Structural Requirements for Agonist Actions at the Benzodiazepine Receptor: Studies with Analogues of 6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester

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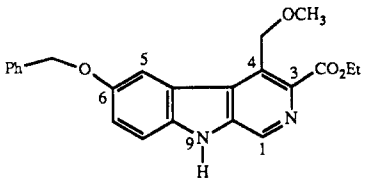
The analogues **2**, **3**, **8a–c**, **9**, **13**, and 14 of the β-carboline 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester (ZK-93423, 1) were synthesized in order to determine which functional groups are required for agonist activity exhibited by the parent compound 1 at the benzodiazepine receptor (BzR). The potencies of these compounds were evaluated in vitro and in vivo; the β-carbolines **2**, **8a–c**, and **13** were found to exhibit high affinity (0.5–22 nM) for BzR, but in vivo studies demonstrated they were not anticonvulsant while analogues **8a** and **8b** antagonized the effects of diazepam. These results are in agreement with the model proposed for the pharmacophore of the agonist site (domain) of the BzR, which requires the interaction of two sites of electron density [N(2) and C(4)], a third at E^+ (electrostatic or steric), and two of lipophilicity [C(3) and C(6)] of the ligand with the BzR protein (see Figure 1). The pharmacophore for the agonist site is derived from the structure–activity relationships of the benzodiazepines, pyrazoloquinolines, and the β-carboline 1, and compared to interactions required for ligand inverse agonist activity.

The identification of stereospecific, high-affinity binding sites for benzodiazepines (Bz) in the mammalian CNS has been described in vivo[1-3] and in vitro.[4-6] The benzodiazepines, which are the most widely prescribed psychoactive drugs in current therapeutic use,[7,8] exhibit a wide range of pharmacological actions which include anticonvulsant,[9] sedative–hypnotic,[9] muscle relaxant,[10] and anxiolytic[11] effects. It has been shown that the benzodiazepine receptor (BzR) is part of a supramolecular complex which also contains discrete but allosterically coupled recognition sites for GABA and barbiturates. The oligomeric units of this supramolecular complex are thought to form a drug and transmitter responsive chloride channel.[12,13] It is generally believed that BzR ligands elicit their pharmacological effects through modulation of this ligand-gated chloride ion channel. The pharmacological properties of these ligands appear to be a continuum,[14a] ranging from a complete mimicry of the 1,4-benzodiazepines (sometimes referred to as "full agonists") to substances that produce actions best described as opposite to the benzodiazepines (that is, convulsions, anxiety, increased muscle tone)[14b] termed inverse agonists.[14c]

There are a wide variety of compounds (Bz) which exhibit full agonist activity; however, compounds which are structurally unrelated to the benzodiazepines, but which possess similar efficacy for the BzR, have also been reported. One of the most important in this series is the β-carboline derivative 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester 1.[15-18]



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This is the only β-carboline reported to date which exhibits full agonist activity.[15-19] The present investigation was initiated to execute a structure–activity relationship (SAR) study of analogues of 1 to determine the functional substituents necessary to elicit an agonist profile of activity. Furthermore, there is controversy as to the nature of the binding sites for inverse agonist/antagonist and agonist ligands at BzR[20-22] and the relationship between the β-

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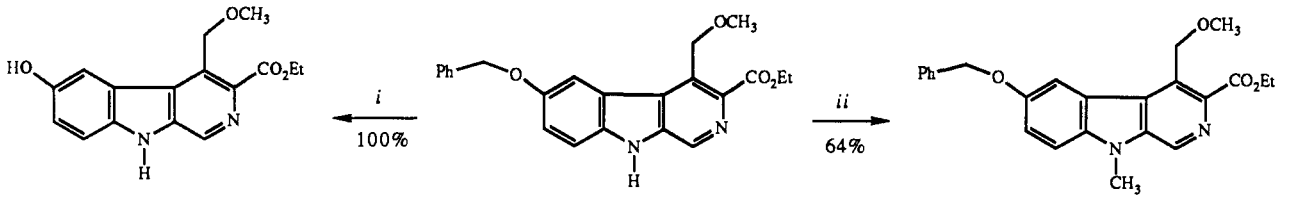
Scheme I[a]



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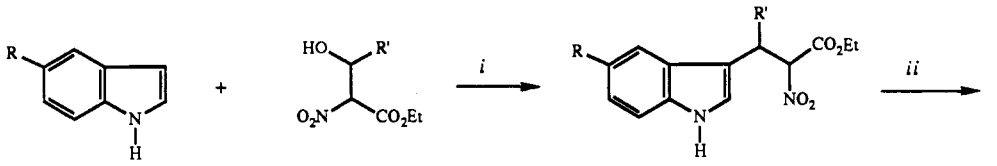
[a] Reagents: i, 10% Pd–C, H_2, 760 mmHg, EtOH; ii, NaH, CH_3I, DMF.

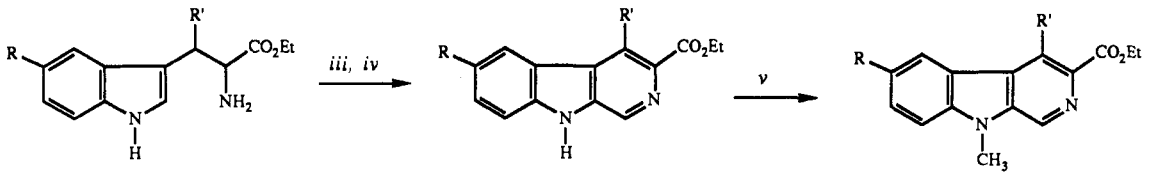
Scheme II[a]



4a: R = OCH_3
4b: R = H
4c: R = OCH_2Ph

5a: R' = CH_2OCH_3
5b: R' = CH_2CH_3

6a: R = OCH_3, R' = CH_2OCH_3
6b: R = H, R' = CH_2OCH_3
6c: R = OCH_2Ph, R' = CH_2CH_3



7a: R = OCH_3, R' = CH_2OCH_3
7b: R = H, R' = CH_2OCH_3
7c: R = OCH_2Ph, R' = CH_2CH_3

8a: R = OCH_3, R' = CH_2OCH_3
8b: R = H, R' = CH_2OCH_3
8c: R = OCH_2Ph, R' = CH_2CH_3

9: R = OCH_2Ph, R' = CH_2CH_3

[a] Reagents: i, $PhCH_3$, AcOH, Δ;[26] ii, Raney nickel, H_2, EtOH, 760 mmHg, 25 °C; iii, $OHCCO_2H$, $PhCH_3$, reflux;[27] iv, xylenes, Pd–C, reflux;[28] v. NaH, CH_3I, DMF.

carboline 1 (an agonist) and inverse agonists such as methyl β-carboline-3-carboxylate (β-CCM) may provide a link between these sites at BzR. The recent report by Pritchett et al.[23] that at least three distinct cDNA's must be expressed in vitro for a fully functional (i.e. BzR modulated, GABA gated) chloride channel indicates the BzR may be constituted by domains formed by the interaction of these proteins. This finding adds another level of complexity to the question of the elements necessary to form BzR as defined by classical neurochemical techniques. For this reason we have continued to treat the inverse agonist/antagonist and agonist sites as separate entities, although different interactions in the same domain related to the hypothesis of Ehlert[22] is also consistent with this approach.

