Molecular Yardsticks. Rigid Probes To Define the Spatial Dimensions of the Benzodiazepine Receptor Binding Site

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A series of rigid planar azadiindoles (**8a**, **8b**, and **8d**), benzannelated pyridodiindoles (**11a**, **11b**, and **11d**), and indolopyridoimidazoles (**11c**, **20**, and **24**) were synthesized from 4-oxo-1,2,3,4-tetrahydro-β-carboline **5** via the Fischer indole cyclization with the appropriate arylhydrazines. These analogues were employed as probes ("molecular yardsticks") to define the spatial dimensions of the lipophilic regions of the benzodiazepine receptor (BzR) binding cleft. Benzannelated indoles **11a–d** and indolopyridoimidazoles **20** and **24** were important in establishing an area of negative interaction (S_1, see Figure 6, part b) in the binding cleft common to the interactions of both inverse agonists and agonists. Data from this chemical and computer-assisted analysis of the pharmacophore (see Figure 6) indicates that inverse agonists and agonists bind to the same binding region, but the pharmacophoric descriptors required for the two activities are different, in keeping with previous studies with these planar ligands. However, the hydrogen bond donating site H_1 and the lipophilic region L_1 in the receptor binding site are common interactions experienced by both series of ligands. The low affinities of both indolo[3,2-*c*]carbazole (**3a**) and indolo[3,2-*b*]isoquinoline (**3b**) for the BzR are consonant with the requirements of a hydrogen bond acceptor interaction at donor site H_1 and a hydrogen bond donor interaction at acceptor site A_2 for potent inverse agonist activity in the β-carboline series. The hydrochloride salts of 1-aza- **8a** (IC_{50} 10.6 nM), 2-aza- **8b** (IC_{50} 51.5 nM), and 4-azadiindole **8d** (IC_{50} 11.2 nM) were found to be much more soluble in water than the corresponding salt of the parent diindole **2**. Moreover, aza analogues **8a** and **8b** were shown to be partial inverse agonists with proconvulsant potencies comparable to that of the parent diindole **2**.

Introduction

Since the introduction of Librium in 1960[1] and the subsequent discovery in 1977[2,3] of its mode of action via the γ-aminobutyric acid (GABA)/benzodiazepine receptor (BzR) system, the benzodiazepines have enjoyed widespread use rivaled by few other classes of compounds. Benzodiazepine agonists effect a variety of pharmacological actions, but are far less toxic than, for example, barbiturates. Benzodiazepine receptor ligands produce their pharmacological effects by modulating the gating action of GABA on neuronal chloride ion channels which have recently been shown to be constituted by at least three distinct proteins (α,β,γ).[4-9] Previously, a tetrameric protein channel lumen had been proposed (2α2β); however, coexpression of cloned α and β protein subunits in *Xenopus* oocytes failed to produce benzodiazepine-modulated ion flow as monitored by single channel patch-clamp techniques, although GABA activation was observed. Only coexpression of all three cloned proteins (α,β,γ) provided a system which produced a pharmacology consistent with that of native BzR receptors.

A barbiturate binding site (BbR) is intimately associated with the BzR/$GABA_A$/ion channel complex.[10,11] This association is underscored by the recent observation that the chloride ion channel "cage convulsant" isopropylbicyclophosphate (IPPO) reduced the mortality of pentobarbital overdose in mice.[12] Although less toxic than picrotoxin in this regard, the clinical utility of this finding was nonetheless overshadowed by the potent convulsant properties of IPPO. As a result, the ability to antagonize barbiturate overdose remains unresolved, although antagonists do exist to reverse the effects of opiate and benzodiazepine central nervous system (CNS) depression.

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‡ National Institutes of Health.

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A potential step toward resolving this problem rests in the ability of inverse agonists (BzR ligands which effect actions opposite to those of agonists) to retard ion flux through the channel lumen via their own affect on the $GABA_A$/ion channel.[12-15] In this regard, the partial inverse agonist 3-ethoxy-β-carboline (1, 3-EβC) (see Table I) is currently under study.[16,17] In order to define the structural features required of ligands that exhibit nonconvulsant, partial inverse agonist activity, a better understanding of the BzR recognition site(s) is needed. Despite recent advances, the exact structural nature of the site(s) which accommodate agonist and inverse agonist/antagonist ligands remains unresolved.[18-22] Of the ligand skeletal classes which exhibit affinity for the BzR, the β-carbolines offer a unique opportunity for study since their pharmacological activities span a continuum of in vivo profiles (agonist, antagonist, and inverse agonist).[23-31] The series based on

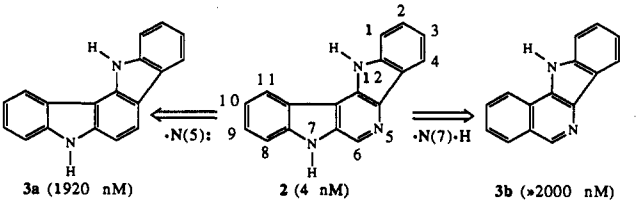

Figure 1. Importance of pharmacophoric descriptors N(5): and N(7)-H for in vitro affinity of diindole **2**.

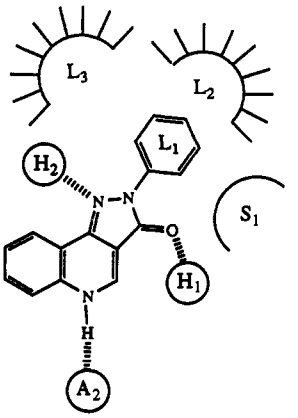

Figure 2. The pyrazoloquinoline ligand CGS-8216 (**4a**) fitted to a two-dimensional schematic representation of the BzR binding site. H_1 and H_2 designate hydrogen bond donating sites on the receptor protein required for agonist activity, in addition to regions L_1 and L_2/L_3. H_1 and A_2 represent hydrogen bond donor and acceptor regions on the receptor protein, respectively, required for inverse agonist activity, in addition to L_1. L_1–L_3 are lipophilic regions of steric attraction, and S_1 is a region of steric repulsion. A three-dimensional schematic of this site has been reported in ref 24 and 25.

7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole (**2**) (see Figure 1) is intriguing, for it can be viewed as an indole-homologated β-carboline and has a fully aromatic, planar topography. This latter attribute provides a tool through which the actual spatial dimensions of the Bz binding cleft can be probed by modification of the diindole nucleus and subsequent biological screening, while retaining the rigid, spatially fixed character of the ligand. Areas of lipophilicity and steric repulsion can then be accurately defined through structure–activity relationships (SAR) together with molecular modeling.[24-26,30,31]

A comprehensive "template" approach towards mapping the inverse agonist/antagonist site has been recently reported.[30,31] Pharmacophoric descriptors (see Figure 2) which interact at A_2 and H_1 of the receptor protein have been defined. As illustrated in Figure 1, removal of either of these hydrogen bonding descriptors resulted in ligands **3a** [-N:(5)] and **3b** [-N(7)-H], both of which did not bind to the BzR.[30,31] Ligand interaction at A_2 and H_1, as well as occupation of the lipophilic region L_1, is proposed as a requirement for potent inverse agonist activity. A similar analysis of the BzR agonist pharmacophore has been reported recently.[24-26] Pharmacophoric descriptors (see Figure 2) necessary for agonist activity were derived by superposition of several families of agonist ligands. These descriptors include two receptor hydrogen bond donating groups designated H_1 and H_2, where H_1 would interact

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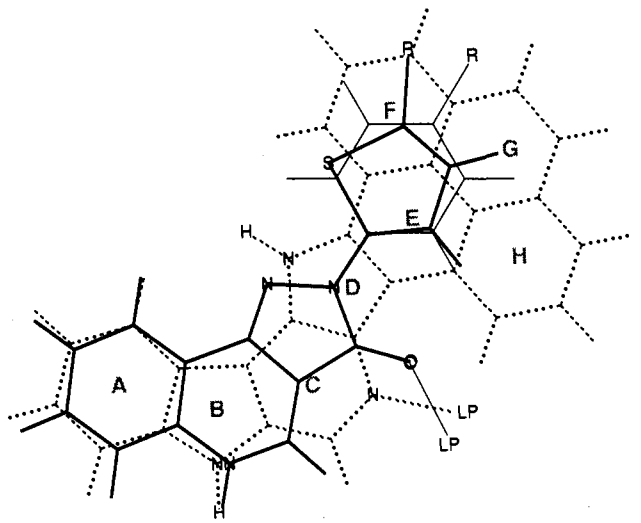

Figure 3. Superposition of the parent 7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole (**2**) (dashed line, rings A–E), the 1,2-benzannelated diindole **11a** (dashed line, rings A–E and F), the 3,4-benzannelated diindole **11b** (dashed line, rings A–E and H), 2-arylpyrazolo[3,4-*c*]quinolin-3-one (**4a**) (thin line), and thien-2-ylpyrazolo[3,4-*c*]quinolin-3-one (**4b**) (thick line). Ring H interacts with the repulsive region S_1 depicted in Figures 2 and 6.

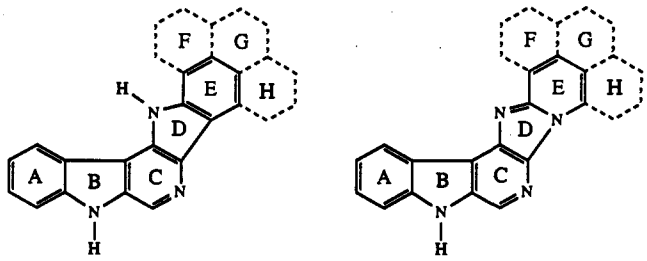

Figure 4. Benzannelated pyridodiindoles and indolopyridoimidazoles.

with the carbonyl oxygen of the agonists CGS 9896/9895 or with the pyridine N:(5) of diindole **2**. Three areas of lipophilic interaction (L_1, L_2, and L_3) were also defined. Recent SAR results[24-26] suggest that ligand interaction at H_1 and H_2, as well as occupation of the lipophilic regions L_1 and L_2, results in partial agonist activity, while an additional interaction with region L_3 is required for full agonist activity (i.e. diazepam[24]). Replacement of the indole N(12)-H moiety (a hydrogen bond donor) of the pyridodiindole inverse agonist **2** with an electron-rich atom (a hydrogen bond acceptor) would provide a site of interaction with H_2 in this series. S_1 represents an area of negative steric interaction, a region occupied by the receptor protein which is inaccessible to ligands (see refs 24–26, 30, and 31 for full details).

With regard to the potency of partial inverse agonists and partial agonists (defined here as compounds which do not possess the complete spectrum of pharmacological actions that full agonists [e.g. diazepam] and inverse agonists [e.g. DMCM] exhibit),[16,24-26,30,31] the definition of the spatial limitations of the lipophilic regions L_2 and L_3 and their affect on in vivo activity is of paramount importance. In order to accurately determine the physical boundaries of the BzR binding cleft, higher homologues of the rigid Bz ligands are needed (see Figures 3 and 4). Since the β-carbolines display a continuum of in vivo profiles, this class of ligands offers potential as topological probes ("molecular yardsticks"), especially in the rigid, planar diindole series. A number of these diindole analogues have been proposed (see Figure 5), and their syntheses as well as their biological activity form the subject of this report.

