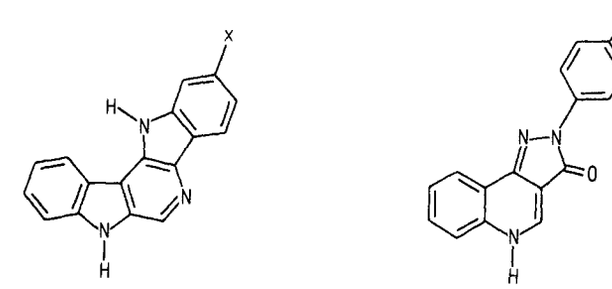
Synthesis of 7,12-Dihydropyrido[3,4-*b*:5,4-*b*']diindoles. A Novel Class of Rigid, Planar Benzodiazepine Receptor Ligands

Sir:

The practice of incorporating biofunctionality into a rigid framework to enhance activity or selectivity of action is by no means new. The early work of Bentley et al.[1] in the morphine area serves as an excellent example. While structure–activity relationship studies[2a–f] suggest that one necessary criteria for high-affinity binding of ligands to benzodiazepine receptors (Bz R) is the ability of these molecules to assume a planar or pseudoplanar topography,[2a–f] to our knowledge no completely rigid compounds with a planar geometry have been shown to bind with high affinity to Bz R. We now report the synthesis of high-affinity ligands of the Bz R that originated from studies directed toward this goal. These molecules are based on the 7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole system **2** and have been shown to possess high affinity (5–15 nM, see Table I) for Bz R. Moreover, substitution on the E ring of the pyridodiindoles has a marked effect on both the in vitro binding affinity and the pharmacological activity of these compounds: replacement of either hydrogen or methoxyl at position 2 of **2** by a chlorine atom results in a change in the activity from an inverse agonist to an antagonist.

High-affinity, stereoselective binding sites for benzodiazepines in the mammalian central nervous system are currently thought to mediate the principal pharmacological actions of the benzodiazepines.[3] Since the discovery of these receptors in 1977, at least one-half dozen unique classes of compounds have been shown to bind to these sites. Such compounds have been shown to produce a wide range of pharmacologic actions that vary from those that are virtually indistinguishable from benzodiazepines (e.g., zopiclone)[4] to "inverse agonists" that produce convulsions,[5] reduce sleep,[6] and produce a syndrome resembling fear or anxiety in primates.[7] Other compounds, such as the pyrazoloquinolinones[8] CGS-9896, CGS-9895, and CGS-8216

Scheme I



2, X = H, inverse agonist

4e, X = OCH_3

CGS-8216, X = H, inverse agonist

CGS-9895, X = OCH_3

have been shown to possess both selective agonist (benzodiazepine-like), mixed agonist/antagonist, and partial inverse agonist properties,[9] respectively. Our data indicate that **2** and the analogues **4d–f** block the anticonvulsant activity of diazepam. Furthermore, **2** and **4e** (see Table I) are proconvulsants, which suggests these compounds may function as "partial" inverse agonists at Bz R. The structural similarities between **2** and CGS-8216 are clearly illustrated in Scheme I and are based on molecular models and electron-density maps.[10] Examination of the in vitro binding data for the pyridodiindoles indicates that position 2 (X = H, F, Cl, OCH_3) is the most compatible with potent affinity in agreement with the location of similar groups in the CGS series; derivatives **2**, **4c**, **4d**, and **4e** possess IC_{50} values ≤10 nM, whereas the other analogues have a lower affinity (see Table I) for these receptors. Thus, these rigid, planar pyridodiindoles represent a new class of high-affinity ligands for Bz R.

Chemistry. When 2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (1) was heated in a 25-fold excess of phenylhydrazine at 150 °C, **2** was obtained as the sole product in 88% yield, as illustrated in Scheme II.[11] The substituted derivatives **4a–i** were prepared by heating 1 in a two- to fivefold excess of the appropriately substituted phenylhydrazine for 4 h at 160 °C. This gave the intermediate benzamide **3** via a Fischer indole cyclization (Scheme II). Generally, in this procedure a 25-fold excess of hydrazine was then added to the reaction mixture, and without isolating intermediate **3** the solution was then allowed to reflux for 12 h. This furnished the desired substituted pyridodiindoles **4a–i**, in yields ranging from 50% to 90%. The analogues **4c–e** were prepared from meta-substituted phenylhydrazines as described above; however, the Fischer indole cyclization gave two isomeric derivatives from each reaction, as expected. In each case the 2-substituted isomer predominated over the corresponding 4-substituted analogue and the isomers could be obtained in pure form via flash chromatography (CH_3OH/CH_3CN, SiO_2).