The specific β-carbolines necessary for this study were prepared by systematic variation of the substituents at C(4), C(6), and N(9), after which data from in vitro and in vivo (anticonvulsant) paradigms were evaluated.

Chemistry

The initial phase of this study centered on alteration of the electronic character of the ligand at C(4) without loss of ligand topology (i.e. replacement of 4-CH_2OCH_3 with CH_2CH_3). Moreover, variation of both the electronic and lipophilic nature of the ligand at C(6) was carried out, while holding all other parameters constant.

Initially, the agonist 1 was converted into the phenolic β-carboline **2** by catalytic hydrogenation over Pd–C in quantitative yield (Scheme I). Although **2** demonstrated potent affinity for BzR (IC_{50} = 0.9 nM), administration (ip) of up to 40 mg/kg did not block pentylenetetrazole (PTZ) induced seizures; moreover, this analogue did not produce a proconvulsant action nor antagonize the anticonvulsant effects of diazepam. In order to determine if the ligand hydrophilicity retarded penetration across the blood–brain barrier, the octanol/water partition coefficient[24,25] was calculated. Indeed, the partition coefficient (log P = 1.44) of **2** suggests a much higher hydrophilicity than 1 (log P = 3.97) and indicates that sufficient concentrations of **2** may not be achieved in vivo. Consequently, the corresponding 6-OCH_3 derivative (**8a**) of 1 was synthesized (see Scheme II). Thus, 5-methoxyindole (**4a**) was allowed to react with ethyl 4-methoxy-3-hydroxy-2-nitrobutanoate (**5a**)[26] according to the method of Neef et al.,[26] to provide the corresponding nitro ester **6a**. Raney nickel reduction of the nitro function afforded 3-(5-methoxyindol-3-yl)-2-amino-4-methoxybutanoic acid ethyl ester (**7a**) in 48% overall yield from **4a**. Cyclization of **7a** with glyoxylic acid was achieved via a modification of the Pictet–Spengler reaction[27] to provide the tetrahydro-β-

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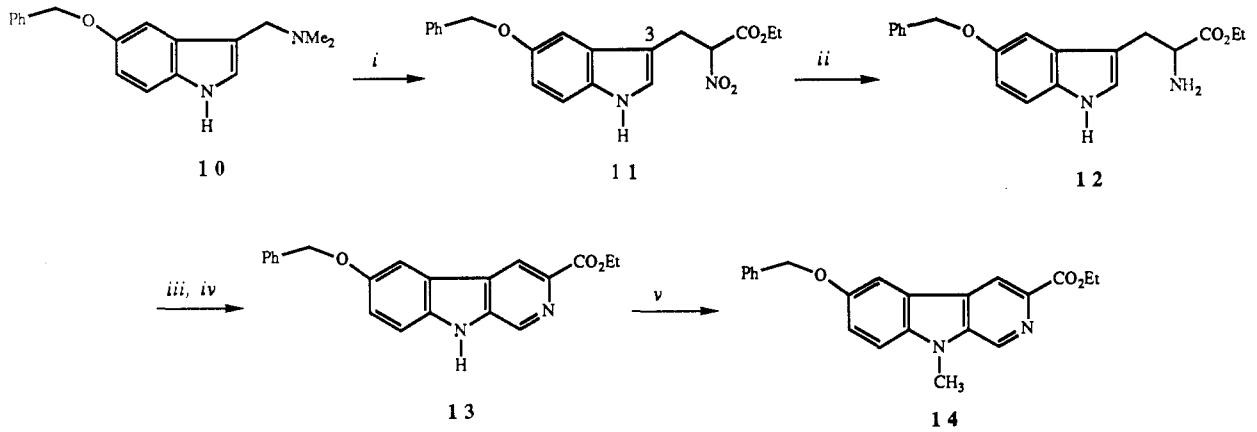
Scheme III[a]



[a] Reagents: i, $O_2NCH_2CO_2Et$, $PhCH_3$, Δ;[29] ii, Raney nickel, H_2, EtOH, 760 mmHg, 25 °C; iii. $OHCCO_2H$, $PhCH_3$, reflux;[27] iv, xylenes, Pd–C, reflux;[28] v, NaH. CH_3I, DMF.

carboline. Subsequent oxidation[28] of the intermediate over Pd–C in mixed xylenes provided the fully aromatic β-carboline **8a**. This ligand had a high affinity for BzR (IC_{50} = 0.5 nM), but unlike the parent **1**, it did not antagonize the convulsant effects of PTZ. However, **8a** antagonized the anticonvulsant effects of diazepam (EC_{50} = 1.25 mg/kg), indicating this compound was capable of penetrating the blood–brain barrier. A related ligand **8b** was prepared from indole **4b** and hydroxy ester **5b** via a series of reactions (Scheme II) analogous to those reported for **8a** above. This β-carboline **8b** (IC_{50} = 2.3 nM), which is devoid of a substituent at C(6), was not an anticonvulsant (Table I), but it antagonized the anticonvulsant actions (EC_{50} = 15 mg/kg) of diazepam.

The effect of altering the substituent at C(4) of the β-carboline agonist **1** was then examined. In order to retain the same topology, the electronic nature of this substituent was altered by synthesis of the corresponding 4-ethyl analogue, 5-(benzyloxy)-4-ethyl-β-carboline-3-carboxylic acid ethyl ester (**8c**, Scheme II). This ligand possessed an IC_{50} value of 22 nM, but was not an anticonvulsant.

In addition, ethyl 6-(benzyloxy)-β-carboline-3-carboxylate (**13**) was synthesized since this ligand is completely devoid of a substituent at C(4). This material was prepared by reaction of ethyl nitroacetate with 5-(benzyloxy)gramine (**10**), according to the method of Lyttle[29] (Scheme III). The nitro ester **11** which resulted was then subjected to a reaction sequence similar to that previously described [Raney nickel reduction (→ **12**), Pictet–Spengler reaction,[27] and oxidation (→ **13**)[28]]. This ligand, 6-(benzyloxy)-β-carboline-3-carboxylic acid ethyl ester (**13**), exhibited a high affinity for BzR (IC_{50} = 8.9 nM), but was neither anticonvulsant or proconvulsant, nor did it antagonize the anti-PTZ action of diazepam. The related 6-benzylamino β-CCM prepared by Hagen[18] was also devoid (IC_{50} = 10 nM)[18] of agonist activity, but demonstrated low potency as a Bz antagonist. The in vivo data above provide additional support for the requirement of an electronegative substituent at C(4) to effect an agonist profile of activity in this series.

