata, S.; Takahara, Y.; Shintaku, M. *J. Med. Chem.* **1988**, *31*, 1738. (b) *Ibid.* **1987**, *30*, 454.

(39) Gardner, C. R. *Prog. Neurobiol.* **1988**, *31*, 425.

(40) Shannon, H. E.; Guzman, F.; Cook, J. M. *Life Sci.* **1984**, *35*, 2227.

(41) (a) Katzman, N.; Shannon, H. E. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 589. (b) Brown, C.; Martin, I. *Eur. J. Pharmacol.* **1985**, *106*, 167. (c) Petrack, B.; Czernick, A.; Cassidy, J.; Bernard, P.; Yokoyama, N. In *Benzodiazepine Recognition Site Ligands. Biochemistry and Pharmacology*; Biggio, G., Costa, E., Eds.; Raven Press: New York, 1983; p 129.

doses up to 40 mg/kg (Table III) demonstrated anticonvulsant actions inherent to BzR agonists. Proconvulsant actions were determined by employing a subthreshold dose of PTZ (40 mg/kg). The pyridodiindoles were also tested for benzodiazepine antagonist activity by assessing their ability to disrupt the anticonvulsant actions of diazepam (2.5 mg/kg) against PTZ (80 mg/kg). The in vivo data for the pyridodiindole derivatives is summarized in Table III.

The parent compound **2** was found to be proconvulsant with an ED_{50} value of 15.5 mg/kg.[31] In the proconvulsant paradigm, **2** produced a maximum of 60% seizures in the test animals. This compound also blocked the anticonvulsant effects of diazepam with an ED_{50} value of 21.3 mg/kg. 2-Methoxypyridodiindole **19a** (ED_{50} = 7.0 mg/kg) was found to be nearly twice as potent a proconvulsant as **2**. In addition, **19a** was also shown to be a potent antagonist of the anticonvulsant effects of diazepam (ED_{50} = 3.9 mg/kg).[31] In contrast, in the proconvulsant paradigm, 2-chloropyridodiindole **16a** did not exhibit proconvulsant actions even at the highest dose tested (30 mg/kg).[31]

3-Fluoropyridodiindole **4** was also found to be devoid of proconvulsant activity but did antagonize the anticonvulsant effects of diazepam, albeit with lower potency (ED_{50} = 24 mg/kg) than the other pyridodiindoles, **2**, **16a**, and **19a**.[31] In vivo data was obtained for several other substituted pyridodiindoles and is also listed in Table III. The 2-fluoro- (**15**), 2-bromo- (**17a**), 2-hydroxy (**21a**) and the 3-hydroxy (**10**), derivatives of pyridodiindole were not proconvulsant even at doses up to 40 mg/kg nor did they antagonize the anticonvulsant effects of diazepam at these doses. The loss of efficacy of these 2-substituted pyridodiindoles was unexpected since these derivatives all exhibited high potency in vitro (IC_{50}'s <20 nM; Table I). Of particular significance was the lack of activity observed for the 2-hydroxy analogue **21a** since this agent was anticipated to be a potent inverse agonist analogous to the 2-methoxy derivative **19a** based on the similar steric and electronic character of the two derivatives.

In order to better understand why derivatives **15**, **17a**, **18a**, and **21a** were inactive in vivo, octanol/water partition coefficients were determined for all of the 2-substituted pyridodiindoles. Octanol/water partition coefficients have often been successfully employed to measure the ability of a drug to cross the blood–brain barrier.[42,43] In this vein, the crude octanol/water partition coefficients were calculated for the pyridodiindoles from tabulated fragmentation constants.[43] The partition coefficient of 2-hydroxypyridodiindole **21a** was also determined experimentally since the calculation using the fragmentation method can often lead to erroneous values for anilinic and phenolic compounds.[43] The partition coefficient for 2-hydroxypyridodiindole **21a** (log P = 0.95)[44] was determined to be much smaller than the partition coefficients for the other pyridodiindole congeners (Table III). On the basis of the log P values the 2-hydroxy derivative **21a** is nearly 1000-fold less lipophilic than the active analogues. This may indicate that the hydroxy compound **21a** does not penetrate the blood–brain barrier. The generally low in vivo potency of many of the pyridodiindoles may also be attributed to the low solubility of this class of ligands in aqueous media (pH = 7.4). The octanol/water partition coefficients of the pyridodiindoles were compared to those calculated or previously reported for the β-carbolines (β-carboline-3-carboxylic acid ethyl ester, log P = 2.26)[45] and

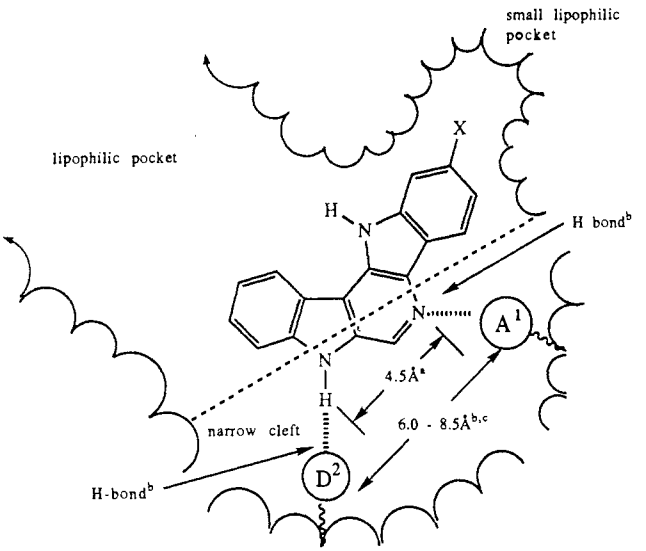


Figure 1. Proposed model of the benzodiazepine receptor inverse agonist/antagonist active site. (a) Average interatomic distance between binding sites on ligand. (b) Intermolecular hydrogen bond lengths were obtained from crystal structures of ligands. (c) Distance between binding-site residues on receptor are dependent upon hydrogen bond lengths.

