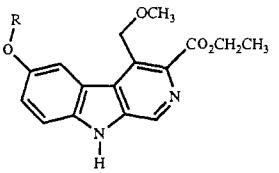
The Agonist Pharmacophore of the Benzodiazepine Receptor. Synthesis of a Selective Anticonvulsant/Anxiolytic

The benzodiazepines exhibit a wide range of pharmacological actions which include anxiolytic, anticonvulsant, sedative/hypnotic, and myorelaxant effects mediated by specific binding sites in the central nervous system.[1] The benzodiazepine receptor (BzR) is one constituent of a supramolecular complex which also contains discrete but allosterically coupled recognition sites for GABA and barbiturates. The oligomeric units of this supramolecular complex form a drug and transmitter responsive chloride channel.[2,3] The pharmacological properties of BzR ligands appear to be a continuum,[4] ranging from a complete mimicry of 1,4-benzodiazepines (such as diazepam, **2**) to substances termed inverse agonists that produce actions best described as opposite to the benzodiazepines.[5]

Despite advances at the molecular level,[2,3] the search continues for selective anxiolytics which are devoid of the other effects typical of the 1,4-benzodiazepines.[6] Recently, a computer-assisted analysis of the pharmacophore for inverse agonists at the BzR has been carried out,[7] which has resulted in the synthesis of the long-lived inverse agonist, 3-ethoxy-β-carboline.[8] In order to employ a rational drug design to prepare selective agonists at the BzR,[6] a similar approach has been employed with ligands defined as BzR agonists. As a result of these studies we wish to report the synthesis of a new anxiolytic/anticonvulsant, 6-(*n*-propoxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester (6-PBC, **1**) which is devoid of myorelaxant and ataxic effects.

A pharmacophore and an alignment rule were established for agonist BzR ligands.[9] Despite the previously reported pharmacophore models[10] there was a need to define the pharmacophoric descriptive points which permit the design of ligands with selective agonist[9] or inverse agonist[7] properties. Briefly, the ligands (see supplementary material) used in the study belong to several families: 1,4-benzodiazepines,[11] 1,2-annelated 1,4-benzodiazepines,[12] *s*-triazolo-, imidazo-, and thienobenzodiazepines,[12] 1- and 2-benzazepines,[12] 2-arylpyrazoloquinolines,[13] 2-thienylpyrazoloquinolines,[14] 1,3-diarylpyrazoloquinolines,[15] 1-benzopyranopyrroles, -pyrazoles, and -1,2,3-triazoles, pyrazolopyridines,[16,17] (aminoaryl)-1,2,4-triazolophthalazines,[18] 3,6-disubstituted pyridazinoisoquinolines,[19] (imidazopyrimidinyl)phenylmethanones,[20] and β-carbolines.[21] The determination of the low-energy conformations of these ligands and superimposition of the common sites of electron density as well as lipophilic regions were performed as described elsewhere.[7,9] The resulting pharmacophore is illustrated in Figure 1. This pharmacophore requires two hydrogen bond donating groups termed H_1 and H_2 and a lipophilic area with two zones essential for agonist activity defined as L_2 and L_3. Regions of repulsive steric interaction which reduce ligand affinity for the receptor termed S_1 and S_2 were also defined.[9] The optimum distance between H_1 and H_2 was found to be 6.5 Å.



1 R=$CH_2CH_2CH_3$
4 R=CH_2Ph
5 R=CH_3

The few β-carbolines which effect agonist actions (i.e. ZK-93423, **4**)[21] are the most challenging ligands to model since the multiple rotamers produced by the substituents at positions 3, 4, and 6 present difficulties.[10] Nevertheless, a low-energy conformation was determined that permitted **4** to fit compatibly into the agonist pharmacophore,[9] as illustrated in Figure 1. SAR[11-21] and modeling[7,9] studies

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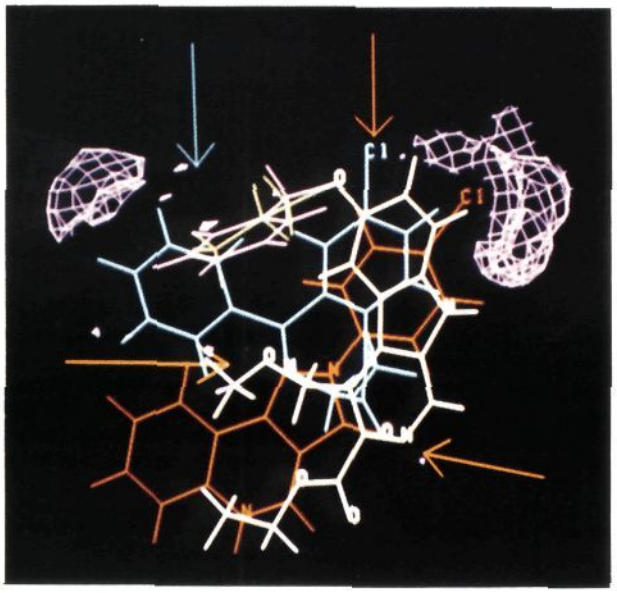