Controversy exists as to the nature of the binding sites for agonist and inverse agonists/antagonists at BzR. Skolnick[20a] and Fujimoto[20b] have advanced the domain (one site) theory, while Hirsch et al. favor the existence of two distinct binding sites.[21] Ehlert[22] has postulated that there are two distinct conformations of the same receptor protein, a ground state and an activated state.[23] In an attempt to shed light on this controversy, the N_a-methyl-β-carboline congeners (**3**, **9**, and **14**) were prepared, since it is known that N_a-methyl-β-carbolines do not bind tightly to the inverse agonist site.[30-32] For example, agonist **1** was converted into the corresponding N_a-methyl analogue **3** by treatment with NaH/MeI in dimethylformamide (Scheme I). This ligand **3** demonstrated a low affinity at BzR (945 nM, Table I). Similarly, the analogous N_a-methyl-4-ethyl derivative **9** was synthesized from **8c** (Scheme II) in 64% yield, and N_a-methyl-6-benzyloxy β-CCE **14** (65%) was prepared from **13** (Scheme III). These analogues exhibited IC_{50} values of >5000 nM (Table I). The low affinities of these N_a-methyl analogues (**3**, **9**, and **14**) when compared to their N_a–H counterparts suggest that agonists and inverse agonists may interact in different regions of the same binding domain; however, further work is necessary before this is resolved.

Discussion

A systematic modification of the ligand **1** at C(4) and C(6) resulted in a series of β-carbolines (**2**, **8a–c**, **13**) with high affinities for BzR (0.5–22 nM) (Table I). These modifications, however, resulted in a loss of the pharmacological actions of "full agonists" since, unlike the parent β-carboline **1**, these compounds lacked anticonvulsant activity against a standard challenge dose of pentylenetetrazole, while **8a** and **8b** only blocked the anticonvulsant actions of diazepam (Table I). These findings are in

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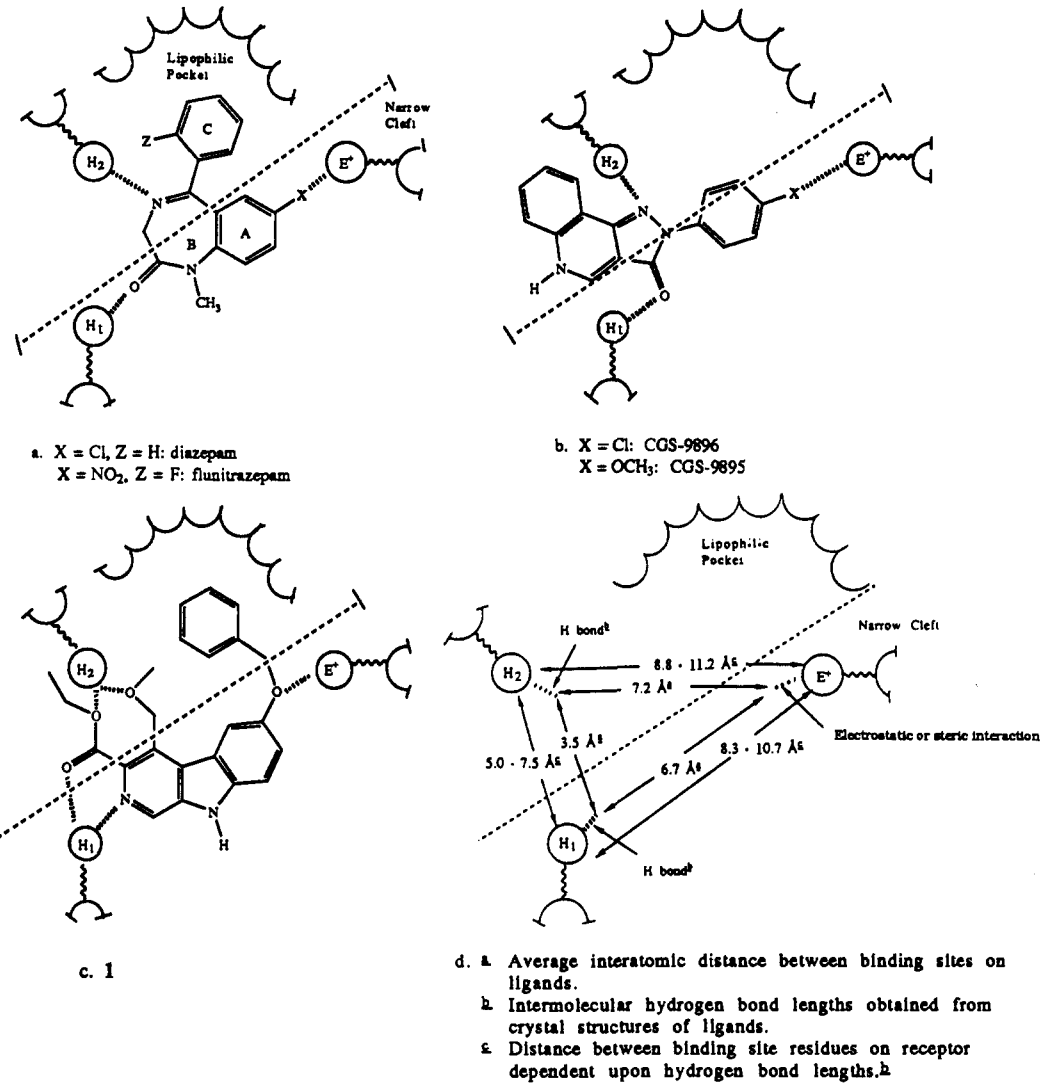

Figure 1. Interactions at the proposed agonist pharmacophore for (a) the benzodiazepines, (b) the pyrazoloquinolin-3-ones, and (c) 1. The full model of the pharmacophore is outlined in (d).

agreement with the requirements of the pharmacophore for the agonist site developed here (Figure 1)[32] and elsewhere (see below).[33] The model of the pharmacophore of the inverse agonist/antagonist binding site (domain) is documented[30,32] and was formulated independent of the agonist site from the SAR and in vivo efficacy of the pyridodiindole, β-carboline, and the 2-arylpyrazoloquinoline ligands.[30,32] It has been proposed[30,32,34] that the inverse agonists, partial inverse agonists, and antagonists interact at the same active site (domain) of the BzR, and a proposed model of this pharmacophore is illustrated in Figure 2.[30,32]

The essential features necessary for high affinity ligand binding to the inverse agonist/antagonist site are a hydrogen bond acceptor site H_1 (perhaps three-centered) and a hydrogen bond donor site D_2, both of which lie at the base of a narrow lipophilic cleft.[30,32] Hence, only planar ligands (i.e. pyridodiindoles) or ligands which can assume