Chemistry

The parent diindole **2** originated from the Fischer indole cyclization of phenylhydrazine and the acylindole 2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (**5**) (see Scheme I). The β-carboline skeleton of ketobenzamide **5** was constructed via a Pictet–Spengler cyclization of tryptamine and glyoxylic acid under classical, aqueous acid conditions.[27-29] The pivotal step in the synthesis of ketobenzamide **5** was the incorporation of the acyl functionality into the side chain attached at position-3 of the indole nucleus. This was accomplished with the selective oxidizing agent 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in aqueous THF at reduced temperature (−78 °C).[32-35] Phenylhydrazone **6a** was formed by heating ketobenzamide **5** in a 25-fold excess of phenylhydrazine at 150 °C (see Scheme I).[29,31] After phenylhydrazone **6a** tautomerized to ene-hydrazine **6b**, electrophilic aromatic substitution via a [3,3] sigmatropic rearrangement occurred to yield diimine **6c**. Rearomatization and intramolecular nucleophilic attack of the aniline lone pair of electrons on the unsaturated imine resulted in the formation of the pentacyclic system **6d**. Irreversible elimination of ammonia yielded the second indole nucleus present in bisindole **6e**. Finally, hydrazine-mediated deamidation and oxidation (presumably by air) afforded the fully aromatic 7,12-dihydropyrido[3,2-*b*:5,4-*b'*]diindole (**2**) in 88% yield. The steps involved in the conversion of ketobenzamide **5** into diindole **2** are important, for this specific procedure will be employed in a general fashion to synthesize a number of pyridoindole "molecular yardsticks" depicted in Figure 5.

Analysis of the spectrum of diindole **2** revealed a characteristic proton NMR signal pattern for these diindoles: two broad singlets (indole NH resonances, δ 10–14) of low intensity appeared downfield in the proton spectrum.[28] When screened, compound **2** displayed an affinity (IC_{50} 4 nM) at the BzR comparable to that of diazepam in vitro and it was proconvulsant (ED_{50} 15.5 mg/kg) in vivo.[29,31] Unfortunately, the highly lipophilic nature of diindole **2** imparted upon it a low aqueous solubility (<2 mg/mL H_2O),[29,31] which rendered the compound difficult to administer in vivo in drug discrimination studies (see Table I). Measures to avert this solubility problem are described below.

It appeared possible that the central ketobenzamide intermediate **5** would provide access to a series of water-soluble, higher homologues of the parent diindole **2** through variation of the starting arylhydrazines. Proposed BzR ligands produced by the reaction of ketobenzamide **5** with pyridyl-, naphthyl-, quinolyl-, isoquinolyl-, and phthalazinylhydrazines are depicted in Figure 5.

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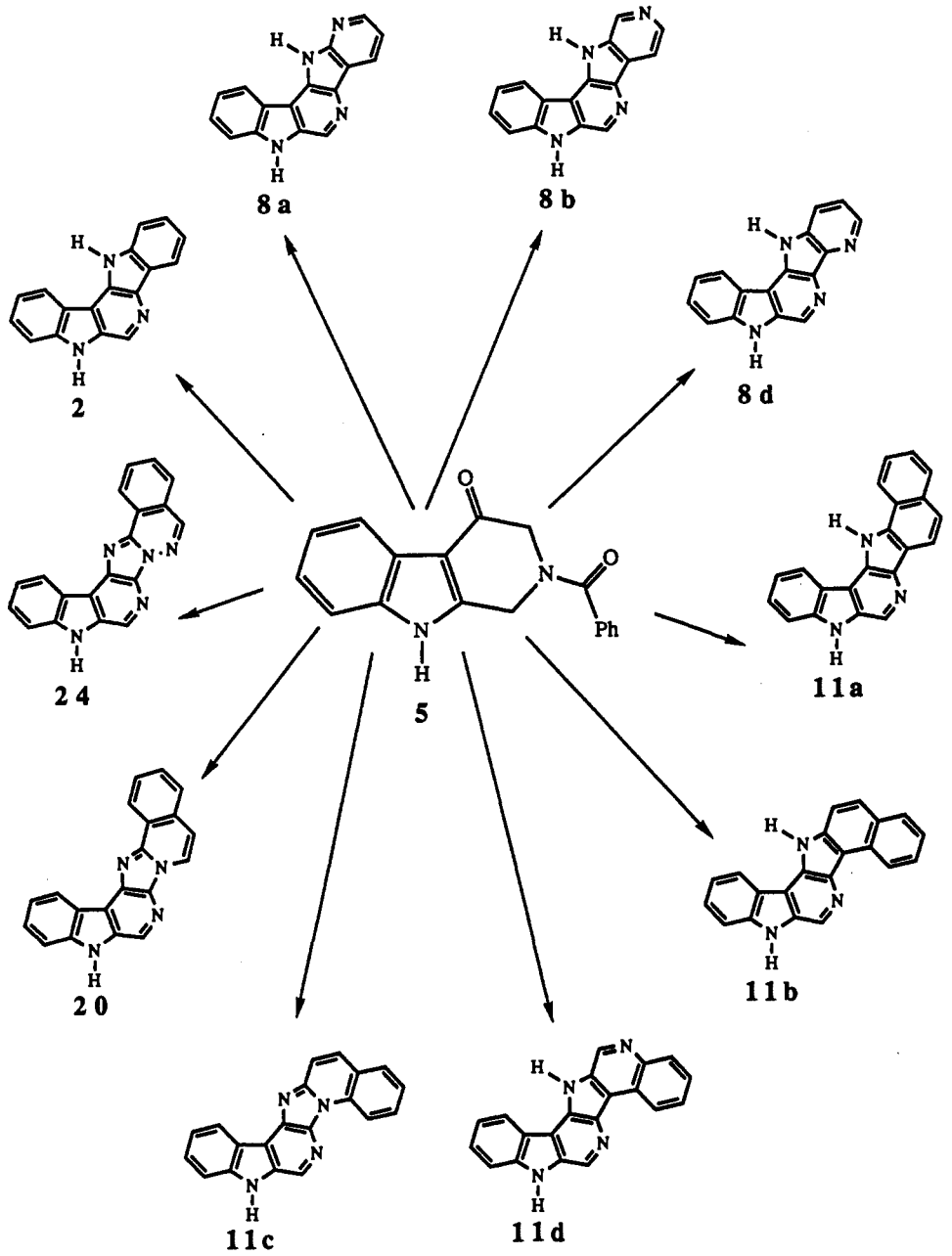

Figure 5. Molecular yardsticks via the Fischer indole route.

A straightforward approach towards increasing water solubility without altering the rigid, planar topography of the diindole nucleus centered on the incorporation of an additional heteroatom into the skeleton. Incorporation of nitrogen into diindole **2** could be readily accomplished in this series by Fischer indole cyclization of hydrazinopyridines with ketobenzamide **5**. Replacement of carbon with nitrogen in ring E of the newly formed indole moiety would alter the electron density distribution within that ring, as indicated by MNDO calculations,[36,37] and would increase water solubility. Unfortunately, the lack of reactivity of pyridylhydrazones in the Fischer indole cyclization reaction under acid catalysis (Brønsted or Lewis; classical conditions) was well known.[38] However, the thermally-induced Fischer indole cyclization to provide azaindoles (either in glycols or as a neat solution) was also known, and the scope included a variety of substrates which reacted in moderate yield.[38] Consequently, ketobenzamide **5** was reacted with 2-hydrazinopyridine **7a** (available commercially) under standard thermal conditions[29,31,36,37] to furnish 7,12-dihydropyrido[3″,2″:4′,5′]pyrrolo[2′,3′:5,6]-pyrido[3,4-*b*]indole (**8a**) (1-azadiindole) in 71% yield (see Scheme II). Examination of the proton NMR spectrum of **8a** revealed the characteristic indole signals at δ 12.95 and 13.80. The details of the structure determination of **8a** are included in the Experimental Section.

For the preparation of azadiindoles **8b** and **8d**, the required 3-hydrazinopyridine (**7b**) was synthesized by the method of Binz and Räth.[39] The diazonium salt of 3-aminopyridine was generated (aqueous HCl/$NaNO_2$, 0

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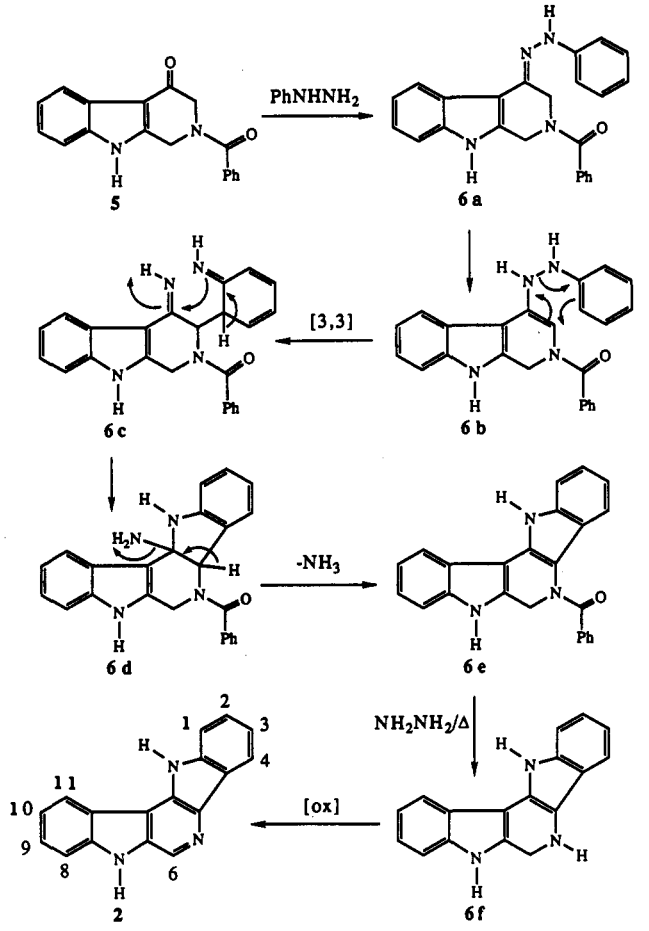
Scheme I



°C) and reduced in situ with tin(II) chloride. Fischer indole cyclization of hydrazine **7b** with ketobenzamide **5** under standard thermal conditions[29,31,36,37] afforded a mixture of the isomeric 2-aza- **8b** and 4-azadiindoles **8d** in moderate yield (see Scheme II). These were subsequently separated by flash chromatography. Again, examination of the proton NMR data (see Experimental Section for details) for both compounds revealed the existence of two indolic protons downfield for each molecule: δ 12.60/13.75 (**8b**) and δ 12.80/13.65 (**8d**). These isomeric 2-aza- and 4-azadiindoles arise from electrophilic attack from either side of the ene-hydrazine (position-4 → 2-aza, position-2 → 4-aza), in agreement with the accepted mechanism[38] of Fischer indole cyclizations.

The required 4-hydrazinopyridine (**7c**) was prepared from 4-chloropyridine by treatment with hydrazine by the method of Mann et al.[40] All attempts to react ketobenzamide **5** with hydrazine **7c** (acid catalysis and thermal induction) to provide the 3-azadiindole analogue **8c** failed uniformly (see Schemes II and III). This attempted Fischer indole cyclization always provided instead 4-amino-β-carboline (**8e**) which was identical in all respects with an authentic sample prepared by an alternate route.[28] It is believed that the nitrogen atom at position-4 of the pyridine ring of **8f** readily accepts electron density from the ene-hydrazine intermediate, deactivating it toward cyclization in favor of N–N bond scission. The dissociation constants of 2-amino- and 3-aminopyridine are nearly neutral (2-NH_2: 6.86, 3-NH_2: 5.98), while that of 4-aminopyridine is significantly more basic (4-NH_2: 9.17).[41] This implies that in the case of 4-hydrazinopyridine (**7c**) the pyridine nitrogen atom readily accepts electron density from the ene-hydrazine intermediate and promotes N–N bond cleavage. The predominance of aminopyridine formation upon attempted cyclization of 4-pyridylhydrazones has been documented.[38] In the 2-hydrazino- (**7a**) and 3-hydrazinopyridine (**7b**) cases, the negative charge which develops is not effectively delocalized into the pyridine ring, and the [3,3] sigmatropic rearrangement proceeds. Trudell has previously shown that when the aromatic ring of phenylhydrazine is substituted with a strongly deactivating group (i.e. 4-nitrophenylhydrazine), attempted Fischer indole cyclization with ketobenzamide **5** resulted in the exclusive formation of 4-amino-β-carboline (**8e**).[29,36]

All the aza analogues **8a**, **8b**, and **8d** bound with high affinity at the BzR in vitro (see Table I). Moreover, both **8a** and **8b** exhibited inverse agonist activity in vivo, in addition to improved water solubilities versus diindole **2** (see Table I).