diazepam (log P = 2.87).[43] In general, the pyridodiindoles are 1 order of magnitude more lipophilic (less water soluble) than diazepam and BCCE. Therefore, on the basis of the QSAR data described above, the lack of efficacy for many of the pyridodiindoles may be the result of low solubility in aqueous media. This may lead to poor absorption and bioavailability of these halo-substituted diindoles after parenteral administration. This problem has been circumvented in a number of cases by the preparation of water-soluble azapyridodiindoles as reported.[49]

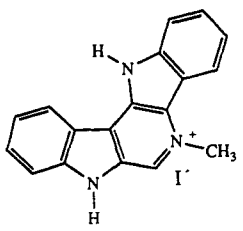
The problem of drug transport across the blood–brain barrier has traditionally been solved with prodrug derivatives.[34,42] In this vein, 2-hydroxy derivative **21a** was converted into pivaloyl ester analogue **22**. Preliminary studies have shown that **22** elicits potent proconvulsant activity (ED_{50} = 17.5 mg/kg). It is believed that the activity of **22** is derived from the 2-hydroxy derivative **21a**, which is released upon hydrolysis once across the blood–brain barrier.[34] It is unlikely that the observed pharmacologic activity stems from **22**, since the ester **22** exhibits such low potency in vitro relative to the 2-hydroxy analogue **21a**. However, at this time it is unclear as to the actual concentration of the 2-hydroxypyridodiindole **21a** present at BzR. Further studies are currently underway to establish the maximum proconvulsant activity and the mechanism of action of prodrug **22**.

Model of the Inverse Agonist/Antagonist Active Site

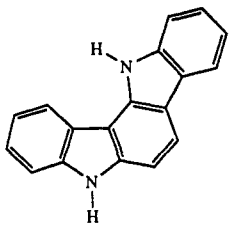
The derivation of the inverse agonist active site was based on the rigid planar pyridodiindole derivatives as "templates" for the identification of possible sites of ligand–receptor interaction. One of the primary binding sites of the pyridodiindoles is believed to be the pyridyl nitrogen atom N(5). It is proposed that the nonbonded lone-pair of electrons on the pyridyl nitrogen atom N(5) interact with an acidic hydrogen of an amino acid residue (i.e., OH, NH, or SH) at the active site of the receptor to form a hydrogen bond. This hypothesis is supported by the SAR

(42) Bradbury, M. *The Concept of the Blood Brain Barrier*; Wiley and Sons: New York, 1979.
(43) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.
(44) Martin, M. J.; Trudell, M. L.; Cook, J. M., unpublished results.
(45) Pietrogrande, M. C.; Borrea, P. A.; Lodi, G.; Bighi, C. *Chromatographia* **1987**, *23*, 713.

of methiodide salt **38a** (IC_{50} > 3000 nM)[46] and indolo-



38a



38b

carbazole **38b** (IC_{50} = 1920 nM),[27] which exhibit very low affinity for BzR due to the absence of a hydrogen-bonding interaction between the receptor and N(5). In the proposed model the hydrogen-bonding site which interacts with the pyridyl nitrogen atom N(5) is a hydrogen bond acceptor site (i.e., accepts a pair of electrons from the ligand to form a hydrogen bond[27,47] and is designated A^1 (Figure 1).

Interaction of the pyridodiindole with a second site is also believed to be necessary for binding. It is proposed that the indole N(7)–H moiety of the ligand interacts with an amino acid residue at the active site to form a hydrogen bond. The binding site on the receptor is a hydrogen bond donor site (i.e., donates a pair of electrons to the ligand to form a hydrogen bond)[27,47] and is designated D^2. The evidence for the existence of this site is derived from the SAR of the methylated pyridodiindole derivatives **34–36**. Of these compounds, only 12-methylpyridodiindole **35** demonstrated moderate affinity. Simple steric constraints do not appear to be the principal cause for the low affinities observed for **34** and **36**, since the 6-ethyl analogue **33** is nearly 5-fold more potent. This is in agreement with the in vitro data reported for 9-methyl-BCCM (IC_{50} > 50 000 nM) and other β-carboline analogues.[30]

Since the pyridodiindoles are completely rigid planar molecules, the distance between the proposed binding sites on the ligand are fixed. The distance between N(5) and N(7)–H was determined from geometry-optimized structures obtained from MNDO[48] and was found to be 4.5 Å. Hydrogen-bond lengths of 1.5–4.0 Å were employed to set the distance between hydrogen bond acceptor site A^1 and hydrogen bond donor site D^2 at approximately 6.0–8.5 Å (Figure 1).

The dramatic difference observed for the in vitro potencies of the various substituted pyridodiindoles can be primarily attributed to steric effects. The 1- and 3-substituted pyridodiindoles exhibit low affinities, predominately because of steric repulsion between the substituents at position 1 and 3 and the BzR binding site. The 2-substituted pyridodiindoles are thought to exhibit high affinity because the substituents at position 2 are more readily accommodated by the small lipophilic pocket in this region of the binding cleft.[28] The size and depth of this pocket is relatively small, since it will only accommodate substituents such as methyl, bromo, and methoxyl, but not the large benzyloxy and pivolyl groups.

Substituents at position-4 are proposed to disrupt the hydrogen-bonding interaction between the receptor (A^1) and the pyridyl nitrogen atom N(5). As a result, an overall reduction in ligand affinity is observed. However, it is believed that substituents which possess nonbonded lone pairs of electrons (**16b**, **19b**, and **21b**) together with the pyridyl nitrogen atom N(5) form an electronegative "edge" and as a result stabilize the interaction at A^1 relative to 4-methyl derivative **18b**. This slight degree of added stabilization may be in the form of simple dipole–dipole interactions or a three-centered hydrogen bond. A similar trend has been observed for the azapyridodiindoles.[49]

A final structural feature of the model of the inverse agonist/antagonist active site is a large lipophilic channel which exists at the mouth of a narrow cleft. It is proposed that this portion of the active site is responsible for correctly orienting potential ligands in the active site. This lipophilic channel permits only molecules of the correct lipophilicity to enter, while the narrow cleft prevents bulky nonplanar molecules from interacting with the active site. The model derived from the SAR of the pyridodiindoles for the inverse agonist/antagonist binding site is illustrated in Figure 1.