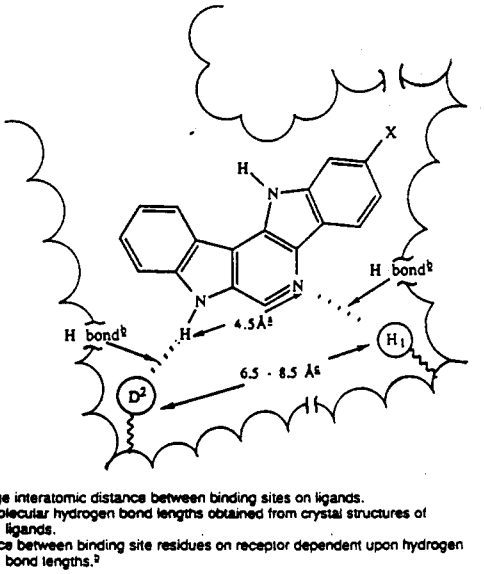

Figure 2. The binding of pyridodiindole to the proposed pharmacophore for the benzodiazepine receptor inverse agonist site.[30,32]

a pseudoplanar topography [i.e. β-carboline 3-acid esters, 2-arylpyrazoloquinolin-3-one (CGS-8216)][28,30] may be capable of penetrating sufficiently far into the cleft to in-

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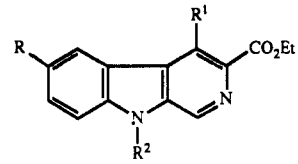
Table I. In Vitro and in Vivo Data of β-Carboline Ligands to BzR



drug	R	R^1	R^2	in vitro[a] IC_{50}, nM	in vivo (ip)[b]
1	OCH_2Ph	CH_2OCH_3	H	1	agonist[15-19]
2	OH	CH_2OCH_3	H	0.9	no effect at 40 mg/kg
3	OCH_2Ph	CH_2OCH_3	CH_3	945	no effect at 40 mg/kg
8a	OCH_3	CH_2OCH_3	H	0.5	antagonist (EC_{50} = 1.25 mg/kg)
8b	H	CH_2OCH_3	H	2.3	antagonist (EC_{50} = 15 mg/kg)
8c	OCH_2Ph	CH_2CH_3	H	22	no effect at 40 mg/kg
9	OCH_2Ph	CH_2CH_3	CH_3	>5000	
13	OCH_2Ph	H	H	8.9	no effect at 40 mg/kg
14	OCH_2Ph	H	CH_3	>5000	

[a] Standard radioligand binding assay employing [^{3}H]flunitrazepam binding vs ligand (see Experimental Section for details). [b] Male NIH/Swiss mice (Harlan/Sprague–Dawley (20–25 g)) were injected with 0.1 mL of drug (dissolved or suspended in 20% diluted Emulphor/80% saline) or vehicle. The anticonvulsant, convulsant/proconvulsant activity *was* determined as previously described[43] (see Experimental Section for full details of dose range and protocol). [c] Values represent the mean of at least two determinations (≤20% differences between experiments), with r^2 values for active compounds in individual experiments of ≥0.98.

teract with the hydrogen bonding sites H_1 and D_2 of the active site of the pharmacophore. Consideration of the SAR of the planar pyridodiindole, coupled with other SAR studies, resulted in the requirements for ligand interactions with the inverse-agonist site outlined in Figure 2.

The model of the agonist portion of the pharmacophore was developed independent of the inverse agonist/antagonist model. It was formulated by comparison of the SAR of the benzodiazepines, the 2-aryl-2,5-dihydro-5*H*-pyrazolo[4,3-*c*]quinolin-3-ones (CGS-9896/95[35] and CGS-17,867A,[36,37] a saturated analogue), and the β-carboline 1. Examination of the SAR of the benzodiazepines (agonists) indicates that the active site of the agonist pharmacophore interacts with two sites of high electron density and a third site represented by E^+ which is either electrostatic[33a] or steric[33c] in nature. For the benzodiazepines it is believed that hydrogen bonding between ligand and receptor takes place at the oxygen atom of the carbonyl carbon [C(2)] and the imine nitrogen [N(4)] function of the molecule. Two amino acid residues which behave as hydrogen bond acceptor sites would then be required at the active site.[38] In the proposed model these are designated H_1 and H_2 (Figure 1a). A third interaction is also thought to be important and is also considered necessary for agonist efficacy. This interaction is either electrostatic or steric in character and occurs between a substituent on the ligand [for example, Cl of diazepam (Figure 1a)] and a site on the receptor pharmacophore (designated E^+) which may be necessary for a ligand to function as an agonist. These proposals are consistent with the previous models of Loew[33a] and Gilli.[33c] In a similar manner, the 2-aryl-2,5-dihydro-5*H*-pyrazolo-[4,3-*c*]quinolin-3-ones are also believed to interact at these sites (H_1, H_2, and E^+) of the proposed pharmacophore (Figure 1b). On comparison of the SAR of the benzodiazepines and pyrazoloquinolin-3-ones, two additional features of the model may be derived. Since the pyrazoloquinolin-3-ones can assume a near-planar topography and bind to Bz receptors with higher in vitro affinities than the benzodiazepines, it is proposed that the binding sites on the receptor, H_1 and E^+, are located inside a narrow cleft. As a result, the pseudoplanar pyrazoloquinolines can access these binding sites better than the benzodiazepines, hence the greater binding affinities in vitro. Secondly, it is proposed that a lipophilic pocket exists near the mouth of the cleft. It is believed that occupation of this "pocket" by ring C of the benzodiazepines leads to full agonist efficacy. Since the pyrazoloquinoline agonists do not occupy this region, this interaction is not present; consequently, only a partial agonist response is elicited.[14a,37] In addition, the chloro and methoxyl substituents of the 2-aryl-2,5-dihydro-5*H*-pyrazolo[4,3-*c*]quinolin-3-ones (see Figure 1b) are required to direct these agonist ligands into the agonist domain; consequently a ligand devoid of a substituent at this position interacts at D^2 and H_1 of the inverse agonist site (Figure 2) as observed for the parent 2-aryl-2,5-dihydro-5*H*-pyrazolo[4,3-*c*]quinolin-3-one.