Depicted in Figure 4 are the templates **2** and **9** proposed for the compounds designated as molecular yardsticks. Selective annelation of rings F, G, and H to the diindole **2** nucleus provides a series of BzR ligands to probe the spatial dimensions of the receptor binding cleft. The rigid, planar requirement is retained, as are the H_1 and A_2 hydrogen bond descriptors (see Figure 2).[24-26,30,31] The obvious difference among the structure of diindole **2** and the structures of the proposed ligands is based in the benz-fused annelation. Consequently, any reduction in in vitro affinity presumed to arise from a negative interaction with the receptor will necessarily define the spatial limitations of the receptor binding cleft, data critical for accurate molecular modeling.

Fischer indole cyclizations involving naphthyl- and quinolylhydrazones have been reported.[38] Since the direction in which the sigmatropic rearrangement proceeds can be predicted, selective ring annelation to diindole **2** can be controlled by the position of the hydrazine substituent on the naphthalene ring. On this basis, the diindole annelated at ring F resulted from Fischer indole cyclization of ketobenzamide **5** with 1-hydrazinonaphthalene (**10a**) (see Scheme IV), which had been prepared from 1-aminonaphthalene by the method of Binz and Räth.[39] As depicted, the reaction of ketobenzamide **5** and hydrazine **10a** under standard thermal conditions[29,31,36,37] furnished 9,14-dihydroindolo[2″,3″:5′,4′]pyrido[2′,3′:4,5]-pyrrolo[2,3-*a*]naphthalene (**11a**) in 40% yield. The characteristic diindole signals [N(9)-H and N(14)-H] appeared in the proton NMR spectrum of this indolic base as singlets at δ 12.86 and 13.44, respectively (see Experimental Section for details).

Unlike 1-hydrazinonaphthalene (**10a**), the sigmatropic rearrangement which would involve 2-hydrazinonaphthalene (**10b**) could have proceeded through either the α-position (position-1) or the β'-position (position-3) of the naphthalene ring. The required 2-hydrazinonaphthalene (**10b**) was prepared from 2-aminonaphthalene by standard methods.[39] However, reaction of ketobenzamide **5** and hydrazine **10b** (see Scheme IV) under standard

(40) Mann, F. G.; Prior, A. F.; Willcox, T. J. The Structure and Properties of Certain Polycyclic Indolo- and Quinolino-Derivatives. Part XIII. The Cyclisation of Certain 4-Pyridyl- and 4-Quinolyl-hydrazones. *J. Chem. Soc.* **1959**, 3830–3834.

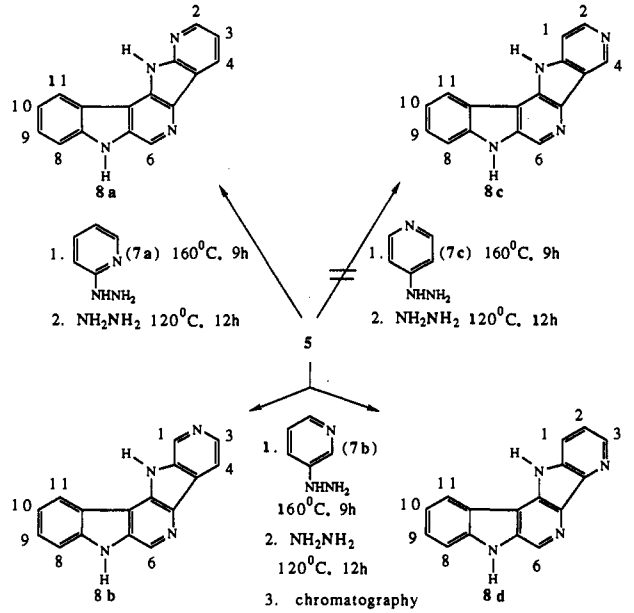
(41) Schofield, K. *Heteroaromatic Nitrogen Compounds*; Butterworths: London, 1967; p 146.

Table I. Physical and Biological Data of β-Carboline Inverse Agonists

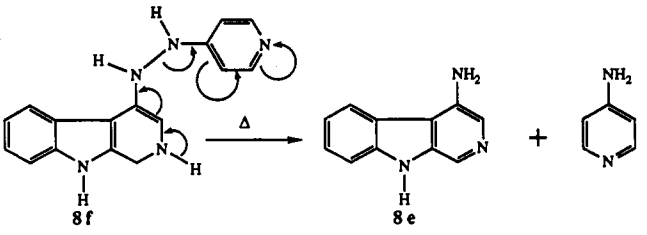
agent[a]	IC_{50} (nM)	ED_{50} (mg/kg)	in vivo activity	solubility (mg/mL H_2O)	CLOGP[b,c] partition coefficients
DMCM[d,e]	1	3-4	potent convulsant	<0.2	3.89
βCCE[d,f]	5	3.2	proconvulsant anxiogenic[g]	<0.2	3.27
βCCE·HCl[d]				1	
diindole 2[h]	4	15.5	proconvulsant	<2	4.32
1-azadiindole 8a	10.6 ± 1.9	11	proconvulsant[i]	7.6	3.09
2-azadiindole 8b	51.5 ± 7.2	25	proconvulsant[i]	13	3.09
4-azadiindole 8d	10.2 ± 0.8	-	inactive[j]	11	3.30
3-EβC(1)[k]	24	7	proconvulsant[i] anxiogenic[i]	12	3.41

[a] All new compounds were screened as the hydrochloride salts; see refs 16, 30, 31 and 58 and text for biological protocols. [b] CLOGP partition coefficients were calculated using fragment method; see refs 59 and 60. [c] CLOGP values were calculated using MedChem version 3.54 (Daylight Chemical Information Systems, Inc., Irvine, CA). [d] Hydrolysis of β-carboline esters in vivo occurs rapidly which influences ED_{50} values. [e] See Braestrup, C.; Schmiechen, R.; Neef, G.; Nielsen, M.; Petersen, E. N. Interaction of Convulsive Ligands with Benzodiazepine Receptors. *Science* **1982**, *216*, 1241-1243. [f] See Tenen, S. S.; Hirsch, J. D. β-Carboline-3-Carboxylic Acid Ethyl Ester Antagonizes Diazepam Activity. *Nature* **1980**, *288*, 609-610. Due to its low solubility, this compound was administered as a suspension in PG/Tween-80. [g] Although only proconvulsant in rats at doses up to 75 mg/kg, potent anxiogenic activity was observed in Rhesus monkeys at a dose of 2.5 mg/kg; see Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. Benzodiazepine Receptor-Mediated Experimental Anxiety in Primates. *Science* **1982**, *218*, 1332-1334. [h] See ref 29. [i] Dose necessary to induce convulsions in 50% of the mice that had been previously given a subconvulsant dose of pentylenetetrazole (40 mg/kg). 3-4 doses of drug were employed in 10 animals at each dosage. [j] No proconvulsant, antagonist, or anticonvulsant action was observed at the highest dose tested (40 mg/kg). [k] See refs 16 and 58. [l] Anxiogenic activity at the highest dose used (20 mg/kg) was measured as a 45% decrease in the mean time animals spent in the open arms of the elevated plus-maze, a 32% decrease in the total number of arm entries, and a 24% decrease in the number of entries into the open arms.

Scheme II

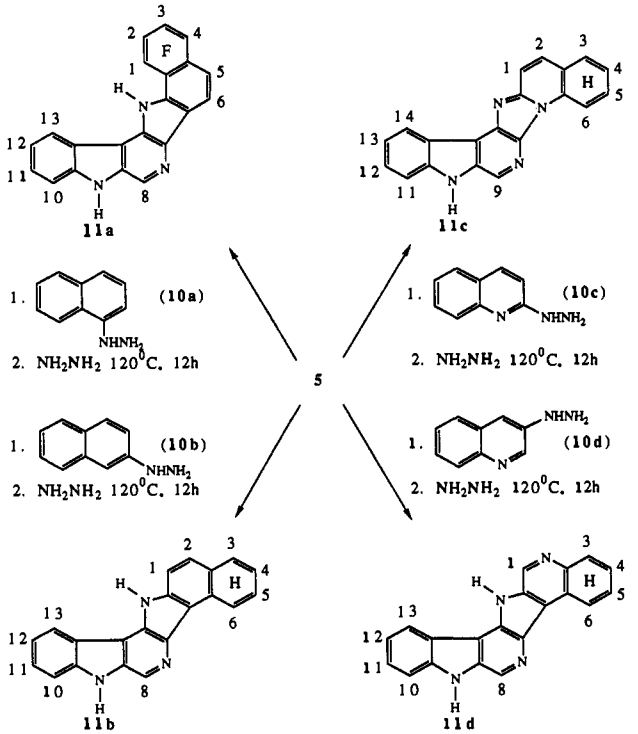


Scheme III



thermal conditions[29,31,36,37] furnished only one product in 60% yield. The structure of 9,14-dihydroindolo[2″,3″:5′,4′]pyrido[2′,3′:4,5]pyrrolo[3,2-*a*]naphthalene (**11b**) was assigned based on proton decoupling and NOE experiments, as well as comparison with the proton spectrum of **11a**. The diindole signals were identified as two indole NH resonances at δ 12.50 and 13.50, respectively. Also of interest was the doublet strongly shifted downfield (1 H, δ 9.45, J = 8.0 Hz) which indicated a close proximity of this proton to the pyridine N:(7); it was correspondingly

Scheme IV



assigned as the proton at C(6) and verified the direction of cyclization to yield the diindole annelated at ring H. Although attack from the β′-position of the naphthalene ring would yield the more thermodynamically stable intermediate,[42,43] the sigmatropic rearrangement followed by concomitant loss of NH_3 is irreversible (see Scheme I), so thermodynamic equilibrium is never attained. Instead, an increased reaction rate for electrophilic substitution at the α-position[42,43] resulted in attack to yield the kinetically favored angular 3,4-benzodiindole **11b**. Formation of the linear 2,3-isomer was not observed. It has been

(42) Morrsion, R. T.; Boyd, R. N. *Organic Chemistry*, 4th ed.; Allyn and Bacon, Inc.: Newton, MA, 1983; pp 1241-1242.
(43) March, J. *Advanced Organic Chemistry*, 3rd ed.; J. Wiley and Sons, Ltd.: New York, 1985; p 454.

reported[44-46] that the hydrazones of β-hydrazinonaphthalenes and β-tetralones undergo Fischer indole cyclization to yield exclusively angular indolocarbazoles. Furthermore, Murakami et al.[47] have recently demonstrated that Fischer indole cyclizations of β-naphthylhydrazones failed to yield benz[f]indoles (linear benzannelation), even when α-substituted hydrazones were employed. These findings are in agreement with our observed results.