Biological Activity. The biological profiles of compounds **2** and **4a–i** are illustrated in Table I. The ability of these agents to inhibit [^{3}H]diazepam binding to rat

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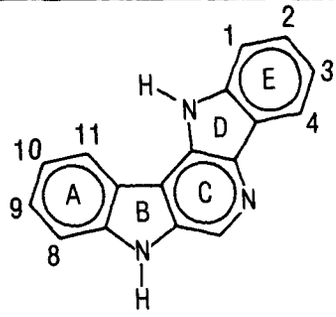
Table I. Biological Activity of 7,12-Dihydropyrido[3,2-*b*:5,4-*b'*]diindoles



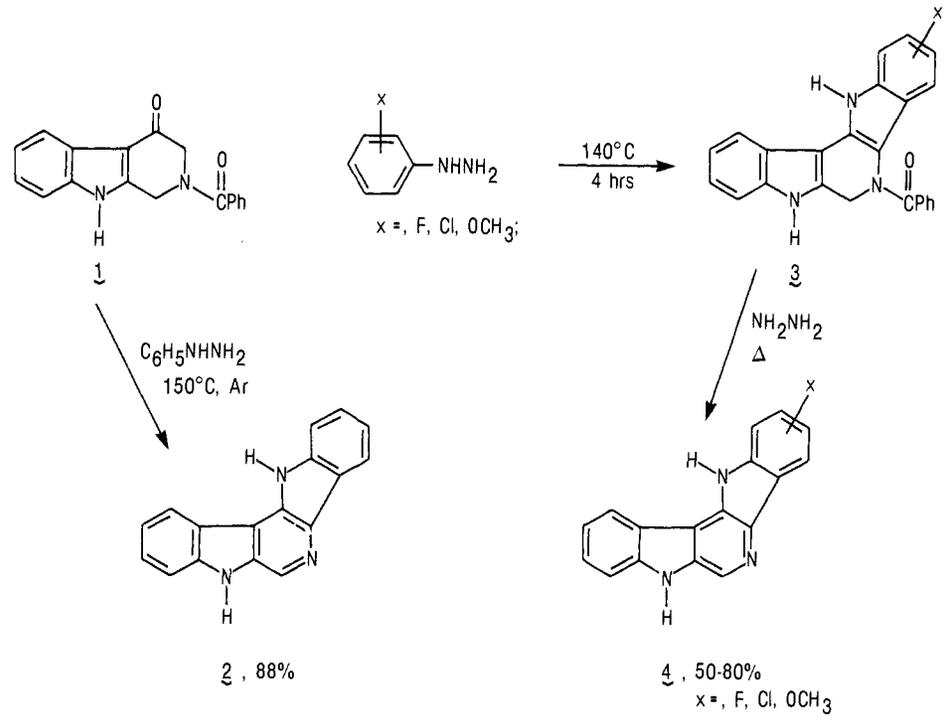
Agent[a]	Substitution 1	2	3	4	*in vitro*[b] IC_{50}, nM	Proconvulsant Actions[b] ED_{50}, mg/Kg[c]	Diazepam Antagonism[b] ED_{50}, mg/Kg[c]	Yield %	Analyses[f]
2	**H**	**H**	**H**	**H**	**4**	15.1	21.3	88	$C_{17}H_{11}N_3 \cdot 1\ 1/8\ H_2O$[g]
4a	F	H	H	H	12			51	$C_{17}H_{10}N_3F$[h]
b	Cl	H	H	H	80			73	$C_{17}H_{10}N_3Cl$
c	**H**	**F**	**H**	**H**	**7**			36	$C_{17}H_{10}N_3F$
d	**H**	**Cl**	**H**	**H**	**10**	—[e]	2.5	44	$C_{17}H_{10}N_3Cl$
e	**H**	**OCH_3**	**H**	**H**	**8**	7.0	3.9	52	$C_{18}H_{13}N_3O \cdot HCl \cdot 3/4\ H_2O$
f	H	H	F	H	16	—[e]	24	69	$C_{17}H_{10}N_3F$
g	H	H	Cl	H	2150			72	$C_{17}H_{10}N_3Cl$
h	H	H	OCH_3	H	890			90	$C_{18}H_{13}N_3O \cdot HCl \cdot 1/4\ H_2O$
i	H	H	H	Cl	715			15	$C_{17}H_{10}N_3Cl$

[a] All dihydropyridodiindoles were administered as the HCl salts. [b] See: Cain, M.; Weber, R.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081 and references cited therein. Also, see 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 anticonvulsant effects of diazepam (2.5 mg/kg) in mice that had not been given a convulsant dose of PTZ (80 mg/kg). [e] Did not produce convulsions at the highest dose tested (30 mg/kg). [f] Analyzed for C, H, N. Structures were determined by NMR (250 MHz), IR, and mass spectroscopy. [g] H: calcd, 4.78; found, 4.37. [h] C: calcd, 74.17; found, 73.62.

cerebral cortical membranes was performed as previously described.[2b] On the basis of the in vitro data it is clear that both steric and electronic constraints may be placed on the rigid pyridodiindole ligands by the receptor site. Since the receptor site will tolerate the fluoro substituent at any position of the E ring of **2** with little or no loss in affinity, it would appear that steric rather than electronic constraints are of primary importance. However, when the larger chloro and methoxyl substituents were moved from the 2-position to the adjacent 1-, 3-, or 4-positions, the affinities for Bz R decreased dramatically. For example, the 3- (**4g**, **h**) and 4- (**4i**) substituted derivatives have IC_{50} values 2–3 orders of magnitude lower than the corresponding 2-substituted analogues. Consequently, as mentioned above, both steric and electronic effects contribute to the in vitro affinities of these compounds.