Any model of the agonist pharmacophore must accommodate 1.[15-17] It is believed that this compound does not evoke a response at exactly the same site (domain) as do the β-carboline inverse agonists β-CCM, β-CCE, 2EBC, etc.[30] The 4-methoxymethyl group of 1 is thought to be of critical importance and directs the ligand into the active site (domain) of the agonist pharmacophore through the formation of a hydrogen bond between the ether oxygen and H_2 of the active site (Figure 1c). In addition, a hydrogen bond is formed between H_1 and the pyridyl nitrogen atom [N(2)] of the β-carboline. This hydrogen bond may be stabilized by the interaction of the carbonyl group at C-3 in a three-center hydrogen bond analogous to that proposed for the other β-carbolines.[30,34] An electrostatic or steric interaction is believed to be formed between E^+ of the site (model) and the 6-benzyloxy (OCH_2) fragment. The large phenyl group of the benzyloxy substituent and the ethyl group of C(3) are proposed to occupy lipophilic pockets (Figure 1c). The SAR study strongly indicates that the combined effect of these substituents in 1 is critical for "full" agonist activity. Thus, replacement of the 6-benzyloxy group in β-carboline 1 for a 6-methoxy group [8a (0.5 nM)], results in a high-affinity ligand with no anti PTZ action, but the ligand antagonizes the anticonvulsant

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(38) For an explanation of the terms "donor" and "acceptor", see ref 1b in Allen, M, S.; Hagen, T. J.; Trudell, M.; Codding, P. W.; Skolnick, P. W.; Cook, J. M. *J. Med. Chem.* **1988**, *31*, 1854.

effects of diazepam. Similarly, removal of the functionality at the 6-position of 1 also provides a ligand **8b** (2.3 nM) with an antagonist action. This data supports the hypothesis that the lipophilic group of 1 fits into the same agonist pocket as the phenyl group of the benzodiazepines (see Figure 1). Moreover, both of these interactions are believed necessary to elicit a full agonist response. As reported,[15,39,40] movement of the benzyloxy group from position 6 of 1 to position 5 considerably decreases the agonist efficacy of the ligand and results in a partial agonist response.

Furthermore, replacement of the 4-methoxymethyl group of agonist 1 with an ethyl group, as in **8c** (22 nM), provides a high-affinity ligand for BzR, yet appears inactive in the tests employed to evaluate an agonist/inverse agonist profile (anti PTZ) of activity. The corresponding compound **13** (8.9 nM), which has no substituent at C(4), is also inactive, and these results, together, support the requirement for agonist activity of an electronegative substituent at C(4), which directs the ligand into the active site (domain) of the agonist domain of the pharmacophore and forms a hydrogen bond with H_2 (Figure 1c). The N_a-methyl congeners **3**, **9**, and 14 demonstrated poor affinity for BzR [**3** (980 nM), **9**, and 14 (>5000 nM)], and this information should be useful in the correlation between the inverse agonist and agonist sites at BzR. Despite the high affinities of **2**, **8c**, and **13** for BzR in vitro, these compounds were not anticonvulsant, proconvulsant nor did they block the anti-PTZ actions of pentylenetetrazole (Table I). While the partition coefficient of **2** (see Experimental Section) strongly indicates limited penetration across the blood–brain barrier in comparison to 1, the calculated partition coefficients (log P_{oct/H_2O}) of the other two compounds and structural similarities to 1 suggest ready penetration across this barrier in vivo. Nonetheless other pharmacokinetic factors such as limited absorption or extensive metabolism may explain their lack of effect.[14a] For example, the presence of a substituent at position 4 of β-carbolines retards the rate of hydrolysis[42a] of the ester substituent [C(3)] in vivo. This is in agreement with the lack of activity for **13** observed here in vivo and weak antagonist response of 6-benzylamino β-CCM.[18] It is important to note that 6-benzylamino β-CCM, a bioisostere of **13**, was absorbed and was clearly devoid of agonist activity[18] in agreement with the model presented here. Moreover, the lack of activity in vivo for **8c** (IC_{50} 22 nM) correlates well with the activity in vivo or dimunition thereof for **8b**, as illustrated in Table I (compare **8a–c**). Alternatively, these compounds may have pharmacological effects if a different biological endpoint (e.g. a test of anxiety) were employed. The possibility that these bases possess a "partial agonist" profile (e.g. anticonflict actions) or a partial inverse agonist spectrum of activity cannot be discounted. Such selectivity has been documented for other BzR ligands[14a] and will require more detailed studies in vivo.

In summary, this model of the agonist pharmacophore requires the interaction of two sites of electron density [N(2) and C(4)], a third interaction at E^+ (electrostatic or steric), and two of lipophilicity [C(3) and C(6)] in the agonist (1) series with the BzR protein (see Figure 1). This has been demonstrated by the synthesis and biological evaluation of the ligands **2**, **3**, **8a–c**, **9**, **13**, and 14. This has permitted the electronic character of 1 to be altered without loss of ligand topology at C(4) (i.e. replacement of 4-CH_2OCH_3 with CH_2CH_3 and H) and allowed variations of electronic and lipophilic character at C(6), while holding all other parameters constant (i.e. replacement of 6-OCH_2Ph with OH, OCH_3, and H). The in vitro and in vivo data outlined in Table I are consistent with this model. Further studies are currently in progress[42b] to determine whether the ligands described herein exhibt partial agonist activity[42b] and will be reported in due course. It is clear, however, that the ligands described in this report do not demonstrate an agonist profile of activity related to that of 1. More importantly, the ligand–receptor interactions necessary for an agonist response are clearly different from those necessary for inverse agonist/antagonist activity although a hydrogen bond donor site in one ligand class may overlap with that of the other class. This observation is consistent with the report of Pritchett et al.,[23] data from which suggest that the BzR may constitute a binding domain (cleft) dependent upon more that one protein rather than a binding site localized on a single protein.

Experimental Section

Melting points were taken on a Thomas-Hoover melting apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker 250 MHz spectrometer. IR spectra were taken on a Matteson Polaris instrument while mass spectral data were obtained on a Hewlett Packard 5855 GC–mass spectrometer. Microanalyses were performed on an F and M Scientific Corp. Model 185 carbon, hydrogen, and nitrogen analyzer. Analytical TLC plates used were E. Merck Brinkmann UV-active silica gel on plastic. All reactions were performed under an atmosphere of nitrogen, unless otherwise stated. The proconvulsant, convulsant, anticonvulsant data were determined as previously reported[28,30,41] and are briefly described below.

Methods. The effects of ligands on radioligand binding to BzR were examined in washed membranes prepared from rat cerebral cortex. 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, and disrupted in 100 vol of buffer with a Polytron (setting 6–7, 15 s). The tissues were then centrifuged at 20000*g* (4 °C) for 20 min. The supernatants were discarded, and the suspension/centrifugation was repeated two more times. Following the last centrifugation, membranes were resuspended in 50 vol of buffer. [^{3}H]Flunitrazepam binding was determined in a total volume of 1 mL consisting of 0.3 mL of tissue, 0.1 mL of [^{3}H]flunitrazepam (final concentration, 0.9–1.1 nM), 0.1 mL of solution of test compound, and 0.5–0.6 mL of Tris-HCl buffer. Reactions were initiated by addition of radioligand and terminated after 120 min (4 °C) by addition of 5 mL of ice-cold buffer and rapid filtration over GF/B filters followed by an additional 5-mL wash with buffer. The radioactivity retained by the filters was measured in a Beckman LS 5801 liquid scintillation spectrometer. Nonspecific binding was determined by replacing 0.1 mL of buffer with an equal volume of Ro 15-1788 (10 μM) and represented <5% of the total binding under these conditions. Six to eight concentrations of inhibitor were routinely employed in these studies. IC_{50} values were estimated by fitting data to a sigmoid curve (GraphPad, ISI software). Values represent the mean of at least two determinations (≤20% difference between experiments), with r^2 values for active compounds in individual experiments ≥0.98.