Low water solubilities for **11a** and **11b** were anticipated; consequently, the same transformations were performed with 2-hydrazinoquinoline (**10c**) and 3-hydrazinoquinoline (**10d**), respectively (see Scheme IV). The required 2-hydrazinoquinoline (**10c**) was prepared from 2-chloroquinoline by the method of Mann et al.,[40] while 3-hydrazinoquinoline (**10c**) was prepared from 3-aminoquinoline by the method of Binz and Räth.[39] In the case of 3-quinolylhydrazine (**10d**), Fischer indole cyclization of ketobenzamide **5** and hydrazine **10d** under standard thermal conditions[29,31,36,37] provided the expected attack from the α'-position (position-4 of the quinoline ring) to furnish the angular 9,14-dihydroindolo[2'',3'':5',4']pyrido-[2',3':4,5]pyrrolo[2,3-*c*]quinoline (**11d**) in 25% yield. Low yields of Fischer indole cyclizations which employ hydrazinopyridine systems were expected due to their low reactivity toward electrophilic aromatic substitution.[38] Examination of the proton NMR spectrum of **11d** again identified the diindole signals at δ 12.40 and 13.20. The complete assignments are detailed in the Experimental Section.

An important analogue resulted from the Fischer indole cyclization of ketobenzamide **5** and 2-hydrazinoquinoline **10c** under standard thermal conditions.[29,31,36,37] Attack occurred from the α-position of the quinoline ring of **10c** through the quinoline nitrogen atom to afford 10*H*-indolo-[2'',3'':5',4']pyrido[3',2':4,5]imidazo[1,2-*a*]quinoline (**11c**) (see Schemes IV and V). This thermally-induced aza Fischer indole cyclization provided a means through which the N(12)-H hydrogen bond donating group of diindole **2** could be altered to an N:(12) hydrogen bond acceptor group, necessary if interaction at H_2 was desired (see Figure 2). This route also provided access to ligands with enhanced water solubility as their corresponding polyhydrochloride salts. Examination of the proton NMR spectrum of indolopyridoimidazole **11c** revealed the absence of the characteristic diindole signals; only one indole NH resonance was observed at δ 12.25. This confirmed the expected course of reaction. Decoupling and NOE experiments were employed to assign the ring A [C(10)-C(13), $J \simeq 8$ Hz] and β-carboline [C(8); s (1 H), δ 9.10] protons. Of the multiple resonances which remained (2 t and 4 d, $J = 8.0$ Hz), the strongly shifted doublet at δ 10.10 was of particular significance. This highly deshielded resonance was assigned as the proton at C(6) of ring H of **11c**. Comparison of the signals which remained with the spectra of **8b** and **8d** using similar arguments and additional decoupling experiments suggested the structure which resulted from annelation at ring H.

Scheme V

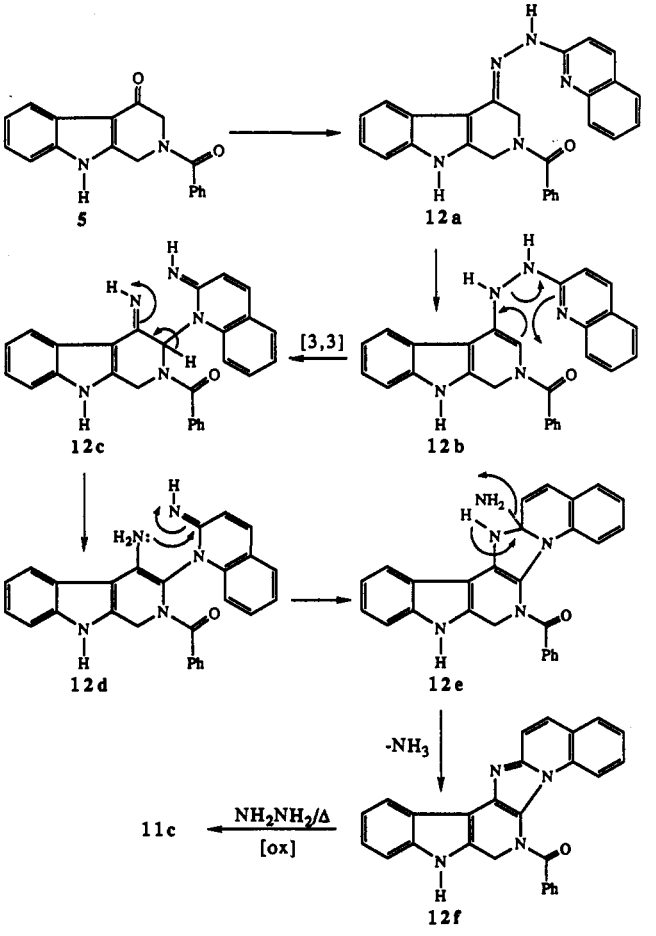


Table II. In Vitro Binding Data of Molecular Yardsticks at the BzR

agent[a]	IC_{50} (nM)[b]
2[c]	4
9[d]	39
11a	≫125[e]
11b	≫125[e]
11c	>3000
11d	>1000
20	>2000
24	200 ± 87
diazepam[f]	5

[a] All compounds were screened as the hydrochloride salts. [b] Measured against [^{3}H]diazepam (**11a–c**) or [^{3}H]flunitrazepam (**11d**, **20**, and **24**), with nonspecific binding determined using flunitrazepam (10 μM). Values represent the average of three or more experiments. Those compounds with an IC_{50} of >1000 nM were only tested twice. See Experimental Section and refs 16 and 58 for full details. [c] See ref 29. [d] See ref 48. [e] The poor water solubility of this ligand precluded an accurate evaluation of the IC_{50} value. [f] See ref 24.

As illustrated in Scheme V, the steps (**12a** → **12f**) involved in the aza Fischer indole cyclization to yield **11c** may differ slightly from those of the classical reaction. It is possible that proton loss from **12c** to provide the 4-amino-2-iminoquinoline intermediate **12d** occurred as illustrated, after which loss of ammonia generated the imidazole **12f**. However, if the quinoline lone pair of **12c** participated in rearomatization of the quinoline ring to provide 2-aminoquinoline, then the formation of the imidazole system would follow the classical Fischer indole cyclization, as illustrated in Scheme I (**6c** → **6d**).

Molecular yardsticks **11a–d** were evaluated for their in vitro affinities at the BzR (see Experimental Section for

(44) Schöpff, M. Zur Constitutionsfrage der 2,3-Oxynaphthoësäure. *Chem. Ber.* **1896**, *29*, 265–270.
(45) Oakeshott, S. H.; Plant, S. G. P. Derivatives of 7,8,9,10-Tetrahydro-α,β-naphthacarbazole and of 8,9,10,11-Tetrahydro-α',β'-naphthacarbazole. *J. Chem. Soc.* **1928**, 1840–1847.
(46) Bryant, S. A.; Plant, S. G. P. The Action of Nitric Acid on Polycyclic Indole Derivatives. *J. Chem. Soc.* **1931**, 93–105.
(47) Murakami, Y.; Watanabe, T.; Ishii, H. Synthesis of Linear Ethyl 9-Methoxy-1*H*-benz[*f*]indole-2-carboxylate. *J. Chem. Soc., Perkin Trans. I* **1988**, 3005–3012.

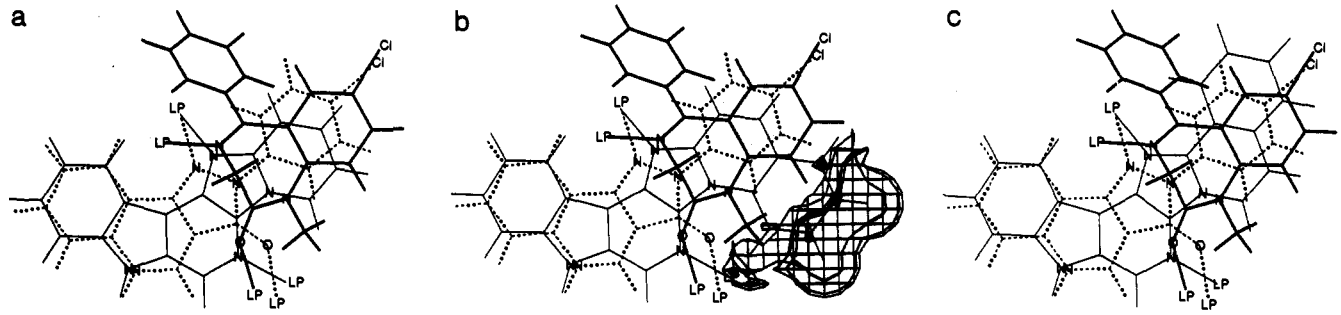
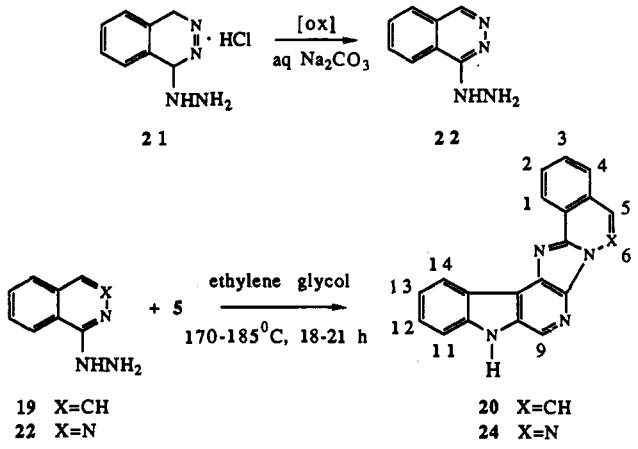
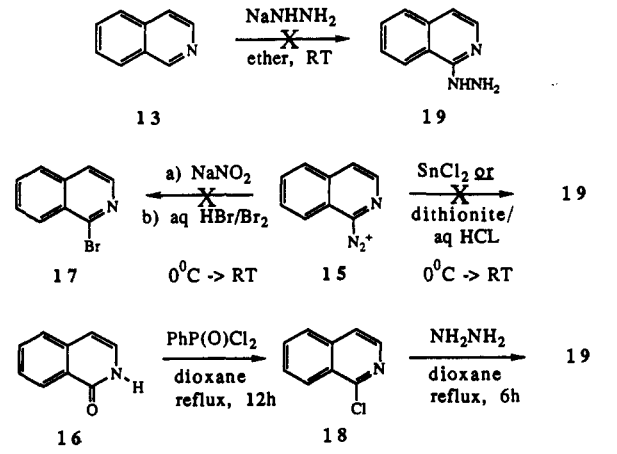


Figure 6. Receptor-excluded volume analysis of the repulsive region S_1. (a) Superposition of the parent indolopyridoimidazole **9** (thin line), 2-arylpyrazoloquinoline **4a** (dashed line), and benzodiazepine **25** (thick line). (b) Superposition of the 3,4-benzannelated indolopyridoimidazole **11c** (thin line), **4a** (dashed line), and **25** (thick line). Excluded volume (molecular volume of **11c** minus the volumes of **2**, **9**, and **25**) is represented by a wire mesh. Occupation of this region by BzR ligands greatly diminishes receptor affinity. (c) Superposition of the 1,2-benzannelated indolopyridoimidazole **20** (thin line), **4a** (dashed line), and **25** (thick line).