The difference in ligand efficacy observed for the 2-substituted pyridodiindoles (Table III) is believed to be due to differences in the electronic character of the ligands which result from inductive effects of the substituent. This proposal is supported by a comparison of the net atomic charge densities (MNDO)[47] on N(5) of **16a** (−0.152), **2** (−0.155), and **19a** (−0.159). The antagonist efficacy of 2-chloropyridodiindole **16a** (see also **4**) is believed to be the result of inductive electron withdrawal from the pyridyl nitrogen atom N(5) through the π-aromatic system of the molecule by the chlorine atom. The decreased electron density at N(5) of **16a** leads to a weaker hydrogen-bonding interaction at A^1. As a result, a smaller allosteric conformational change in the supramolecular BzR complex occurs, which results in weaker efficacy (antagonist rather than inverse agonist). Conversely, the increased electron density at N(5) of **2**, **19a**, and **21a** (delivered as **22**) is believed to lead to the formation of a stronger hydrogen bond at A^1. The stronger hydrogen-bonding interaction increases the allosteric modulation of the supramolecular BzR complex which results in greater efficacy observed for these derivatives relative to the 2-chloropyridodiindole **16a** or its fluoro analogues (**2**,[50] **4**).

This model of the inverse agonist/antagonist binding site is currently being evaluated with an expanded basis set of inverse agonists and antagonists which includes the β-carbolines and 2-aryl-pyrazoloquinolin-3-ones. Computational methods are being employed to provide a quantitative model of the inverse agonist/antagonist active site. The results of this study will be reported in due course.

Experimental Section

Microanalyses were performed on an F and M Scientific Corp. Model 185 carbon, hydrogen, and nitrogen analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are reported uncorrected. Proton NMR spectra were recorded on a Bruker multiprobe 250-MHz spectrometer. Infrared spectra were recorded on a Beckman Acculab-1 or a Mattson Polaris IR-10400 spectrometer. Mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5855 GC-mass spectrometer, while high-resolution mass spectral data (exact mass) were obtained on a Finnigan HR mass spectrometer.

All chemicals were purchased from Aldrich Chemical Co. unless otherwise noted. The analytical TLC plates used were E. Merck

(46) Tan, Y. C. M.S. Thesis, University of Wisconsin—Milwaukee, 1988.

(47) Baker, B. R. *Design of Active-Site Directed Irreversible Enzyme Inhibitors*; John Wiley and Sons: New York, 1967; p 24.

(48) (a) Dewar, M. J. S.; Theil, W. J. *J. Am. Chem. Soc.* **1977**, *99*, 4899. (b) The QCPE program was expanded to 50-atom capability. Sorenson, T.; England, W. B., unpublished results.

(49) Tan, Y. C.; Trudell, M. L.; Cook, J. M. *Heterocycles* **1988**, *27*, 1607.

(50) The 2-fluorodiindole **15** was recently screened in vivo in squirrel monkeys and was found to exhibit antagonist activity. Barrett, J.; Trudell, M. L.; Cook, J. M., unpublished results.

Brinkmann UV active silica gel (Kieselgel 60 F254) on plastic. "Chromatography" refers to flash chromatography using 230–400 mesh 60-Å silica gel, grade 60 (EM Reagent).[50]

Analytically pure samples of the substituted pyridodiindoles were obtained by either recrystallization of the hydrochloride salt from methanol, recrystallization of the free base from benzene/EtOH (9:1), or by high-vacuum sublimation (0.01 Torr) of the free base.

The hydrochloride salts of the pyridodiindoles were prepared from the corresponding crude free base. The free base was dissolved in dry methanol (5 mL) and was added to a solution of methanolic HCl (10 mL) at 0 °C. The slurry was stirred for 30 min and the solid was filtered from the medium to provide the substituted 7,12-dihydropyridodiindole hydrochloride salt. The experimental details for synthesis of diindoles **34–36** are reported in ref 36.

Biological Methods.[30] In Vitro. Adult (175–250 g) male Sprague–Dawley rats (Taconic Farms, Germantown, NY) were killed by decapitation and the brains were removed and placed in ice-cold 50 mM Tris·HCl buffer (pH = 7.4). The cerebral cortices were dissected, weighed, homogenized in 100 vol of Tris·HCl buffer (pH 7.4) and disrupted with a Brinkmann Polytron (setting 6.5, 15 s). The tissues were then centrifuged at 2000*g* (4 °C) for 20 min. The supernatants were discarded, and the suspension/centrifugation ("washing") procedure was repeated three times. Following the last centrifugation, membranes were resuspended in 50 vol of buffer and used immediately or maintained at −70 °C until use. The assay consisted of 0.3–0.4 mL of tissue suspension, 0.1 mL of drug solution, and 0.1 mL of radioligand solution (2 nM [^{3}H]diazepam, Du Pont-NEN) and was diluted with buffer solution to 1 mL total volume. The assays (0–4 °C) were initiated by addition of the radioligand and terminated after 60–90 min by rapid filtration through Whatman GF/B filters (Brandel M-24R, Gaithersburg, MD) with two 5-mL aliquots of ice-cold buffer. Nonspecific binding was determined with 10 μM flunitrazepam (Hoffmann-LaRoche, Nutley, NJ) and usually represented <10% of the total binding. Potencies were estimated with six or more concentrations of the test compounds, with IC_{50} values estimated from Hill plots. Values represent the mean of at least three determinations for substances with potencies <1000 nM. The SEM for these values were generally ≤15% of the mean. Inactive substances (with IC_{50} values >1000 nM) were generally tested twice.