The anticonvulsant activity of test compounds was examined against a standard convulsant dose (80 mg/kg, ip) of pentylenetetrazole (PTZ) that produces clonic and tonic convulsions in 100% of control mice. Animals were injected ip with test com-

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pound and 15 min later administered PTZ. Mice were observed for an additional 15 min for the appearance of clonic/tonic convulsions. The convulsant/proconvulsant activity was determined as previously described.[43] Male NIH/Swiss mice (Harlan/Sprague–Dawley, Frederick, MD) (20–25 g) were injected (ip) with 0.1 mL of drug (dissolved or suspended in 20% diluted Emulphor/80% saline) or vehicle. Fifteen minutes later, the mice received 0.1 mL of PTZ (40 mg/kg). Animals were observed for an additional 15 min for the presence of clonic and/or tonic convulsions. Groups of 10 mice were injected with various doses of test agents followed 15 min later by diazepam (2.5 mg/kg). Fifteen minutes later, mice were challenged with PTZ (80 mg/kg), and the presence or absence of seizures was followed by an additional 15 min. This dose of diazepam protected >90% of all mice against PTZ. The dose of test compound required to elicit convulsions in 50% of the mice was estimated graphically by plotting percent convulsions versus (log) dose.

6-Hydroxy-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (2). β-Carboline (1; 260 mg, 0.528 mmol) was treated with hydrogen (760 mmHg) in EtOAc/EtOH (1:1, 20 mL total) over 10% Pd–C for 18 h. The catalyst was removed by filtration through Celite, and the solvent was evaporated under reduced pressure to yield **2** as a white foam (158 mg, 100%) which crystallized from EtOAc–Et_2O: mp 232–5 °c; IR (KBr) 3200–3400, 1720 cm^{-1}; ^1H NMR (d^6-DMSO) δ 1.34 (3 H, t, J = 7.1 Hz), 4.34 (2 H, q, J = 7.1 Hz), 5.10 (2 H, s), 7.11 (1 H, dd, J = 2.5, 9.0 Hz), 7.47 (1 H, d, J = 9.0 Hz), 7.57 (1 H, d, J = 2.4 Hz), 8.80 (1 H, s), 9.23 (1 H, br s), 11.72 (1 H, br s); mass spectrum (CI, CH_4), m/e 301 (M + 1, 100); high-resolution mass spectrum, m/e 300.1095 ($C_{16}H_{16}N_2O_4$ requires 300.1110). Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

9-Methyl-6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (3). Sodium hydride (8 mg of 80% dispersion in oil, 0.169 mmol; 1.1 equiv) was added to a stirred mixture of **1** (60 mg, 0.154 mmol) and iodomethane (24 mg, 11 μL, 0.169 mmol; 1.1 equiv) in dry DMF (3 mL) at ambient temperature. After 2 h the solvent was removed under high vacuum (Kugelrohr). The residue was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ after which the organic layer was separated and dried and the solvent removed under reduced pressure. Flash chromatography on silica gel afforded **3** as a white solid (40 mg, 64%): mp 110–13 °C; IR (KBr) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (3 H, t, J = 7.1 Hz), 3.42 (3 H, s), 3.84 (3 H, s) 4.51 (2 H, q, J = 7.1 Hz), 5.20 (2 H, s), 5.32 (2 H, s), 7.24–7.50 (7 H, m), 7.88 (1 H, s), 8.80 (1 H, s); mass spectrum (CI, CH_4), m/e 405 (M + 1, 100); high-resolution mass spectrum, m/e 404.1710 ($C_{24}H_{24}N_2O_4$ requires 404.1736). Anal. ($C_{24}H_{24}N_2O_4$) C, H, N.

3-(5-Methoxyindol-3-yl)-2-amino-4-methoxybutanoic Acid Ethyl Ester (7a). 5-Methoxyindole (**4a**) (6 g, 40.77 mmol) and ethyl 4-methoxy-3-hydroxy-2-nitrobutanoate (**5a**)[26] (16.8 g) were heated to reflux in a mixture of toluene–glacial acetic acid (9:1, 180 mL total) for 16 h. The solution was cooled, poured into aqueous $NaHCO_3$, and extracted with $PhCH_3$. The organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue was flash chromatographed on silica gel (20:1 $CHCl_3$–Et_2O, eluent) to afford 16.4 g of a red gum which was comprised of a mixture of the nitro ester **6a** and unreacted hydroxyl ester **5a**. This mixture was used in the next step without further purification: mass spectrum, m/e (EI) 336 (M^+, 68), 245 (100). The mixture was treated with hydrogen at atmospheric pressure over Raney nickel (20 g) in EtOH. After 4 days the presence of starting material was no longer observed by TLC. The catalyst was removed by filtration through Celite and washed with hot EtOH. Flash chromatography of the residue on silica gel (9:1, $CHCl_3$–CH_3OH eluent) afforded the amine **7a** (5.98 g, 48%) as a white solid which was homogeneous by TLC (9:1 $CHCl_3$–CH_3OH): mp 127–8 °C; IR (KBr) 3362, 3320, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (3 H, t, J = 7.1 Hz), 3.39 (3 H, s), 3.76–3.88 (4 H, m), 3.90 (3 H, s), 4.12 (2 H, q, J = 7.0 Hz), 6.78–7.25 (4 H, m), 8.06 (1 H, br s); mass spectrum, m/e (CI. CH_4) 307 (M + 1, 100); high-resolution mass spectrum, m/e 306.1580 ($C_{16}H_{22}N_2O_4$ requires 306.1570).