Scheme VI



Scheme VII



details). As depicted in Table II, ligands **11b–d** displayed low affinities due to the repulsive interaction of the 3,4-benzannelated fragment with the receptor protein, in comparison to the higher affinities exhibited by the parent systems, diindole **2** (IC_{50} 4 nM) and indolopyridoimidazole **9** (IC_{50} 39 nM)[48] (see Figure 4, rings A–E only). Examination of the receptor-excluded volume analysis of the repulsive region S_1 (see Figure 6, part b) offers an explanation for the low affinities of **11b–d**. Any substituent which interacts with region S_1 will decrease the affinity of the ligand at the BzR. The low affinities of **11b–d** confirm the previous notion that S_1 is an inaccessible, restricted region.[24-26] The data for these rigid, planar ligands can now be employed to better define the exact boundaries of S_1 via molecular modeling. The 1,2-benzodiindole **11a** also exhibited low affinity ($IC_{50} \gg 125$ nM) at the BzR. The 1,2-benzannelated fragment of this ligand appears to interact at the boundary (see Figure 2) between the lipophilic regions designated L_2 and L_3 in the receptor cleft (see refs 24–26 for details); consequently, ligand **11a** does not bind in relation to the parent **2**. The dimensions of this rigid, planar probe **11a** can be employed in future modeling studies to determine where this boundary exists relative to the depth of L_2 and L_3. The influence on the biological efficacy of ligands through the occupation of L_2 and/or L_3 by lipophilic substituents may be paramount to the design of novel partial agonists and new partial inverse agonists at the BzR.

Preliminary results gleaned from the molecular yardsticks provided further insights into the requirements for inverse agonist/antagonist binding. In agreement with previous reports,[24-26,30,31] the inverse agonist/antagonist site cannot tolerate substitution at positions 3 and 4 of the diindole **2** nucleus, while substitution at positions 1 and 2 resulted in only a minimal decrease in affinity. Substitution at position-2 has already been shown to be well tolerated.[29] Additionally, the route to ligands with increased water solubility via pyridyl- and quinolylhydrazines offers an alternate approach for the syntheses of molecular yardsticks. Since an aza Fischer indole cyclization has been successfully achieved, the desired analogue **20** of **9** annelated at ring F (see Scheme VI, X = CH) can be obtained by inversion of the disposition of the hydrazine and nitrogen moieties of the arylhydrazine. The rigid, planar indolophthalazine **24** is also of interest due to its potential for enhanced water solubility (see Scheme VI, X = N).

Synthesis of the required 1-hydrazinoisoquinoline (**19**), as depicted in Scheme VII, proved to be less than straightforward[49] since the chloro compound **18** was not readily available. Attempted hydrazination ($NaNHNH_2$)[50] of isoquinoline **13** according to the method of Kauffmann et al. and reduction ($SnCl_2$,[39] dithionite[51]) of the diazonium salt **15** generated from 1-aminoisoquinoline **14** were uniformly unsuccessful in our hands. Attempts to displace the diazonium function of **15** with bromine ($NaNO_2$/HBr/Br_2)[52] to generate the 1-bromo derivative **17** also failed. However, 1-chloroisoquinoline **18** was prepared by the

(48) Martin, M. J.; Dorn, L. J.; Cook, J. M. Manuscript in preparation.

(49) Martin, M. J.; Cook, J. M. Unpublished results.

(50) Kauffmann, T.; Hansen, J.; Kosel, C.; Schoeneck, W. Direkte Hydrazinierung von Heterocyclen zu Hydrazino-Verbindungen. *Liebig's Ann. Chem.* **1962**, *656*, 103–113.

(51) Hentschel, M.; Gewald, K. 2-Hydrazinothiophen-3-Carbonsäureester. *J. Prakt. Chem.* **1974**, *316*, 878–880.

method of Chang et al.[53,54] through the reaction of phenylphosphonic dichloride on isocarbostyril (16).[55]

Treatment of 18 with hydrazine by the method of Mann et al.[40] (a patented procedure[56] specific for 19) furnished 1-hydrazinoisoquinoline (19). Ketobenzamide 5 and hydrazine 19 were then reacted (see Scheme VI) under standard thermal conditions[29,31,36,37] to afford 10*H*-indolo-[3″,2″:4′,5′]pyrido[3′,2′:4,5]imidazo[2,1-*a*]isoquinoline (20), albeit in low yield. Examination of the proton NMR data for indolopyridoimidazole 20 revealed the presence of only one indolic resonance at δ 11.97, which confirmed the direction of an aza Fischer indole cyclization (see Experimental Section for details).

For the synthesis of the indolopyridophthalazine 24, the 1-hydrazinophthalazine (22) skeleton was available commercially in a lower oxidation state as the hydralazine hydrochloride 21 (see Scheme VI). Fischer indole cyclization of ketobenzamide 5 and hydralazine 21 would not be expected to occur. Consequently, hydrochloride salt 21 was treated with aqueous base, and the subsequent facile air oxidation furnished the required aromatic 1-hydrazinophthalazine (22).[57] The hydrazone 23 was prepared by heating ketobenzamide 5 and hydrazine 22 in ethanol under acidic conditions, followed by cyclization in ethylene glycol (see Scheme VI) under standard thermal conditions[29,31,36,37] to furnish 10*H*-indolo[3″,2″:4′,5′]pyrido[3′,2′:4,5]imidazo[2,1-*a*]phthalazine (24) in 45% yield. Examination of the proton NMR spectrum of indoloimidazophthalazine 24 revealed three downfield singlets (1 H each): the indole N(10)-H resonance at δ 12.15, the β-carboline C(9) proton resonance at δ 8.98, and a resonance indicative of a terminal proton adjacent to an aromatic heteroatom at δ 9.26. After assignment of the ring-A [C(11)-C(14), J = 8.0 Hz] protons, the signals that remained consisted of two triplets and two doublets (1 H each), which were demonstrated as arising from the protons of ring F through decoupling, NOE, and 2D-COSY experiments.

Biological Results and Discussion

All ligands were evaluated for their in vitro affinities at the BzR by methods previously reported.[16,30,31,58] As illustrated in Table I, all of the azapyridodiindole analogues 8a (IC_{50} 10.6 nM), 8b (IC_{50} 51.5 nM), and 8d (IC_{50} 10.2 nM) potently displaced [^{3}H]diazepam from the BzR. In addition, the water solubilities of these ligands (7.6–13 mg/mL of H_2O) were significantly improved compared to the parent diindole 2 (<2 mg/mL of H_2O). Furthermore, as illustrated in Table I, ligands 8a–d are much more soluble in water than either DMCM (<0.2 mg/mL of H_2O) or βCCE (<0.2 mg/mL of H_2O). For this reason, ligands 8a–d were tested in vivo (mice) for their affect on PTZ-induced (80 mg/kg) convulsions.[30,31] Under these conditions, none of the compounds tested at doses up to 40 mg/kg were anticonvulsant. This is not surprising, for ligands 8a–d lack a hydrogen bond acceptor site at N(12) which is proposed as a requisite for agonist activity (see H_2, Figure 2).[24–26] In fact, azadiindoles 8a–d contain instead a hydrogen bond donor site at this position.

Proconvulsant actions were determined with a subthreshold dose of PTZ (40 mg/kg) via methods described previously.[16,30,31,58] The ED_{50} values for the 1-aza- 8a and 2-azadiindole 8b analogues in the proconvulsant paradigm were 11 mg/kg and 25 mg/kg, respectively. In addition to an improved proconvulsant potency versus the parent 2 (15.5 mg/kg), the enhanced water solubility of 8a may render this ligand useful in discriminative stimulus paradigms or in cognitive enhancement studies. Although doses of the 4-azadiindole 8d up to 40 mg/kg were employed, this ligand was inactive in anticonvulsant, proconvulsant, and antagonist paradigms (see Experimental Section for details); however, some behavioral changes in the animals were observed. Examination of IC_{50} values, partition coefficients,[59,60] and solubilities for the agents depicted in Table I does not account for the lack of in vivo activity for 8d. Presumably this inactivity stems from a transport problem.

The IC_{50} values for 9, 11a–d, 20, and 24 are shown in Table II. As mentioned, the 1,2-benzannelated diindole 11a ($IC_{50} \gg 125$ nM) and both the 3,4-benzannelated systems 11b ($IC_{50} \gg 125$ nM) and 11d ($IC_{50} > 1000$ nM) exhibited poor affinities for the BzR, in contrast to the parent 2 (4 nM) [the structures of these ligands are illustrated in Figure 5]. Superposition of ligands 2, 11a, and 11b over the 2-arylpyrazolo[3,4-*c*]quinolin-3-ones 4a and 4b is illustrated in Figure 3. As indicated, the 3,4-benzannelated rings of 11b and 11d clearly undergo a negative interaction at S_1 (see Figures 2 and 6) which resulted in low affinity. Comparison of the IC_{50} values of these ligands to diindole 2 fully supports the pharmacophore depicted in Figures 2 and 6. Comparison of the IC_{50} values for the parent indolopyridoimidazole 9 (IC_{50} 39 nM) with that of the 3,4-benzannelated analogue 11c ($IC_{50} > 3000$ nM) defines the existence of the region designated S_1. Since ligand 11c is a rigid, planar analogue of 9 and the dimensions of the 3,4-benzfused ring are fixed (Å), the receptor-excluded volume represented by the wire mesh in Figure 6 appears accurate. The 1,2-benzannelated indolopyridoimidazole 20 ($IC_{50} > 2000$ nM) and the indoloimidazophthalazine 24 (IC_{50} 200 nM) both possess a rigid, planar benzene ring which principally occupies the lipophilic region L_2; however, this substituent interacts with the boundary between regions L_2 and L_3. The reduced affinity of these ligands at the BzR is the result of a negative steric interaction at this boundary, consistent with the phenomenon observed with 1,2-benzannelated diindole 11a ($IC_{50} \gg 125$ nM) described above. It is known that 1-methylpyridodiindole binds to the BzR with an IC_{50} of 83 nM, while the 2-methyl congener of 2 exhibits an IC_{50} value of 8 nM. It now remains to compare the differences in distance among molecular yardsticks 11a, 20, and 24,

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as well as their corresponding potencies, versus the same parameters in the 1- and 2-methyldiindole series to define the distance from L_1 (see Figure 2) to the receptor boundaries between lipophilic regions L_2 and L_3.

Although the region S_1 in Figure 6 was originally derived for agonists,[24-26] the poor affinity of diindoles which bear substituents at positions 3 and 4 [for example, 3-methylpyridodiindole (IC_{50} 224 nM),[31] 4-methylpyridodiindole (IC_{50} > 5000 nM),[31] **11b** ($IC_{50} \gg 125$ nM) and **11d** (IC_{50} > 1000 nM)] indicates that inverse agonists bind to the same region as agonists.[24,25] In fact, diindole **2** can be superimposed over indolopyridoimidazole **9** (see Figure 6); however, the pharmacophoric descriptors required for agonist (H_1, H_2, L_1, L_2 and/or L_3) and inverse agonist (H_1, A_2, L_1) activities are clearly different (see Figure 2).[24-26] A number of other molecular yardsticks must be prepared before the full impact of the occupation of L_2 and L_3 on in vivo activity can be assessed. It is important to note that the partial inverse agonist 3-EβC (1) (ED_{50} 7 mg/kg) has been prepared.[16,58] As illustrated in Table I, the partition coefficient[59,60] of 3-EβC (1) is similar to that of βCCE; however, 3-EβC (1) is much more soluble in water than either DMCM or βCCE. Since 3-EβC (1) does not cause convulsions even at doses up to 50 mg/kg, it is not as efficacious as DMCM and may exhibit therapeutic potential as a partial inverse agonist.[16,17,58] Further work in regard to modeling with molecular yardsticks and in vivo activity will be reported in due course.