Compounds **2**, **4d**, and **4e** were evaluated in vivo because of their structural resemblance to the pyrazoloquinolinones CGS-8216, -9895, and -9896, respectively (Scheme I). The 3-fluoropyridodiindole **4f** was also screened since it was the only 3-substituted derivative to demonstrate potent binding affinity in vitro to the receptor. These compounds were tested in mice for anticonvulsant properties by using the convulsant pentylenetetrazole (PTZ, 80 mg/kg) and for proconvulsant actions by employing a subthreshold dose of PTZ (40 mg/kg).[2b] These diindoles were also tested as benzodiazepine antagonists by assessing their ability to disrupt the anticonvulsant actions of diazepam (2.5 mg/kg) against PTZ (80 mg/kg).[2b] In brief, adult male mice (NIH colony, 20–25 g) were injected intraperitoneally with graded doses of the pyridodiindoles **2**, **4d–f** or an equal volume of vehicle (0.1 mL) 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 agents or vehicle, followed 15 min later by administration of diazepam (2.5 mg/kg, ip). Fifteen minutes after injection of diazepam, animals were injected with PTZ (80 mg/kg). In vehicle-pretreated mice, 80 mg/kg produced tonic–clonic convulsions in 100% of the animals, while the 40 mg/kg dose was subconvulsant. The dose of diazepam used in these studies protected >90% of the mice tested against PTZ. Under these conditions none of the compounds tested demonstrated anticonvulsant actions (results not shown).[12] The parent diindole **2** was found to be a proconvulsant with an ED_{50} value of 15.5 mg/kg (see Table I). At the

Scheme II



highest dose tested (40 mg/kg) in the proconvulsant paradigm, **2** produced a maximum of 60% seizures. This compound also blocked the anticonvulsant effects of diazepam with an ED_{50} value of 21.3 mg/kg. In contrast, in the proconvulsant paradigm, 2-chloro-7,12-dihydropyrido[3,4-*b*:5,4-*b*']diindole (**4d**) potently antagonized the anticonvulsant actions of diazepam (ED_{50} = 2.5 mg/kg), but did not have a proconvulsant action, even at the highest dose tested (30 mg/kg). It is noteworthy that replacement of the hydrogen at position 2 of the parent **2** with a chlorine atom resulted in **4d**, in which inverse agonist activity was completely lost. Likewise, in the CGS series (X = H), the parent compound (8216) has partial inverse agonist actions, while the chloro analogue (9896) loses inverse agonist qualities and becomes a selective agonist.[8,9] The 2-methoxy derivative **4e** was found to be a proconvulsant (ED_{50} = 7.0 mg/kg) and also potently antagonized the anticonvulsant effects of diazepam (ED_{50} = 3.4 mg/kg). Substitution of the pyridodiindole series at position 2 does change the pharmacological profile, but not as drastically as in the CGS series. The 3-fluoro analogue **4f** was not a proconvulsant, but antagonized the anticonvulsant effects of diazepam, albeit with a lower potency (ED_{50} = 24 mg/kg) than the 2-substituted derivatives **4d** or **4e**.

These findings indicate that **2** and **4e** are behaving as partial inverse agonists at the Bz R, while the 2-chloro analogue **4d** is an antagonist that lacks inverse agonist activity in the paradigm described here. Further examination of the pharmacological profile (e.g., in conflict/proconflict, muscle relaxant, and motor activity paradigms) will better define the nature of these compounds. Nonetheless, the preliminary spectrum of activity for **2** suggests that this compound may behave as a partial inverse agonist, while **4d** possesses antagonist actions similar to those of Ro 15-1788.

The significance of the results described herein rest on the rigid, planar nature of these pyridodiinole ligands which have no degrees of conformational freedom, bind with high affinity to Bz R, and have different pharmacological profiles (**2** and **4e** vs. **4d**). Rigid analogues related to the structures of **4d–f** will be important in searching for agents to selectively effect one type of Bz receptor in the presence of another, as well as providing rigid ligands to probe the structure of the pharmacophore for Bz R.

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(12) **4d** (60 mg/kg, ip) was ineffective in protecting mice against maximal electroshock (MES). In constrast, diazepam (38 mg/kg) protected 100% of mice against MES-induced convulsions.

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