6-Methoxy-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (8a). The amine **7a** (5.66 g, 18.5 mmol) and glyoxylic acid monohydrate (1.87 g, 20.3 mmol) were heated at reflux, under a Dean–Stark trap, in $PhCH_3$–dioxane (10:1, 200 mL total) for 2–3 days.[27] The reaction mixture was cooled after which the solvent was removed under reduced pressure. The residue was then dissolved in dry mixed xylenes containing solid 10% Pd–C (1 g). The mixture was heated to reflux for 2 days, and the catalyst was removed by filtration through Celite followed by a wash with hot xylenes. The filtrate was evaporated and the residue flash chromatographed on silica gel (4:1 EtOAC–$CHCl_3$ eluent) to afford the title compound **8a** as a white solid (600 mg, 10%): mp 174–5 °C (lit.[26] mp 175–7 °C); IR (KBr) 3325, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (3 H, t, J = 7.1 Hz), 3.48 (3 H, s), 3.85 (3 H, s), 4.49 (2 H, q, J = 7.0 Hz), 5.32 (2 H, s), 7.12 (1 H, dd, J = 2.4, 8.9 Hz), 7.38 (1 H, d, J = 8.9 Hz), 7.69 (1 H, d, J = 2.5 Hz), 8.65 (1 H, s), 10.30 (1 H, br s); mass spectrum, m/e (CI, CH_4) 315 (M + 1, 100); high-resolution mass spectrum, m/e 314.1267 ($C_{17}H_{18}N_2O_3$ requires 314.1264).

Ethyl 4-Methoxy-3-indol-3-yl-2-aminobutanoate (7b). Indole **4b** (3.8 g, 32.4 mmol) and ethyl 4-methoxy-3-hydroxy-2-nitrobutanoate (**5a**) (8.8 g)[26] were combined and heated as previously described for the preparation of **6a**/**7a**. After 17 h a further 2 g of hydroxy ester **5a** was added and the mixture heated to reflux for an additional 24 h. Workup and flash chromatography on silica gel ($CHCl_3$ → 9:1 $CHCl_3$–Et_2O, gradient elution) afforded **7b** (2.56 g) as a brown oil which was used without further purification in the next step. This residue was hydrogenated over Raney nickel (10 g), as described above. Workup and flash chromatography on silica gel (9:1 $CHCl_3$–CH_3OH eluent) provided **7b** (1.58 g, 17%) as a white foam. This material was homogeneous by TLC (9:1 $CHCl_3$–CH_3OH), and was used without further purification in the next step (see below); mass spectrum, m/e (CI, CH_4), 277 (M + 1, 100).

4-(Methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (8b). The amine **7b** (1.28 g, 4.63 mmol) and glyoxylic acid monohydrate (0.49 g, 5.10 mmol) were heated at reflux, as described above for the preparation of **8a**, for 3 days. Oxidation over 10% Pd–C (0.5 g) and flash chromatography on silica gel (4:1 EtOAc–$CHCl_3$) afforded the β-carboline **8b** as a solid (123 mg, 9%): mp 180–1 °C (lit.[26] mp 180–3 °C); IR (KBr) 3200, 1725 cm^{-1}; ^1H NMR (d^6-DMSO) δ 1.41 (3 H, t, J = 7.1 Hz), 3.32 (3 H, s), 4.32 (2 H, q, J = 7.1 Hz), 5.20 (2 H, s), 7.30 (1 H, m), 7.62 (2 H, m), 8.30 (1 H, m), 8.91 (1 H, s), 12.05, (1 H, br s); mass spectrum, m/e (CI, CH_4) 285 (M + 1, 100); high-resolution mass spectrum, m/e 284.1152. ($C_{16}H_{16}N_2O_3$ requires 284.1161). Anal. ($C_{16}H_{16}N_2O_3$) C, H, N.

Ethyl 3-Hydroxy-2-nitropentanoate (5b). To a cooled (0 °C) stirred solution of propionaldehyde (0.5 g, 8.61 mmol) in EtOH was added NaOAc (14 mg in 0.5 mL of H_2O) followed by dropwise addition of ethyl nitroacetate (1.15 g, 8.61 mmol). Stirring was maintained for 14 h at ambient temperature. The solvent was removed in vacuo (<30 °C) and the residue dissolved in Et_2O, which was washed with 10% aqueous NaH_2PO_4 followed by brine. The organic layer was dried (Na_2SO_4), filtered, and removed under reduced pressure to provide **5b** as an oil (0.99 g), which was used in the following steps without any further purification.

3-[5-(Benzyloxy)indol-3-yl]-1-aminopentanoic Acid Ethyl Ester (7c). 6-(Benzyloxy)indole (**4c**) (3.3 g, 14.8 mmol) and hydroxy ester **5b** (6.6 g) were combined and heated for 4 h, as previously described, to yield **6c** (5.4 g) as a brown oil, which was used without further purification: mass spectrum, m/e (CI, CH_4), 397 (M + 1, 17.8), 264 (100). Hydrogenation over Raney nickel (8 g), and flash chromatography on silica gel (9:1 $CHCl_3$–CH_3OH) provided **7c** (4.5 g, 83%) as a foam. This material was homogeneous by TLC (9:1 $CHCl_3$–CH_3OH): IR (KBr) 3400, 1730 cm^{-1}; mass spectrum, m/e (CI, CH_4) 367 (M + 1, 100). This was taken onto the next stage without further purification.

6-(Benzyloxy)-4-ethyl-β-carboline-3-carboxylic Acid Ethyl Ester (8c). The amine **7c** (4.9 g, 13.4 mmol) and glyoxylic acid monohydrate (1.2 g, 13.4 mmol) was combined and heated as described above. Oxidation of the tetrahydro-β-carboline which resulted over 10% Pd–C provided the β-carboline **8c** (540 mg, 11%) as a white solid after flash chromatography on silica gel (4:1 EtOAc–$CHCl_3$, eluent). This material was homogeneous by TLC (4:1 EtOAc–$CHCl_3$): mp 180–2 °C (EtOAc); IR (KBr) 3400, 1725

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cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (3 H, t, J = 7.1 H), 1.44 (3 H, t, J = 7.1 Hz), 3.48 (2 H, q, J = 7.2 Hz), 4.49 (2 H, q, J = 7.1 Hz), 5.18 (2 H, s), 7.27–7.51 (8 H, m), 7.70 (1 H, d, J = 2.3 Hz), 8.81 (1 H, s); mass spectrum, m/e (CI, CH_4) 375 (M + 1, 100).

9-Methyl-6-(benzyloxy)-4-ethyl-β-carboline-3-carboxylic Acid Ethyl Ester (9). The β-carboline **8c** (193 mg, 0.516 mmol), NaH (16 mg of an 80% dispersion in oil, 0.568 mmol), and iodomethane (81 mg, 0.568 mmol) was reacted under conditions analogous to the procedure described for the preparation of **3**. Workup and flash chromatography on silica gel (3:1 EtOAc–$CHCl_3$ eluent) afforded the title compound **9** (128 mg, 64%) as a white solid: mp 110–11 °C; IR (KBr) 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (3 H, t, J = 7.1 Hz), 1.46 (3 H, t, J = 7.2 Hz), 3.52 (2 H, q, J = 7.2 Hz), 3.95 (3 H, s), 4.50 (2 H, q, J = 7.2 Hz), 8.72 (1 H, s); mass spectrum, m/e (CI, CH_4) 389 (M + 1, 100); high-resolution mass spectrum, m/e 388.1789 ($C_{24}H_{24}N_2O_3$ requires 388.1787). Anal. ($C_{24}H_{24}N_2O_3$) C, H, N.