Experimental Section

Receptor Binding. The potencies of test compounds at BzR in rat cerebral cortical membranes were determined through a modification of previously described procedures.[16,30,31,58] In brief, rats were killed by decapitation, and the cerebral cortex was removed. Tissue was disrupted in 100 volumes of Tris-HCl buffer (50 mM, pH 7.4) with a Polytron homogenizer (15 s, setting 6–7, Brinkman Instruments, Westbury, NY) and centrifuged (4 °C) for 20 min at 20000*g*. Tissue was resuspended in an equal volume of buffer and recentrifuged. This procedure was repeated a total of three times, and the tissue was resuspended in 50 volumes of buffer. Tissue was either used fresh or stored at −70 °C until used. Incubations (1 mL) consisted of tissue (0.3 mL), drug solution (0.1 mL), buffer (0.5 mL), and radioligand (0.1 mL). Incubations (4 °C) were initiated by addition of [^{3}H]diazepam (final concentration, ≃2 nM; specific activity, 76 Ci/mmol; Du Pont-NEN, Boston, MA) or [^{3}H]flunitrazepam (final concentration, ≃1 nM; specific activity, 90 Ci/mmol; Du Pont-NEN, Boston, MA) and terminated after 120 min by rapid filtration through GF/B filters and washing with two 5-mL aliquots of ice-cold buffer with a Brandel M-24R filtering manifold. Nonspecific binding was determined by substituting nonradioactive flunitrazepam (final concentration, 10 μM) for the drug solution and represented <10% of the total binding. Specific binding was defined as the difference in binding obtained in the presence and absence of 10 μM flunitrazepam. The IC_{50} values were estimated with GraphPad LnPlot using at least six concentrations of inhibitor. Values represent the mean of at least three determinations for substances with potencies <1000 nM. The SEM for these values were generally ≤10% of the mean. Inactive substances (IC_{50} > 1000 nM) were generally tested only twice. Compounds **2**, **3a**, **8a–d**, and **11a–c** were screened against [^{3}H]-diazepam. Compounds **3b**, **11d**, **20**, and **24** were screened against [^{3}H]flunitrazepam.

In Vivo Activity. Adult male NIH mice (25–30 g) were injected intraperitoneally (ip) with graded doses of the azadiindoles (diluted Emulphor/saline, 1:9) or an equal volume of vehicle (0.1 mL; diluted Emulphor/saline, 1:9), followed 15 min later with PTZ to assess anticonvulsant (80 mg/kg) and proconvulsant (40 mg/kg) actions. Groups of 10 mice were injected ip with 3–4 graded doses of drug or vehicle, followed 10 min later by administration of diazepam (2.5 mg/kg, ip). After 5 min, the 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.

Molecular Modeling. The starting geometries of the parent heterocyclic systems used in the modeling studies were constructed from the fragment library of SYBYL version 5.32 (Tripos Associates, St. Louis, MO) with the exception of benzodiazepine **25**, for which the X-ray geometry was used.[61] The N-N-aryl torsional angles of the pyrazoloquinolinones **4a** and **4b** were set to 0°. All the bond lengths and valence angles of these structures were in turn fully optimized with Gaussian 90[62] ab initio calculations (Gaussian Inc., Carnegie-Mellon University, Pittsburgh, PA) using the 3-21G basis set running on a Cray X-MP supercomputer. All torsional angles were held fixed in order to speed convergence. The least squares deviation of non-hydrogen atoms between these ab initio optimized geometries and available X-ray crystallographic structures was less than 0.05 Å.[63] In the case of pyrazoloquinolinones **4a** and **4b**, the atoms contained in the A, B, and C rings were compared separately from the atoms contained in the D ring, since the torsional angles between the C and D rings varied considerably in the X-ray crystal structures. The alignment of ligands was performed by least squares fitting of points using version 5.41c of SYBYL. Extended electron lone pair vectors, in addition to ligand ring centroids and heteroatoms, were used in the fitting procedure. The ends of these lone pair vectors approximate the expected location of receptor protein atoms capable of undergoing a hydrogen bonding interaction with the ligand. The lengths of the electron lone pair vectors were set to 1.8 Å, and the C-N-LP and C-O-LP angles were set to 120° and 135°, respectively, which correspond to ideal hydrogen bond geometries.[64] The alignment of ligands **2**, **4a**, **4b**, and **11a,b** (Figure 3) and **4a**, **9**, **11c**, and **20** (Figure 6) was obtained by a least squares fit of four points which include (1) the ring-A centroid, (2) the ring-B nitrogen and attached hydrogen atom, (3) an extended electron lone pair vector attached to either the ring-C nitrogen atom of indoles **2**, **9**, and **11a–c** or the carbonyl oxygen atom of pyrazoloquinolinones **4a** and **4b**, and (4) the ring-E (**2**, **9**, and **11a–c**) or ring-D (**4a** and **4b**) centroid. The RMS deviation of these fitted points was 0.54 Å. A three-point least squares fit was also used to align the structure of the pyrazoloquinolinone **4a** to that of benzodiazepine **25** (Figure 6). The points used in this alignment included (1) the ring-D (**4a**) or ring-A (**25**) centroid, (2) the end of the extended electron lone pair vector attached to either the ring-C nitrogen atom of **4a** or the ring-B amide oxygen atom of **25**, and (3) the end of the extended electron lone pair vector attached to the ring-C imidazole nitrogen atom of **4a** or the ring-C imine nitrogen atom of **25**.

Methods. Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal model IA8100 digital melting point apparatus and are reported uncorrected.

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Proton NMR spectra were recorded on a Bruker 250-MHz or a GE 500-MHz instrument. Infrared spectra were recorded on a Beckman Acculab-1, a Mattson Polaris IR-10400, or a Nicolet FT-IR spectrophotometer. Mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5855 or 5985B GC-mass spectrometer, and high-resolution mass spectral data were obtained on a Finnigan HR mass spectrometer. Microanalyses were performed on an F and M Scientific Corp. Model 185 or Perkin-Elmer 240C carbon, hydrogen, and nitrogen analyzer. Analytical TLC plates employed were E. Merck Brinkman UV active silica gel (Kieselgel 60 F254) on plastic support. The preparations of compounds 1 and 9 are described in refs 16 and 57, respectively.

Cyclohexane-1,3-dione Monophenylhydrazone. Cyclohexane-1,3-dione (10.38 g, 92.8 mmol) was dissolved in H_2O (600 mL), and an aqueous solution of phenylhydrazine (10.00 g, 92.7 mmol in 200 mL of H_2O) was added with stirring. Within minutes a precipitate had formed. The mixture was allowed to stand (2 h), and the solid was filtered, washed with H_2O (2 × 100 mL), and dried to furnish cyclohexane-1,3-dione monophenylhydrazone (16.29 g, 87.1%): mp 175–177 °C (lit.[65] 176–177 °C); MS (CI CH_4) *m/e* (rel inten) 203 (M + 1, 100).

4-Oxo-1,2,3,4-tetrahydrocarbazole. Cyclohexane-1,3-dione monophenylhydrazone (2.69 g, 13.3 mmol) was dissolved in dilute H_2SO_4 (40% (v/v), 0 °C). Black tar was filtered from the solution, and then H_2O (110 mL) was added which precipitated a green-gray solid. The precipitate was filtered and washed with H_2O (5 × 50 mL) to furnish 4-oxo-1,2,3,4-tetrahydrocarbazole as a light green solid (0.7433 g, 30%): mp 225–227 °C (lit.[65] 223 °C); MS (CI CH_4) *m/e* (rel inten) 186 (M + 1, 100).

4-Oxo-1,2,3,4-tetrahydrocarbazole Phenylhydrazone Hydrochloride. 4-Oxo-1,2,3,4-tetrahydrocarbazole (1.51 g, 8.18 mmol) was suspended in EtOH (80 mL) to which phenylhydrazine (0.88 g, 8.19 mmol) and catalytic acetic acid (0.1 mL) had been added. The solution was heated (reflux, 24 h) and then filtered hot to remove unreacted starting material. Ethanolic HCl was added, and the solution was cooled. The white solid which precipitated was filtered, dried, and recrystallized from EtOH to furnish 4-oxo-1,2,3,4-tetrahydrocarbazole phenylhydrazone hydrochloride (2.28 g, 89.4%): mp 234–236 °C (lit.[65] 233–235 °C).

Indolo[3,2-*c*]carbazole (3a). 4-Oxo-1,2,3,4-tetrahydrocarbazole phenylhydrazone hydrochloride (0.300 g, 0.963 mmol) was placed in a sublimation apparatus, and the apparatus was evacuated (1 mmHg) and heated (360 °C, 15 min). The sublimed product was washed with water to remove any starting material and dried to furnish **3a** (0.11 g, 46%): mp 297–298 °C (lit.[65] 299–300 °C); MS (CI, CH_4), *m/e* (rel inten) 257 (M + 1, 100).

1,2,3,4-Tetrahydroisoquinolin-4-ol. 2-Benzyl-1,2,3,4-tetrahydroisoquinol-4-one hydrochloride[66] (600 mg, 2.19 mmol) was suspended in glacial acetic acid (12 mL), and palladium on activated carbon (10%, 60 mg) was added to the mixture. The slurry which resulted was stirred (14 h) under hydrogen (1 atm, 27 °C). The solvent was removed under reduced pressure to furnish a brown oil, which was then washed with diethyl ether to provide a gray precipitate. This precipitate was collected by filtration and then partitioned between Na_2CO_3 and $CHCl_3$ (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure to furnish crude product as the free base. The free base was then recrystallized from EtOAc to furnish 1,2,3,4-tetrahydroisoquinolin-4-ol (235 mg, 72%): mp 83–84 °C; IR ($CDCl_3$) 3606, 3065, 3030, 2945, 2826, 1447, 1223 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 2.26 (broad, 2 H), 3.03 (dd, J_1 = 12.8 Hz, J_2 = 3.0 Hz, 1 H), 3.92 (s, 2 H), 4.54 (t, J = 2.8 Hz, 1 H), 6.99–7.38 (aromatic, 4 H); MS (CI, CH_4) *m/e* (rel inten) 150 (M + 1, 20.3), 132 (M + 1 − 18, 100). This material was employed directly in the next step.

2-Benzoyl-1,2,3,4-tetrahydroisoquinolin-4-ol. 1,2,3,4-Tetrahydroisoquinolin-4-ol (400 mg, 2.68 mmol) was dissolved in dry CH_2Cl_2 (30 mL), and this solution was cooled (−5 to 0 °C, ice–brine). Sodium bicarbonate (220 mg, 2.62 mmol) was then added to the solution. Benzoyl chloride (377 mg, 2.68 mmol, 0.311 mL) was added via syringe under N_2, and the mixture which resulted was stirred (3.5 h, −5 to 0 °C). Precipitated NaCl was removed from the solution by filtration and washed with CH_2Cl_2 (10 mL). The combined organic layers were concentrated under reduced pressure and chromatographed (SiO_2; EtOAc/hexane, 3:2) to furnish 2-benzoyl-1,2,3,4-tetrahydroisoquinolin-4-ol as a colorless oil (475 mg, 70%): IR ($CDCl_3$) 3600, 3064, 3029, 2911, 2853, 1630, 1434, 1272, 1061 cm^{-1}; ^1H NMR (Me_2SO-d_6, 250 MHz) δ 3.40–3.60 (m, 2 H), 4.40–4.80 (m, 2 H), 4.87 (d, J = 17.4 Hz, 1 H), 5.57 (s, 1 H), 7.10–7.60 (aromatic, 9 H); MS (CI, CH_4) *m/e* (rel inten) 282 (M + 29, 20.3), 254 (M + 1, 47.4), 236 (M + 1 − 18, 100). This material was employed directly in the next step.