In Vivo. Adult male NIH mice (25–30 g) were injected intraperitoneally with graded doses of the pyridodiindoles (Emulphor/saline, 1:9) or an equal volume of vehicle (0.1 mL, Emulphor/saline, 1:9) followed 30 min later by PTZ at 80 or 40 mg/kg to assess the anticonvulsant and proconvulsant actions, respectively. Groups of 10 mice were injected with graded doses of drugs or vehicle, followed 15 min later by administration of diazepam, (2.5 mg/kg, ip). Fifteen minutes later animals were injected with a prescribed dose of PTZ. In vehicle-pretreated mice, 80 mg/kg PTZ produced tonic and clonic convulsions in 100% of the animals, while the incidence of seizures in the group receiving the 40 mg/kg dose was ≪10%. The dose of diazepam used in these studies protected >90% of the animals tested against PTZ-induced convulsions.

Procedure A. Acid-Catalyzed Fischer Indole Cyclization. Ketobenzamide 1[32] (500 mg, 1.7 mmol) was dissolved in a solution of dry ethanol (5 mL) and the substituted phenylhydrazine [free base (3.4 mmol)]. Two drops of HCl (concentrated) were added to the ethanolic solution and the mixture was heated to reflux for 20 min. A saturated solution of ethanolic HCl (15 mL) was added and the mixture was heated to reflux overnight. The reaction mixture was cooled and excess solvent was removed under reduced pressure. Cold methanol was then added to the residual oil, which effected the precipitation of the pyridodiindole as its hydrochloride salt. The salt was recrystallized from dry ethanol to furnish the substituted 7,12-dihydropyridodiindole hydrochloride.

7,12-Dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (2): 440 mg, 88%; mp >350 °C; IR (KBr) 3350, 3134, 1384, 729 cm^{-1}; MS (CI, CH_4) *m/e* (relative intensity) 258 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 12.78 (s, H-12), 12.50 (s, H-7), 9.07 (s, H-6), 8.90 (d, *J* = 8.0 Hz, H-11), 8.73 (d, *J* = 8.0 Hz, H-4), 7.61 (d, *J* = 8.0 Hz, H-8), 7.60 (d, *J* = 8.0 Hz, H-1), 7.52 (t, *J* = 7.5 Hz, H-9), 7.40 (t, *J* = 7.5 Hz, H-2), 7.28 (t, *J* = 7.5 Hz, H-10), 7.18 (t, *J* = 7.5 Hz, H-3); exact mass calcd for $C_{17}H_{11}N_3$ (free base) 257.0953, found 257.0933. Anal. ($C_{17}H_{11}N_3$) C, H, N.

Procedure B. Synthesis of 1-Substituted-7,12-dihydropyridodiindoles. Ketobenzamide 1[32] (150 mg, 0.51 mmol) was added to the 2-substituted phenylhydrazine (5.0 mmol), after which the mixture was heated to 140 °C with stirring for 6 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 14 h. The excess hydrazine was removed under reduced pressure and the oil which remained was dissolved in hot chloroform. Upon cooling, the 1-substituted dihydropyridodiindole was precipitated from the solution and collected by vacuum filtration. The crude free base was then converted into the hydrochloride salt.

1-Fluoro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (11): 81 mg; 51%; mp >350 °C; sublimes 210 °C (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 276 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.26 (s, 1 H), 12.83 (s, 1 H), 9.23 (s, 1 H)8, 9.15 (d, *J* = 8.1 Hz, 1 H), 8.42 (d, *J* = 7.7 Hz, 1 H), 7.86 (d, *J* = 8.3 Hz, 1 H), 7.78 (dt, *J* = 7.0 Hz, *J* = 1.0 Hz, 1 H), 7.53 (m, 2 H), 7.40 (dt, *J* = 8.0 Hz, *J* = 4.7 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3F$ 275.0859. Found 275.0843. Anal. ($C_{17}H_{10}N_3F$) H, N, C: calcd, 74.17; found, 73.56.

1-Chloro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (12): 85 mg; 51%; mp >350 °C; sublimes 210 °C (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 292 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 12.85 (s, 1 H), 12.82 (s, 1 H), 9.31 (d, *J* = 8.0 Hz, 1 H), 9.23 (s, 1 H), 8.56 (d, *J* = 7.9 Hz, 1 H), 7.79 (dd, *J* = 6.9 Hz, *J* = 1.0 Hz, 1 H), 7.72 (dd, *J* = 7.7 Hz, *J* = 0.7 Hz, 1 H), 7.51 (dt, *J* = 7.9 Hz, *J* = 0.9 Hz, 1 H), 7.43 (t, *J* = 7.9 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Cl$ 291.0563, found 291.0555. Anal. ($C_{17}H_{10}N_3Cl$) C, H, N.

1-Bromo-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (13): 48 mg; 25%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 338 (M + 3, 100), 336 (M + 1, 100), 258 (57.1); ^{1}H NMR (DMSO-d_6) δ 12.75 (s, 1 H), 12.61 (s, 1 H), 9.32 (d, *J* = 8.0 Hz, 1 H), 9.23 (s, 1 H), 8.55 (d, *J* = 8.0 Hz, 1 H), 7.86 (d, *J* = 7.3 Hz, 1 H), 7.85 (d, *J* = 7.5 Hz, 1 H), 7.77 (t, *J* = 7.5 Hz, 1 H), 7.51 (t, *J* = 7.3 Hz, 1 H), 7.37 (t, *J* = 7.8 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Br$ 335.0058, found 335.0056. Anal. ($C_{17}H_{10}N_3Br$·HCl) H, N, C: calcd, 54.79; found, 54.33.