6-(Benzyloxy)tryptophan Ethyl Ester (12). 5-(Benzyloxy)gramine (5 g, 17.8 mmol) and ethyl nitroacetate (2.61 g, 19.6 mmol) were heated to reflux in anhydrous xylene for 18 h. The solution was cooled, evaporated, and flash chromatographed on silica gel ($CHCl_3$) to afford **11** as a golden yellow oil (6.2 g, 94%), which was used without further purification: IR ($CHCl_3$) 1740, 1550, and 1360 cm^{-1}; mass spectrum, m/e (EI) 368 (M$^+$, 79.4), 322 (12.9), 277 (100); high-resolution mass spectrum 368.1358 ($C_{20}H_{22}N_2O_5$ requires 368.1372). This material was subjected to hydrogenation over Raney nickel catalyst (15 g) as previously described. Workup and flash chromatography afforded **12** (4.5 g, 75%) as a white solid, which was used without further purification: IR (KBr) 3200–3450, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (3 H, t, J = 7.0 Hz), 1.62 (2 H, br s), 3.11 (2 H, ABX, J_{AB} = 14.6, J_{AX} = 7.7 and J_{BX} = 4.4 Hz), 3.78 (1 H, dd, J = 4.7, 8.4 Hz), 4.15 (2 H, q, J = 7.0 Hz). 5.11 (2 H, s), 6.91–7.50 (9 H, m), 8.05 (1 H, br s); mass spectrum, m/e (EI) 338 (M$^+$, 8.2), 236 (100); high-resolution mass spectrum, m/e 338.1624 ($C_{20}H_{22}N_2O_3$ requires 338.1630).

6-(Benzyloxy)-β-carboline-3-carboxylic Acid Ethyl Ester (13). The amine **12** (2.8 g, 8.3 mmol) and glyoxylic acid monohydrate (0.76 g, 8.3 mmol) were reacted as previously described above for the preparation of **8** followed by oxidation (aromatization) over 10% Pd–C (0.8 g) in mixed xylenes. Flash chromatography on silica gel (4:1 EtOAc–$CHCl_3$, eluent) afforded the β-carboline **13** (230 mg, 8%) as a white solid: mp 260–3 °C (lit.[44] mp 261–3 °C); IR (KBr) 3350–3200, 1735 cm^{-1}; ^{1}H NMR (d^6-DMSO) δ 1.38 (3 H, t, J = 7.1 Hz), 4.34 (2 H, q, J = 7.0 Hz), 5.22 (2 H, s), 7.25–7.61 (8 H, m), 8.14 (1 H, d, J = 2.3 Hz), 8.91 (1 H, d, J = 2.9 Hz), 10.91 (1 H, br s); mass spectrum, m/e (CI, CH_4) 347 (M + 1, 100); high-resolution mass spectrum, m/e 346.1291 ($C_{21}H_{18}N_2O_3$ requires 346.1307).

9-Methyl-6-(benzyloxy)-β-carboline-3-carboxylic Acid Ethyl Ester (14). The β-carboline (177 mg, 0.51 mmol), NaH (19 mg of a 60% dispersion in oil, 0.56 mmol), and iodomethane (80 mg, 0.56 mmol) was reacted together as described above. Chromatography on silica gel (9:1 $CHCl_3$–Et_2O, eluent) gave **14** as a white solid (120 mg, 65%) which was homogeneous by TLC (9:1 $CHCl_3$–Et_2O): mp 180–2 °C; IR (KBr) 1725 cm^{-1}; mass spectrum, m/e (CI, CH_4) 361 (M + 1, 100); ^{1}H NMR ($CDCl_3$) δ 1.48 (3 H, t, J = 7.5 Hz), 3.98 (3 H, s), 4.52 (2 H, q, J = 7.5 Hz), 5.20 (2 H, s), 7.40–7.55 (7 H, m), 7.70 (1 H, d, J = 2.5 Hz), 8.81 (1 H, s), 8.92 (1 H, s); mass spectrum, m/e (CI, CH_4) 389 (M + 1, 100). The partition coefficients were either determined experimentally[45] or calculated from tabulated fragmentation constants:[24,25] log P(octanol/water) = 3.97 (**1**), 1.44 (**2**); 2.10 (**8a**), 1.88 (**8b**), 5.79 (**8c**), 4.53 (**13**).

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Registry No. 1, 83910-44-5; **2**, 96136-88-8; **3**, 124563-47-9; **4a**, 1006-94-6; **4b**, 120-72-9; **4c**, 1215-59-4; **5a**, 87824-17-7; **5b**, 104586-20-1; **6a**, 124563-48-0; **6c**, 124563-49-1; **7a**, 124563-50-4; **7b**, 124601-04-3; **7c**, 124563-51-5; **8a**, 83910-32-1; **8b**, 83910-28-5; **8c**, 96136-56-0; **9**, 124563-52-6; **10**, 1453-97-0; 11, 124563-53-7; **12**, 124563-54-8; **13**, 91164-52-2; 14, 124563-55-9; $OHCCO_2H$, 298-12-4; O_2NCH_2COOEt, 626-35-7.

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Synthesis and Structure–Activity Relationship of C5-Substituted Analogues of (±)-10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine [(±)-Desmethyl-MK801]: Ligands for the NMDA Receptor-Coupled Phencyclidine Binding Site

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A series of eight C5-substituted analogues of (±)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (**1**) have been prepared by the directed lithiation–alkylation (and acylation) of its (±)-N-*tert*-butylformamidinyl derivative **2** followed by formamidine solvolysis. An additional 10 analogues were prepared by elaboration of the C5-ethyl ester derivative. Analogues possessing large (e.g. propyl and larger) lipophilic substituents displace [^{3}H]-1-(1-thienylcyclohexyl)piperidine ([^{3}H]TCP) from the high-affinity phencyclidine (PCP) binding site in rat brain homogenates only at high concentrations (K_i > 1000 nM); however, the presence of a polar amino functionality (e.g. 2-aminoethyl) offsets this effect (K_i = 20 nM). Thus, the boundary condition for lipophilic substituents larger than ethyl appears to be polar in nature. Interaction of the 11 relatively small (MR < 14) C5-substituted analogues of **1** with the high-affinity PCP binding site associated with the *N*-methyl-D-aspartate (NMDA) receptor is best described by the equation log $(1/K_i) = -5.83\mathcal{F} + 0.64\pi + 7.41$ (r = 0.90).

The *N*-methyl-D-aspartate (NMDA) subclass of the excitatory amino acid (EAA) glutamate receptor has been the focus of a considerable amount of multidisciplinary research in the field of neuroscience.[1-4] Much, if not all of the interest in the NMDA receptor is due to its postulated involvement in such diverse central nervous system

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