2-Benzoyl-4-oxo-1,2,3,4-tetrahydroisoquinoline. 2-Benzoyl-1,2,3,4-tetrahydroisoquinolin-4-ol (200 mg, 0.79 mmol) was dissolved in CH_2Cl_2 (22 mL), and PDC (253 mg, 0.67 mmol) was added to the solution. The mixture was stirred (11 h, 25 °C) and then filtered to remove salts. The solution was concentrated under reduced pressure to furnish a brown oil which was chromatographed (SiO_2; EtOAc/hexane, 7:3) to provide 1-benzoyl-4-oxo-1,2,3,4-tetrahydroisoquinoline as a yellow oil (160 mg, 81%): IR ($CDCl_3$) 3072, 3037, 2875, 1700, 1638, 1440, 1272 cm^{-1}; ^1H NMR (Me_2SO-d_6, 250 MHz) δ 4.20–4.70 (broad, 2 H), 4.70–5.10 (broad, 2 H), 7.30–7.80 (m, 8 H), 7.92 (d, J = 7.7 Hz, 1 H); MS (EI 15 eV) *m/e* (rel inten) 251 (M^+, 38.7), 146 (M^+ − 105, 99.7), 105 (M^+ − 146, 100). This material was employed directly in the next step.

Indolo[3,2-*b*]isoquinoline Hydrochloride (3b). 2-Benzoyl-4-oxo-1,2,3,4-tetrahydroisoquinoline (180 mg, 0.72 mmol) was dissolved in phenylhydrazine (1 mL), and the solution was heated (170 °C, 12 h) under a nitrogen atmosphere. This solution was allowed to cool, excess hydrazine was added, and heating was continued (120 °C, 24 h). After allowing the solution to cool, excess hydrazine was removed under reduced pressure, and excess phenylhydrazine was removed via Kugelrohr distillation (150 °C, 1 mmHg). The oil which remained was chromatographed (SiO_2; EtOAc/hexane, 1:2) to furnish **3b** as the free base. This material was then converted into the hydrochloride salt by treatment with ethanolic HCl (35 mg, 19%): mp >300 °C; ^1H NMR (Me_2SO-d_6, 250 MHz) δ 7.30 (t, J = 7.1 Hz, 1 H), 7.47 (t, J = 7.1 Hz, 1 H), 7.67 (d, J = 7.0 Hz, 1 H), 7.70 (t, J = 7.0 Hz, 1 H), 7.90 (t, J = 7.1 Hz, 1 H), 8.21 (d, J = 7.5 Hz, 1 H), 8.27 (d, J = 7.2 Hz, 1 H), 8.49 (d, J = 7.1 Hz, 1 H), 9.11 (s, 1 H), 12.18 (s, 1 H); MS (EI 15 eV) *m/e* (rel inten) 218 (M^+, 100). Anal. ($C_{15}H_{10}N_2$·HCl·$^1/_2H_2O$) C, H, N.

7,12-Dihydropyrido[3″,2″:4′,5′]pyrrolo[2′,3′:5,6]pyrido[3,4-*b*]indole (8a). Ketobenzamide **5**[32-35] (187.6 mg, 0.65 mmol) was dissolved in 2-hydrazinopyridine (**7a**) (1.01 g, 9.26 mmol), and the solution was heated (160 °C, 24 h). This solution was then allowed to cool, excess hydrazine (5 mL) was added, and heating was continued (120 °C, 16 h). After allowing the solution to cool, excess hydrazine was removed under reduced pressure and excess pyridyl hydrazine was removed via Kugelrohr distillation. The product was precipitated from $CHCl_3$ to furnish 1-azadiindole **8a** (118 mg, 71%): mp >300 °C; IR (KBr) 3260, 1600, 1480, 1400, 750 cm^{-1}; ^1H NMR (Me_2SO-d_6, 250 MHz) δ 7.52 (d, J = 5.2 Hz, 1 H), 7.54 (t, J = 5.2 Hz, 1 H), 7.80 (t, J = 8.1 Hz, 1 H), 7.87 (t, J = 8.3 Hz, 1 H), 8.75 (d, J = 5.2 Hz, 1 H), 9.10 (d, J = 7.6 Hz, 1 H), 9.15 (d, J = 7.5 Hz, 1 H), 9.31 (s, 1 H), 12.95 (s, 1 H), 13.80 (s, 1 H). The ring-A [C(8)-C(11); t (δ 7.80), t (δ 7.87), d (δ 9.10), d (δ 9.15), all $J \simeq 8$ Hz] and the β-carboline [C(6), s (1 H), δ 9.31] protons were assigned through standard decoupling experiments, and the remainder of the spectrum consisted of a multiplet (2 H, J = 5.2 Hz) and a doublet (1 H, J = 5.2 Hz). The disparate chemical shifts (>1 ppm) of these signals suggested aromatic protons [C(4) and C(3), δ 7.50] and a terminal proton adjacent to an aromatic heteroatom [C(2), δ 8.75]. These assignments are consistent with the structure of 1-azadiindole **8a**; MS (CI CH_4) *m/e* (rel inten) 259 (M + 1, 100), 287 (M + 29, 30); high-resolution MS *m/e* 258.0906 ($C_{16}H_{10}N_4$ requires 258.0905). Anal. ($C_{16}H_{10}N_4$·2HCl·$^5/_4H_2O$) C, H, N.

7,12-Dihydropyrido[4″,3″:4′,5′]pyrrolo[2′,3′:5,6]pyrido[3,4-*b*]indole (8b) and 7,12-Dihydropyrido[2″,3″:4′,5′]pyrrolo-[2′,3′:5,6]pyrido[3,4-*b*]indole (8d). Ketobenzamide **5** (500 mg, 1.73 mmol) was dissolved in 3-hydrazinopyridine (**7b**)[39] (1 g, 9.16

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mmol), and the solution was heated (160 °C, 9 h). This solution was allowed to cool, excess hydrazine (5 mL) was added, and heating was continued (120 °C, 12 h). After allowing the solution to cool, excess hydrazine was removed under reduced pressure and excess pyridyl hydrazine was removed via Kugelrohr distillation. The residue was chromatographed (SiO_2; CH_3CN/EtOH, 7:3) to yield 2-azadiindole **8b** and 4-azadiindole **8d**. Their hydrochloride salts were prepared by addition of methanolic HCl.

2-Azadiindole hydrochloride **8b**: mp >300 °C; IR (KBr) 3200, 1630, 1380, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6, 250 MHz), δ 7.45 (t, J = 7.5 Hz, 1 H), 7.65 (t, J = 7.5 Hz, 1 H), 7.80 (d, J = 8.0 Hz, 1 H), 8.60 (d, J = 7.3 Hz, 1 H), 8.65 (d, J = 7.2 Hz, 1 H), 8.85 (d, J = 8.0 Hz, 1 H), 9.15 (s, 1 H), 9.35 (s, 1 H), 12.60 (s, 1 H), 13.75 (s, 1 H). Assignment of the ring-A [C(8)-C(11), $J \simeq 8$ Hz] and β-carboline [C(6); s (1 H), δ 9.15] protons was achieved through standard decoupling experiments. An additional downfield singlet at δ 9.35 in the spectrum of **8b** indicated an isolated proton adjacent to an aromatic heteroatom and was assigned as the proton at C(1). These assignments were in agreement with the structure of 2-azadiindole **8b**; MS (CI CH_4) m/e (rel inten) 259 (M + 1, 100), 287 (M + 29, 30); high-resolution MS m/e 258.0905 ($C_{16}H_{10}N_4$ requires 258.0905).

4-Azadiindole hydrochloride **8d**: mp >300 °C; IR (KBr) 3200, 1620, 1500, 1350, 730 cm^{-1}; ^1H NMR (Me_2SO-d_6, 250 MHz) δ 7.52 (t, J = 7.3 Hz, 1 H), 7.73 (t, J = 7.3 Hz, 1 H), 7.85 (m, 2 H), 8.60 (d, J = 7.9 Hz, 1 H), 8.76 (d, J = 5.3 Hz, 1 H), 8.93 (d, J = 7.9 Hz, 1 H), 9.20 (s, 1 H), 12.80 (s, 1 H), 13.65 (s, 1 H). Assignment of the ring-A [C(8)-C(11), $J \simeq 8$ Hz] and β-carboline [C(6); s (1 H), δ 9.20] protons was achieved through standard decoupling experiments. The similarity of the signals which arise from the protons of ring E in the spectrum of diindole **8d** with those in the spectrum of azadiindole **8a** (chemical shift and coupling pattern) suggested the isomeric 4-azadiindole **8d**; MS (CI CH_4) m/e (rel inten) 259 (M + 1, 100), 287 (M + 29, 30); high-resolution MS m/e 258.0913 ($C_{16}H_{10}N_4$ requires 258.0905).

Attempted Reaction To Form 3-Azadiindole 8c. Ketobenzamide **5** was dissolved in 4-hydrazinopyridine (**7c**),[40] and the solution was heated (160 °C, 9 h). This solution was then allowed to cool, excess hydrazine (2 mL) was added, and heating was continued (110 °C, 12 h). After allowing the solution to cool, excess hydrazine was removed under reduced pressure and excess pyridylhydrazine was removed via Kugelrohr distillation. The residue was taken up in $CHCl_3$ from which a solid precipitated. The solid was filtered and dried to furnish 4-amino-β-carboline (**8e**), identical in all respects with an authentic sample from an alternate route. Attempted reaction in ethylene glycol and ethanolic HCl also failed uniformly.

9,14-Dihydroindolo[2″,3″:5′,4′]pyrido[2′,3′:4,5]pyrrolo[2,3-*a*]naphthalene (11a). Ketobenzamide **5** (0.58 g, 2 mmol) and neat 1-hydrazinonaphthalene (**10a**)[39] (1.58 g, 10 mmol) were heated (170 °C, 12 h). This solution was allowed to cool, excess hydrazine (16 mL) was added, and heating was continued (120 °C, 12 h). After the solution was allowed to cool, excess hydrazine was removed under reduced pressure. The residue was treated with ethanolic HCl, and the hydrochloride salt which resulted was filtered, dried, and recrystallized from EtOH (246 mg, 40%): mp >300 °C; ^1H NMR (Me_2SO-d_6, 500 MHz), δ 7.55 (t, J = 8.0 Hz, 1 H), 7.70 (t, J = 8.0 Hz, 1 H), 7.80 (t, J = 8.0 Hz, 2 H), 7.90 (m, 2 H), 8.15 (d, J = 8.0 Hz, 1 H), 8.60 (d, J = 8.0 Hz, 1 H), 9.15 (d, J = 8.0 Hz, 1 H), 9.25 (s, 1 H), 9.30 (d, J = 8.0 Hz, 1 H), 12.86 (s, 1 H), 13.44 (s, 1 H). After assignment of the ring-A [C(10)-C(13), $J \simeq 8$ Hz] and β-carboline [C(8); s (1 H), δ 9.25] protons, the remaining four signals [an upfield doublet, a downfield doublet, a triplet, and a multiplet] were all shown to be coupled through decoupling experiments. The downfield doublet (1 H, δ 9.15, J = 8.0 Hz) was assigned as the proton at C(6) [position-3 of the naphthalene ring], due to the proximity of this position to the pyridine N:(7). The first-order coupled upfield doublet (1 H, δ 8.60, J = 8.0 Hz) was correspondingly assigned to the proton at the adjacent C(5) [position-4]. Chemical shift equivalence presumably resulted in the protons of C(2)/C(3) appearing as a triplet resonance (2 H, δ 7.80, J = 8.0 Hz), in addition to a multiplet resonance (2 H, δ 7.90) for the protons of C(1)/C(4). These assignments dictated the structure of the 1,2-angular benzodiindole **11a**; MS (CI CH_4) m/e (rel inten) 308 (M + 1, 100); high-resolution MS m/e 307.1110 ($C_{21}H_{13}N_3$ requires 307.1109). Anal. ($C_{21}H_{13}N_3 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