1-Methyl-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (14): 98 mg; 71%; mp >400 °C; MS (CI, CH_4) *m/e* (relative intensity) 272 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 12.96 (s, 1 H), 12.45 (s, 1 H), 9.31 (d, *J* = 8.1 Hz, 1 H), 9.14 (s, 1 H), 8.57 (d, *J* = 7.9 Hz, 1 H), 7.86 (t, *J* = 8.2 Hz, 1 H), 7.79 (d, *J* = 8.2 Hz, 1 H), 7.54 (d, *J* = 7.9 Hz, 1 H), 7.47 (t, *J* = 7.2 Hz, 1 H), 7.34 (t, *J* = 7.6 Hz, 1 H), 2.50 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3$ 271.1109, found 271.1099. Anal. ($C_{18}H_{13}N_3O\cdot{}^4/_5H_2O$) C, H, N.

2-Fluoro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (15). Ketobenzamide 1[32] (600 mg, 2.1 mmol) was added to (3-fluorophenyl)hydrazine (1.3 g, 11 mmol), after which the mixture was heated to 140 °C with stirring for 5 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture which resulted was heated to reflux for 24 h. The excess hydrazine was removed under reduced pressure and the oil which remained was chromatographed (SiO_2, CH_3OH/CH_3CN; 3:97) to provide 2-fluoropyridodiindole **15**. The free base of the 2-fluoro analogue was converted into hydrochloride salt **15** (260 mg, 50%): mp >350 °C; sublimes 210 °C, (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 276 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.37 (s, 1 H), 12.81 (s, 1 H), 9.17 (s, 1 H), 8.97 (d, *J* = 8.0 Hz, 1 H), 8.66 (dd, *J* = 8.0 Hz, *J* = 5.4 Hz, 1 H), 7.86 (d, *J* = 8.0 Hz, 1 H), 7.78 (t, *J* = 6.9 Hz, 1 H), 7.59 (dd, *J* = 8.8 Hz, *J* = 1.8 Hz, 1 H), 7.51 (t, *J* = 7.3 Hz, 1 H), 7.30 (dt, *J* = 9.4 Hz, *J* = 1.8 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3F$ 275.0859, found 275.0834. Anal. ($C_{17}H_{10}N_3F$) C, H, N.

2-Chloro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (16a). Ketobenzamide 1[32] (1.3 g, 4.5 mmol) was added to (3-chlorophenyl)hydrazine (2.5 g, 17 mmol), after which the mixture was heated to 140 °C with stirring for 6 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 24 h. The excess hydrazine was removed under reduced pressure and the oil which remained was chromatographed (SiO_2, CH_3OH/CH_3CN; 3:97) to provide the

2-chloropyridodiindole and 4-chloropyridodiindole, respectively. The free base of the 2-chloro analogue was converted into hydrochloride salt **16a** (950 mg, 64%): mp >350 °C; sublimes 220 °C (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 292 (M + 1, 100); ^{1}H 100): ^{1}H NMR (DMSO-d_6) δ 13.34 (s, 1 H), 12.82 (s, 1 H), 9.18 (s, 1 H), 8.84 (d, *J* = 7.6 Hz, 1 H), 8.57 (d, *J* = 9.7 Hz, 1 H), 7.84 (d, *J* = 9.0 Hz, 1 H), 7.82 (s, 1 H), 7.77 (t, *J* = 8.0 Hz, 1 H), 7.51 (t, *J* = 7.1 Hz, 1 H), 7.44 (dd, *J* = 8.5 Hz, *J* = 1.8 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Cl$ 291.0563, found 291.0551. Anal. ($C_{17}H_{10}N_3Cl$) C, H, N.

4-Chloro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (16b): 160 mg; 11%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 292 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.77 (s, 1 H), 12.97 (s, 1 H), 9.13 (s, 1 H), 9.08 (d, *J* = 8.1 Hz, 1 H), 7.89 (d, *J* = 8.3 Hz, 1 H), 7.86 (d, *J* = 8.0 Hz, 1 H), 7.85 (d, *J* = 8.1 Hz, 1 H), 7.79 (dt, *J* = 7.5 Hz, *J* = 0.9 Hz, 1 H), 7.62 (t, *J* = 7.8 Hz, 1 H), 7.50 (t, *J* = 7.1 Hz, 1 H), exact mass calcd for $C_{17}H_{10}N_3Cl$ 291.0563; found 291.0536. Anal. ($C_{17}H_{10}N_3Cl$) C, H, N.

2-Bromo-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (17a). Ketobenzamide 1[32] (350 mg, 1.2 mmol) was added to (3-bromophenyl)hydrazine (1.3 g, 7.2 mmol), after which the mixture was heated to 140 °C with stirring for 6 h. The excess hydrazine was removed under reduced pressure and the oil which remained was dissolved in hot chloroform. Upon cooling, the 2-bromopyridodiindole (free base) precipitated. The free base was converted into hydrochloride salt **17a** (150 mg, 33%): mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 338 (M + 3, 95.9), 336 (M + 1, 100), 258 (57.3); ^{1}H NMR (DMSO-d_6) δ 13.76 (s, 1 H), 12.99 (s, 1 H), 9.21 (s, 1 H), 9.10 (d, *J* = 7.8 Hz, 1 H), 8.67 (d, *J* = 8.8 Hz, 1 H), 8.05 (d, *J* = 1.5 Hz, 1 H), 7.87 (d, *J* = 8.5 Hz, 1 H), 7.79 (t, *J* = 8.5 Hz, 1 H), 7.60 (d, *J* = 8.5 Hz, 1 H), 7.50 (t, *J* = 7.8 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Br$ 335.0058, found 335.0061.