Figure 1. Proposed agonist pharmacophore of the benzodiazepine receptor. 1 in green (IC_{50} = 8 nM), 2 in cyan (IC_{50} = 6 nM), CGS-9896 (3) in redorange (IC_{50} = 0.6 nM), and 4 in magenta (IC_{50} = 1 nM) are superimposed. H_1 and H_2 are hydrogen bond donating groups indicated by the arrows in the left (yellow) and right (orange) hand portions, respectively. L_2 and L_3 are areas of lipophilic interaction indicated by the arrows on the top right (redorange) and left (cyan), respectively. S_1 and S_2 are areas of negative interaction shown in purple.

show that agonists such as **2** and **4** require the presence of substituents that project into the areas L_2 and L_3 and the ligands must form hydrogen bonds with H_1 and H_2 to elicit agonist activity. On the basis of this hypothesis, 6-PBC **1**[22] was prepared (see Figure 1) from 6-propoxyindole, according to the method of Neef[23] and Hollinshead.[24]

The effect of **1** on [^{3}H]flunitrazepam binding to the BzR was examined as previously described.[7,24] Under these conditions, the IC_{50} of **1** was 8.1 ± 1.5 nM (n = 4). In the paradigm for anticonvulsant activity, mice were pretreated with **1** (0.5–40 mg/kg) ip, followed 15 min later by administration of pentylenetetrazole [(PTZ) 80 mg/kg (ip)]. Animals were observed for the presence of tonic/clonic convulsions over the next 15 min. **1** inhibited PTZ induced seizures in a dose-dependent fashion with an ED_{50} of 1.6 mg/kg (Figure 2).

The anxiolytic activity of **1** was evaluated by using an elevated plus-maze as described.[25] The drug was administered 15 min prior to testing, at which time mice were placed in the center of the maze under a "bright light" condition. The number of crosses as well as the time spent in the open and closed arms of the maze for the next 15 min were recorded. Control values for the percentage of entries into the open arms, percentage time spent in the open arms, and total entries were consistent with those previously reported.[25] Diazepam (**2**) (2.5 mg/kg) produced a statistically significant increase in all three measures (see Figure 3). The β-carboline **1** effected dose-dependent increases in both the percentage of time animals spent in ($p < 0.05$ at 20 mg/kg) and the percentage of entries into the open arms of the plus-maze ($p < 0.02$ at 20 mg/kg). These doses of **1** increased total entries which was statistically significant at 10 ($p < 0.005$) but not 20 mg/kg. The latter observation indicates that at doses of much greater than used in the present study **1** might produce sedation. Nonetheless, these findings demonstrate that in an extensively validated animal model, **1** elicits anxiolytic actions.

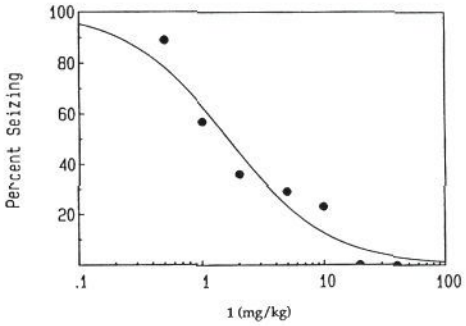

Figure 2. Inhibition of pentylenetrazole (PTZ) induced seizures by **1**. Mice were injected with 0.1 mL of vehicle (10% diluted Emulphor/90% saline) or **1**. PTZ (80 mg/kg) was administered 15 min later, and the animals were observed for an additional 15 min for the presence of tonic and/or clonic seizures. The ED_{50} was 1.6 mg/kg. Each point represents 5–14 mice.

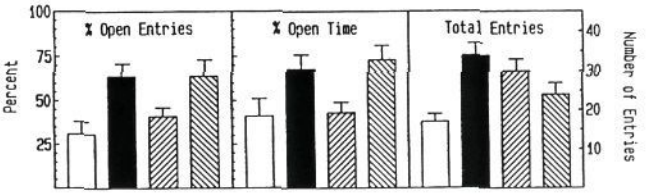

Figure 3. Effects of **2** and **1** in an elevated plus-maze. Mice were injected with vehicle or drug and 15 min later the animals were placed in the center of the maze and tested under a "bright light" condition.[25] **2** effected a significant increase ($p < 0.02$) in all three measures. **1** (20 mg/kg) significantly increased both the percentage entries and percentage of time in the open arms ($p < 0.05$ and $p < 0.02$, respectively) compared to vehicle treated mice. **1** (10 mg/kg) significantly increased the total entries into all arms of the maze. Values represent x ± SEM of 5–8 mice/group. Symbols are as follows: open bars, vehicle; solid bars, **2**, 2.5 mg/kg; left diagonal bar, **1**, 10 mg/kg; right diagonal bar, **1**, 20 mg/kg.