9,14-Dihydroindolo[2″,3″:5′,4′]pyrido[2′,3′:4,5]pyrrolo[3,2-*a*]naphthalene (11b). Ketobenzamide **5** (0.58 g, 2 mmol) and neat 2-hydrazinonaphthalene (**10b**)[39] (1.58 g, 10 mmol) were heated (170 °C, 12 h). This solution was allowed to cool, excess hydrazine (16 mL) was added, and heating was continued (120 °C, 12 h). After the solution was allowed to cool, excess hydrazine was removed under reduced pressure. The residue was treated with ethanolic HCl, and the hydrochloride salt which resulted was filtered, dried, and recrystallized from EtOH (370 mg, 60%): mp >300 °C; ^1H NMR (Me_2SO-d_6, 500 MHz), δ 7.50 (t, J = 8.0 Hz, 1 H), 7.60 (t, J = 8.0 Hz, 1 H), 7.75 (t, J = 8.0 Hz, 1 H), 7.80 (t, J = 8.0 Hz, 1 H), 7.85 (d, J = 8.0 Hz, 1 H), 8.00 (d, J = 8.0 Hz, 1 H), 8.10 (d, J = 8.0 Hz, 1 H), 8.15 (d, J = 8.0 Hz, 1 H), 9.10 (d, J = 8.0 Hz, 1 H), 9.15 (s, 1 H), 9.45 (d, J = 8.0 Hz, 1 H), 12.50 (s, 1 H), 13.50 (s, 1 H); MS (CI CH_4) m/e (rel inten) 308 (M + 1, 100); high-resolution MS m/e 307.1123 ($C_{21}H_{13}N_3$ requires 307.1109). Anal. ($C_{21}H_{13}N_3 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

10*H*-Indolo[2″,3″:5′,4′]pyrido[3′,2′:4,5]imidazo[1,2-*a*]quinoline (11c). Ketobenzamide **5** (0.58 g, 2 mmol) and neat 2-hydrazinoquinoline (**10c**)[40] (1.60 g, 10 mmol) were heated (170 °C, 12 h). This solution was allowed to cool, excess hydrazine (16 mL) was added, and heating was continued (120 °C, 12 h). After the solution was allowed to cool, excess hydrazine was removed under reduced pressure. The residue was treated with ethanolic HCl, and the hydrochloride salt which resulted was filtered, dried, and recrystallized from EtOH (154 mg, 25%): mp >300 °C; ^1H NMR (Me_2SO-d_6, 500 MHz), δ 7.40 (t, J = 8.0 Hz, 1 H), 7.65 (t, J = 8.0 Hz, 1 H), 7.75 (t, J = 8.0 Hz, 1 H), 7.80 (d, J = 8.0 Hz, 1 H), 8.00 (d, J = 8.0 Hz, 1 H), 8.05 (t, J = 8.0 Hz, 1 H), 8.20 (d, J = 7.0 Hz, 1 H), 8.30 (d, J = 8.0 Hz, 1 H), 8.80 (d, J = 8.0 Hz, 1 H), 9.10 (s, 1 H), 10.10 (d, J = 7.0 Hz, 1 H), 12.25 (s, 1 H); MS (EI 15 eV) m/e (rel inten) 308 (M^+, 100). Anal. ($C_{20}H_{12}N_4 \cdot {}^1/_2H_2O$) C, H, N.

9,14-Dihydroindolo[2″,3″:5′,4′]pyrido[2′,3′:4,5]pyrrolo[2,3-*c*]quinoline (11d). Ketobenzamide **5** (0.58 g, 2 mmol) and neat 3-hydrazinoquinoline (**10d**)[39] (1.60 g, 10 mmol) were heated (170 °C, 12 h). This solution was allowed to cool, excess hydrazine (16 mL) was added, and heating was continued (120 °C, 12 h). After the solution was allowed to cool, excess hydrazine was removed under reduced pressure. The residue was treated with ethanolic HCl, and the hydrochloride salt which resulted was filtered, dried, and recrystallized from EtOH (155 mg, 25%): mp >300 °C; ^1H NMR (Me_2SO-d_6, 250 MHz), δ 7.45 (t, J = 8.0 Hz, 1 H), 7.65 (t, J = 8.0 Hz, 1 H), 7.70 (t, J = 8.0 Hz, 1 H), 7.80 (m, 2 H), 8.20 (d, J = 8.0 Hz, 1 H), 8.90 (d, J = 8.0 Hz, 1 H), 9.15 (s, 1 H), 9.42 (s, 1 H), 9.45 (d, J = 8.0 Hz, 1 H), 12.40 (s, 1 H), 13.20 (s, 1 H). After assignment of the ring-A [C(10)-C(13), $J \simeq 8$ Hz] and β-carboline [C(8); s (1 H), δ 9.15] protons by decoupling and NOE experiments, the signals which remained consisted of a multiplet (2 H), a triplet (1 H), and two downfield resonances: a singlet (1 H) and a doublet (1 H) [all J = 8.0 Hz]. The singlet at δ 9.42 presumably corresponded to an isolated proton adjacent to an aromatic heteroatom, similar to the isolated downfield singlet at δ 9.35 of 2-azadiindole **8b**. It was assigned as the proton at C(1), which is consistent with an annelated 2-azadiindole. Chemical shift equivalence arguments and decoupling experiments similar to those above served to resolve the identity of the remaining resonances (d, t, m) as those of the protons of ring H; MS (CI CH_4) m/e (rel inten) 309 (M + 1, 100). Anal. ($C_{20}H_{12}N_4 \cdot 2HCl \cdot 4H_2O$) C, H, N.

10*H*-Indolo[3″,2″:4′,5′]pyrido[3′,2′:4,5]imidazo[2,1-*a*]isoquinoline (20). Ketobenzamide **5** (200 mg, 0.69 mmol) and 1-hydrazinoisoquinoline (**19**)[49,56] (120 mg, 0.75 mmol) were dissolved in triethylene glycol (10 mL) and heated (185 °C, 18 h) accompanied by nitrogen purging. Ammonia evolution was observed after 3 h. The solvent was removed by Kugelrohr distillation, and the residue that remained was subjected to repetitive chromatography (SiO_2; EtOAc/hexane, 1:1) to yield **20** (17 mg, 9%): mp 132–134 °C, 150 °C dec; IR (KBr) 3100, 2950, 1600, 1470, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.40 (t, J = 7.4 Hz, 1 H), 7.46 (d, J = 7.2 Hz, 1 H), 7.61 (t, J = 7.7 Hz, 1 H), 7.72 (d, J = 8.1 Hz, 1 H), 7.82 (m, J = 9.1 Hz, 2 H), 8.04 (m, J = 9.1 Hz, 1 H), 8.61 (d, J = 7.8 Hz, 1 H), 8.81 (d, J = 7.2 Hz, 1 H), 8.85 (m, J = 9.1 Hz, 1 H), 8.86 (s, 1 H), 11.97 (s, 1 H). The assignment

of the ring-A [C(11)-C(14), $J \simeq 8$ Hz] and β-carboline [C(9); s (1 H), δ 8.86] protons was based on decoupling experiments. The signals which remained consisted of two doublets and three multiplet resonances (two long range coupled multiplets and an additional multiplet). The downfield doublet (1 H, δ 8.81, J = 7.2 Hz) suggested the presence of a terminal proton adjacent to an aromatic heteroatom and was assigned as the proton at C(6), with the coupled upfield doublet (1 H, δ 7.46, J = 7.2 Hz) assigned as the adjacent proton at C(5). Analogous to the C(6)-H resonance, the downfield multiplet (1 H, δ 8.85, J = 9.1 Hz) was assigned as the proton at C(1), with its long range coupled resonance (1 H, δ 8.04, J = 9.1 Hz) as the proton at C(4). Coupling and chemical shift equivalence of the protons within ring F of **20** similar to that observed for **11a** presumably resulted in complex resonances for all four protons of ring F; consequently, the multiplet which remained (2 H, δ 7.82, J = 9.1 Hz) was assigned as due to the protons of C(2)/C(3); MS (EI 15 eV) m/e (rel inten) 308 (M^+, 100); high-resolution MS m/e 308.1048 ($C_{21}H_{13}N_3$ requires 308.1062). Anal. ($C_{20}H_{12}N_4 \cdot {}^7/_4H_2O$), C, H, N.

2-Benzoyl-4-(phthalazin-1-ylhydrazo)-1,2,3,4-tetrahydro-β-carboline (23). Ketobenzamide **5** (100 mg, 0.345 mmol) and 1-hydrazinophthalazine (**22**)[57] (60 mg, 0.373 mmol) were dissolved in EtOH (10 mL) to which acetic acid (glacial, 1 mL) was added. The yellow-colored solution which resulted was heated (reflux, 20 h). Upon cooling, a yellow solid precipitated from the solution, which was then filtered and recrystallized from MeOH to furnish **23** (109 mg, 73%): mp 245–247 °C; IR (KBr) 3289, 3254, 1609, 750 cm^{-1}; ^1H NMR (Me_2SO-d_6, 500 MHz) δ 4.80 (s, 1 H), 5.01 (s, 1 H), 5.07 (s, 1 H), 5.23 (s, 1 H), 7.12–7.21 (m, 2 H), 7.46 (m, 7 H), 7.66 (m, 3 H), 7.99 (m, 1 H), 8.51 (d, 1 H), 11.36 (s, 1 H), 11.66 (s, 1 H); MS (EI 15 eV) m/e (rel inten) 433 (M^+, 4.8). Anal. ($C_{26}H_{20}N_6O$) C, H, N.

10*H*-Indolo[3″,2″:4′,5′]pyrido[3′,2′:4,5]imidazo[2,1-*a*]phthalazine (24). Phthalazine hydrazone (**23**) (520 mg, 1.20 mmol) was dissolved in ethylene glycol (25 mL), and the solution was heated (170 °C, 21 h). This solution was allowed to cool, excess hydrazine (5 mL) was added, and heating was continued (reflux, 20 h). After the solution was allowed to cool, excess hydrazine was removed under reduced pressure and excess ethylene glycol was removed by Kugelrohr distillation. The brown oil that remained was chromatographed (SiO_2; EtOAc/MeOH, 85:15) and then treated with ethanolic HCl to furnish **24** (141 mg, 45%): mp 245.1–246.9 °C; IR (KBr) 3416, 1630, 1335, 750 cm^{-1}; ^1H NMR (Me_2SO-d_6, 500 MHz) δ 7.40 (t, $J_{13\text{-}14(12)}$ = 8.0 Hz, 1 H), 7.62 (t, $J_{12\text{-}13(11)}$ = 8.0 Hz, 1 H), 7.68 (d, $J_{11\text{-}12}$ = 8.0 Hz, 1 H), 8.01 (t, $J_{2\text{-}3(1)}$ = 7.5 Hz, 1 H), 8.14 (t, $J_{3\text{-}4(2)}$ = 7.5 Hz, 1 H), 8.30 (d, $J_{1\text{-}2}$ = 7.5 Hz, 1 H), 8.74 (d, $J_{14\text{-}13}$ = 8.0 Hz, 1 H), 8.92 (d, $J_{4\text{-}3}$ = 7.5 Hz, 1 H), 8.98 (s, 1 H), 9.26 (s, 1 H), 12.15 (s, 1 H); MS (EI 15 eV) m/e (rel inten) 309 (M^+, 29); high-resolution MS m/e 309.1000 ($C_{16}H_{11}N_5$ requires 309.1014).

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Supplementary Material Available: The ab initio optimized coordinates, connection tables, and fractional charges of compounds **2**, **4a**, **4b**, **9**, and **11c** (3 pages). Ordering information is given on any current masthead page.