2-Methyl-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (18a). Ketobenzamide 1[32] (600 mg, 2.1 mmol) was added to 3-tolylhydrazine (1.5 g, 11 mmol), after which the mixture was heated to 140 °C with stirring for 6 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 12 h. The excess hydrazine was removed under reduced pressure and the oil which remained was chromatographed (SiO_2, $CH_3OH/CHCl_3$; 10:90) to provide 2-methylpyridodiindole and 4-methylpyridodiindole, respectively. The free base of the 2-methyl analogue was converted into hydrochloride salt **18a** (208 mg, 36%): mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 272 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.08 (s, 1 H), 12.77 (s, 1 H), 9.15 (s, 1 H), 9.01 (d, *J* = 8.0 Hz, 1 H), 8.48 (d, *J* = 8.3 Hz, 1 H), 7.82 (m, 2 H), 7.64 (s, 1 H), 7.53 (t, *J* = 8.0 Hz, 1 H), 7.28 (t, *J* = 8.3 Hz, 1 H), 2.58 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3$ 271.1109, found 271.1117. Anal. ($C_{18}H_{13}N_3$·HCl) C, H, N.

4-Methyl-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (18b): 23 mg; 4%; mp >300 °C; MS (CI, CH_4) *m/e* (relative intensity 272 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.00 (s, 1 H), 12.45 (s, 1 H), 9.03 (s, 1 H), 8.97 (d, *J* = 8.0 Hz, 1 H), 7.86 (d, *J* = 8.3 Hz, 1 H), 7.76 (t, *J* = 7.6 Hz, 1 H), 7.53 (m, 2 H), 7.19 (d, *J* = 7.1 Hz, 1 H), 2.50 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3$ 271.1109; found 271.1112. Anal. ($C_{18}H_{13}N_3$·HCl·$^3/_4H_2O$) C, H, N.

2-Methoxy-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (19a). Ketobenzamide 1[32] (600 mg, 2.1 mmol) was added to (3-methoxyphenyl)hydrazine (1.5 g, 11 mmol; (3-methoxyphenyl)hydrazine hydrochloride purchased from Lancaster Synthesis), after which the mixture was heated to 140 °C with stirring for 6 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 12 h. The excess hydrazine was removed under reduced pressure and the oil which remained was chromatographed (SiO_2, EtOH/EtOAc; 10:90) to provide 2-methoxypyridodiindole and 4-methoxypyridodiindole, respectively. The free base of the 2-methoxy analogue was converted into hydrochloride salt **19a** (400 mg, 66%): mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 288 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.12 (s, 1 H), 12.69 (s, 1 H), 9.08 (s, 1 H), 8.97 (d, *J* = 8.0 Hz, 1 H), 8.50 (d, *J* = 7.5 Hz, 1 H), 7.83 (d, *J* = 8.3 Hz, 1 H), 7.77 (t, *J* = 8.0 Hz, 1 H), 7.50 (t, *J* = 6.8 Hz, 1 H), 7.24 (d, *J* = 2.1 Hz, 1 H), 7.05 (t, *J* = 6.8 Hz, 1 H), 7.01 (dd, *J* = 6.8 Hz, *J* = 2.8 Hz, 1 H), 3.93 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3O$ 287.1058, found 287.1053. Anal. ($C_{18}H_{13}N_3O$·HCl·$^3/_4H_2O$) C, H, N.

4-Methoxy-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (19b): 70 mg; 12%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 288 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.29 (s, 1 H), 12.72 (s, 1 H), 9.02 (d, *J* = 8.3 Hz, 1 H), 8.99 (s, 1 H), 7.90 (d, *J* = 8.3 Hz, 1 H), 7.80 (t, *J* = 7.0 Hz, 1 H), 7.63 (t, *J* = 8.1 Hz, 1 H), 7.53 (t, *J* = 7.5 Hz, 1 H), 7.44 (d, *J* = 7.9 Hz, 1 H), 6.99 (d, *J* = 7.9 Hz, 1 H), 4.16 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3O$ 287.1058, found 287.1051.

2-(Benzyloxy)-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (20a). Ketobenzamide 1[32] (370 mg, 1.3 mmol) was added to [3-(benzyloxy)phenyl]hydrazine[52] (1.5 g, 7.0 mmol), after which the mixture was heated to 140 °C with stirring for 8 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 6 h. The excess hydrazine was removed under reduced pressure and the oil which remained was chromatographed (SiO_2, EtOAc) to provide 2-(benzyloxy)pyridodiindole and 4-(benzyloxy)pyridodiindole, respectively. The free base of the 2-(benzyloxy) analogue was converted into hydrochloride salt **20a** (130 mg, 26%): mp 201–203 °C; MS (EI, 15 eV) *m/e* (relative intensity) 363 (M^+, 100), 272 (91.4); ^{1}H NMR (DMSO-d_6) δ 12.95 (s, 1 H), 12.62 (s, 1 H), 9.10 (s, 1 H), 8.91 (d, *J* = 7.0 Hz, 1 H), 8.43 (d, *J* = 8.8 Hz, 1 H), 7.82 (d, *J* = 7.0 Hz, 1 H), 7.78 (t, *J* = 7.1 Hz, 1 H), 7.48 (d, *J* = 7.5 Hz, 1 H), 7.46 (m, 6 H), 7.15 (d, *J* = 7.8 Hz, 1 H), 5.33 (s, 2 H); exact mass calcd for $C_{24}H_{17}N_3O$ 363.1372, found 363.1375. Anal. ($C_{24}H_{17}N_3O$) C, H, N.

4-(Benzyloxy)-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (20b): 30 mg; 6%; mp 290–291 °C; ^{1}H NMR (DMSO-d_6) δ 13.11 (s, 1 H), 12.61 (s, 1 H), 9.03 (s, 1 H), 8.97 (d, *J* = 8.3 Hz, 1 H), 7.90 (d, *J* = 8.3 Hz, 1 H), 7.80 (t, *J* = 7.3 Hz, 1 H), 7.63 (d, *J* = 7.35 Hz, 1 H), 7.54 (t, *J* = 7.0 Hz, 1 H), 7.51 (t, *J* = 8.0 Hz, 1 H), 7.37 (m, 5 H), 6.97 (d, *J* = 8.0 Hz, 1 H), 5.65 (s, 2 H); exact mass calcd for $C_{24}H_{17}N_3O$ 363.1372, found 363.1361.

Procedure C. Synthesis of 3-Substituted 7,12-Dihydropyridodiindoles. Ketobenzamide 1[32] (0.50 g, 1.7 mmol) was added to the (4-substitutedphenyl)hydrazine (6 mmol), after which the mixture was heated to 140 °C with stirring for 6 h. The solution was cooled and anhydrous hydrazine (5 mL) was added. The mixture was heated to reflux for 14 h. The excess hydrazine was removed under reduced pressure (Kugelrohr) and the oil which remained was dissolved in hot chloroform. Upon cooling, the 3-substituted dihydropyridodiindole precipitated. The crude free base of the 3-substituted analogue was then converted into the hydrochloride salt.

3-Fluoro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (4): 330 mg; 69%; mp >350 °C; sublimes 210 °C (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 276 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 13.50 (s, 1 H), 12.97 (s, 1 H), 9.17 (s, 1 H), 9.06 (d, *J* = 8.1 Hz, 1 H), 8.53 (dd, *J* = 9.0 Hz, *J* = 2.6 Hz, 1 H), 7.87 (d, *J* = 8.9 Hz, 1 H), 7.86 (d, *J* = 8.7 Hz, 1 H), 7.76 (dt, *J* = 7.3 Hz, *J* = 1.2 Hz, 1 H), 7.50 (m, 2 H); exact mass calcd for $C_{17}H_{10}N_3F$ 275.0859, found 275.0834. Anal. ($C_{17}H_{10}N_3F$) C, H, N.

3-Chloro-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (5): 390 mg, 68%; mp >350 °C; sublimes 220 °C (0.025 mmHg) (free base); MS (CI, CH_4) *m/e* (relative intensity) 292 (M + 1, 100); ^{1}H NMR (DMSO-d_6) δ 12.91 (s, 1 H), 12.31 (s, 1 H), 8.92 (s, 1 H), 8.76 (d, *J* = 7.9 Hz, 1 H), 8.20 (d, *J* = 2.0 Hz, 1 H), 7.71 (d, *J* = 8.1 Hz, 1 H), 7.70 (d, *J* = 8.6 Hz, 1 H), 7.60 (t, *J* = 7.1 Hz, 1 H), 7.45 (d, *J* = 8.6 Hz, 1 H), 7.36 (t, *J* = 7.2 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Cl$ 291.0563, found 291.0548. Anal. ($C_{17}H_{10}N_3Cl$) C, H, N.

3-Bromo-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole hydrochloride (6): 250 mg; 40%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 338 (M + 3, 70.0), 336 (M + 1, 70.6), 257 (100); ^{1}H NMR (DMSO-d_6) δ 13.10 (s, 1 H), 12.79 (s, 1 H), 9.30 (s, 1 H), 8.90 (d, *J* = 8.3 Hz, 1 H), 8.65 (s, 1 H), 7.86 (t, *J* = 8.2 Hz, 1 H), 7.78 (d, *J* = 5.3 Hz, 1 H), 7.77 (d, *J* = 5.0 Hz, 1 H), 7.55 (t, *J* =

(51) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(52) Mann, F. G.; Prior, A. F.; Wilcox, T. J. *J. Chem. Soc.* **1959**, 3830.

7.0 Hz, 1 H); exact mass calcd for $C_{17}H_{10}N_3Br$ 335.0058; found 335.0060. Anal. ($C_{17}H_{11}N_3BrCl\cdot{}^1/_2H_2O$) H, N, C: calcd, 51.08; found, 50.67.

3-Methyl-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (7): 216 mg; 58%; mp >300 °C; MS (CI, CH_4) *m/e* (relative intensity) 272 (M + 1, 100); ^1H NMR (DMSO-d_6) δ 13.21 (s, 1 H), 12.88 (s, 1 H), 9.13 (s, 1 H), 9.04 (d, *J* = 8.0 Hz, 1 H), 8.45 (s, 1 H), 7.80 (m, 3 H), 7.54 (t, *J* = 7.8 Hz, 1 H), 7.48 (t, *J* = 8.3 Hz, 1 H), 2.54 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3$ 271.1109, found 271.1130. Anal. ($C_{18}H_{13}N_3\cdot HCl\cdot H_2O$) C, H, N.

3-(Benzyloxy)-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (9): 400 mg, 58%; mp >350 °C; MS (EI, 15 eV) *m/e* (relative intensity) 363 (M^+, 100), 272 (91.6); ^1H NMR (DMSO-d_6) δ 11.84 (s, 2 H), 8.75 (s, 1 H), 8.70 (d, *J* = 8.1 Hz, 1 H), 7.75 (d, *J* = 2.0 Hz, 1 H), 7.65 (d, *J* = 7.4 Hz, 1 H), 7.57 (d, *J* = 7.4 Hz, 1 H), 7.50 (d, *J* = 7.8 Hz, 1 H), 7.30 (m, 7 H), 7.15 (dd, *J* = 8.5 Hz, *J* = 2.6 Hz, 1 H), 5.25 (s, 2 H); exact mass calcd for $C_{24}H_{17}N_3O$ 363.1372, found 363.1364. Anal. ($C_{24}H_{17}N_3O$) C, H, N.

3-Methoxy-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (8): 490 mg; 90%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 288 (M + 1, 100); ^1H NMR (DMSO-d_6) δ 12.96 (s, 1 H), 12.77 (s, 1 H), 9.16 (s, 1 H), 8.94 (d, *J* = 7.9 Hz, 1 H), 8.16 (d, *J* = 2.0 Hz, 1 H), 7.85 (d, *J* = 8.2 Hz, 1 H), 7.76 (t, *J* = 9.2 Hz, 1 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.28 (d, *J* = 8.9 Hz, 1 H), 3.89 (s, 3 H); exact mass calcd for $C_{18}H_{13}N_3O$, 287.1058, found 287.1040. Anal. ($C_{18}H_{13}N_3O\cdot HCl\cdot{}^1/_4H_2O$) C, H, N.

Procedure D. Synthesis of Hydroxypyridodiindoles. The (benzyloxy)pyridodiindole hydrochloride (132 mg, 0.36 mmol) was dissolved in dry ethanol (50 mL). The solution was added to a slurry of 10% Pd on carbon (100 mg) in dry ethanol (20 mL). The heterogeneous mixture was hydrogenated on a Parr hydrogenation apparatus at 45 psi for 4 h. The catalyst was removed by filtration through a pad of Celite. The Celite was washed with dry ethanol (15 mL). The solvent was removed under reduced pressure. The oil which resulted was treated with cold ethanolic HCl (5 mL) to furnish the hydroxy-7,12-dihydropyridodiindole hydrochloride.

2-Hydroxy-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (21a): 100 mg; 90%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 274 (M + 1, 100); ^1H NMR (DMSO-d_6) δ 11.67 (s, 2 H), 9.44 (s, 1 H), 8.70 (s, 1 H), 8.65 (d, *J* = 7.8 Hz, 1 H), 7.92 (d, *J* = 8.1 Hz, 1 H), 7.62 (d, *J* = 6.2 Hz, 1 H), 7.53 (t, *J* = 6.9 Hz, 1 H), 7.31 (t, *J* = 7.3 Hz, 1 H), 7.00 (s, 1 H), 6.75 (d, *J* = 8.1 Hz, 1 H); exact mass calcd for $C_{17}H_{11}N_3O$ 273.0902, found 273.0909.

3-Hydroxy-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (10): 81%; mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 274 (M + 1, 100); ^1H NMR (DMSO-d_6) δ 12.81 (s, 1 H), 12.70 (s, 1 H), 9.55 (s, 1 H), 9.08 (s, 1 H), 8.94 (d, *J* = 8.0 Hz, 1 H), 7.90 (d, *J* = 2.2 Hz, 1 H), 7.84 (d, *J* = 8.3 Hz, 1 H), 7.76 (t, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 8.8 Hz, 1 H), 7.51 (t, *J* = 7.5 Hz, 1 H), 7.16 (dd, *J* = 8.8 Hz, *J* = 2.3 Hz, 1 H); exact mass calcd for $C_{17}H_{11}N_3O$ 273.0902, found 273.0911.

4-Hydroxy-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole hydrochloride (21b): 57%; mp >350 °C; ^1H NMR (DMSO-d_6) δ 12.80 (s, 1 H), 12.07 (s, 1 H), 9.18 (s, 1 H), 8.78 (s, 1 H), 8.70 (d, *J* = 8.3 Hz, 1 H), 7.80 (d, *J* = 6.1 Hz, 1 H), 7.50 (m, 3 H), 7.27 (d, *J* = 8.3 Hz, 1 H), 6.80 (d, *J* = 8.3 Hz, 1 H); exact mass calcd for $C_{17}H_{11}N_3O$ 273.0902, found 273.0922.

6-Ethyl-7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole Hydrochloride (33). 1-Ethyl-2-(trichloroacetyl)-4-oxo-1,2,3,4-tetrahydro-β-carboline[32] (50 mg, 0.23 mmol) was added to phenylhydrazine (2 mL), afterwhich the mixture was heated at 150 °C for 12 h. The mixture was cooled to room temperature and the excess phenylhydrazine was removed by vacuum Kugelrohr distillation. The residual oil was dissolved in chloroform, after which the 6-ethylpyridodiindole precipitated as the free base. The crude free base was treated with cold ethanolic HCl (2 mL), which afforded 6-ethyl-7,12-dihydropyridodiindole hydrochloride **33** (21 mg, 35%): mp >350 °C; MS (CI, CH_4) *m/e* (relative intensity) 286 (M + 1, 100); ^1H NMR (DMSO-d_6) δ 13.05 (s, 1 H), 13.00 (s, 1 H), 8.97 (d, *J* = 8.0 Hz, 1 H), 8.71 (d, *J* = 7.9 Hz, 1 H), 7.80 (m, 3 H), 7.64 (t, *J* = 7.9 Hz, 2 H), 7.54 (t, *J* = 7.4 Hz, 1 H), 7.43 (t, *J* = 7.6 Hz, 1 H), 3.63 (q, *J* = 7.5 Hz, 2 H), 1.52 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{19}H_{15}N_3\cdot HCl\cdot{}^1/_4H_2O$) C, H, N.

7,12-Dihydropyrido[3,2-*b*:5,4-*b'*]diindolyl Pivalate Hydrochloride (22). 2-Hydroxypyridodiindole hydrochloride **21a** (60 mg, 0.194 mmol) was dissolved in trifluoroacetic acid (50 mL) at 50 °C. Pivaloyl chloride (0.287 mL, 2.33 mmol) was added with continuous stirring for 12 h. The solution was allowed to cool to room temperature and the solvent was removed under reduced pressure. The yellow residue was treated with Na_2CO_3 (saturated, 50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic portions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The oil which resulted was chromatographed (SiO_2, ethyl acetate) to afford the free base. The free base was then converted into hydrochloride salt **22** (60 mg, 79%): mp 254–255 °C; ^1H NMR (DMSO-d_6) δ 12.08 (s, 1 H), 11.89 (s, 1 H) 8.82 (s, 1 H), 8.69 (d, *J* = 7.8 Hz, 1 H), 8.15 (d, *J* = 8.2 Hz, 1 H), 7.67 (d, *J* = 7.8 Hz, 1 H), 7.57 (t, *J* = 7.5 Hz, 1 H), 7.34 (t, *J* = 7.5 Hz), 7.33 (s, 1 H), 6.97 (d, *J* = 8.3 Hz, 1 H), 1.37 (s, 9 H); MS (CI, CH_4) *m/e* (relative intensity) 358 (M + 1, 50), 389 (M + 29, 10). Anal. ($C_{22}H_{20}N_3O_2\cdot{}^1/_4H_2O$) C, H, N.

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