The effect of **1** and **2** on the ability of mice to hang suspended by their forepaws from a wire (traction test) was used as an index of myorelaxation.[26] Rotarod performance (Rotamex, Columbus Instruments, Columbus, OH) at 5 rpm[27] was used as a measure of ataxia. After a total of 15 min after administration of **1**, mice were suspended by the forepaws from a 20 gauge wire stretched 50 cm above the laboratory bench between two stands placed 50 cm apart. Mice were given three opportunities to remain suspended from the wire for 30 s. **2** (7.5 mg/kg) impaired this per-

(22) 6-PBC **1**: mp 250–251 °C; ^{1}H NMR (G.E. 500 MHz, $CDCl_3$) δ 10.20 (1 H, s), 8.74 (1 H, s), 7.75 (1 H, s), 7.41 (1 H, d), 7.16 (1 H, d), 5.37 (2 H, s), 4.43 (2 H, c), 4.02 (2 H, t), 3.52 (3 H, s), 1.85 (2 H, s), 1.32 (3 H, t), 1.06 (3 H, t) ppm; ^{13}C NMR (G.E. 125 MHz, $CDCl_3$) δ 10.58, 14.30, 22.68, 58.17, 61.65, 67.83, 70.40, 107.85, 112.60, 119.16, 121.67, 128.74, 129.13, 132.94, 136.14, 137.22, 137.65, 153.99, 167.23 ppm; High-resolution MS (Finnigan HR), m/e 342.1581 ($C_{19}H_{22}N_2O_4$ requires 342.1579). Anal. (Perkin-Elmer 240-c) ($C_{19}H_{22}N_2O_4$) C, H, N.

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Table I. Effects of 1 on Diazepam-Induced Myorelaxation[a]

drug	no. impaired/ no. tested	% impaired
2	10/10	100
1 (10 mg/kg)	0/10	0***
1 (20 mg/kg)	0/10	0***
2 + 1 (10 mg/kg)	5/10	50**
2 + 1 (20 mg/kg)	0/10	0***

[a] Mice were administered vehicle or 2 (2.5 mg/kg), and vehicle or 1 (10 or 20 mg/kg) 15 min later. After a period of 30 min after the first injection, the ability of mice to hang by their forepaws from a suspended wire was measured. Symbols are as follows: **, $p < 0.025$; ***, $p < 0.001$ (Fisher's Exact test).

formance in all mice tested (Table I), while mice pretreated with 1 (10 and 20 mg/kg) were indistinguishable from controls. Moreover 1 (10 mg/kg) reversed diazepam-induced deficits in traction by 50% ($p < 0.05$), while a higher dose of 1 (20 mg/kg) effected a complete reversal of the muscle relaxation effects of 2 in this paradigm (Table I). At a dose of 20 mg/kg mice exhibited no impairment in rotarod performance (data not shown). Thus, not only is 1 devoid of myorelaxant/ataxic activity (20 mg/kg), but it also antagonizes the myorelaxant actions of 2.

Examination of the superimposition of ligands in the agonist pharmacophore (Figure 1) illustrates that the substitution of 1 for ZK-93423 (4) will still fulfill the required lipophilic areas (L_2 and L_3). Further evidence that the lipophilic regions (L_2 and L_3) must be filled to elicit agonist activity has been obtained by the synthesis of 6-methoxy-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester (5) (IC_{50} = 0.5 nM).[24,9] Ligand 5 is a potent antagonist of the anticonvulsant effects of 2 but is devoid of anticonvulsant activity.[24]

The synthesis and biological activity of 1 has important implications for the design of β-carbolines as selective agonists and selective anxiolytics.[28] The proper lipophilic substituents of β-carbolines direct the ligand to the agonist pharmacophore, which is clearly different from that for inverse agonists.[7] More importantly, the new anxiolytic agent derived from this approach exhibits anticonvulsant/anxiolytic activity, but is devoid of the muscle relaxant/ataxic effects often associated with sedation in the benzodiazepine series.[1,6] Furthermore, its design, synthesis, and pharmacological actions, based on computer-assisted analysis of the agonist pharmacophore, provides compelling evidence that the pharmacophore depicted in Figure 1 is valid.[10] Further work is in progress to determine the exact dimensions of the lipophilic areas (L_2 and L_3) required for selective agonist activity at the BzR.

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Supplementary Material Available: Tables of agonist ligands employed in the modeling and methods for the in vivo and in vitro tests (10 pages). Ordering information is given on any current masthead page.

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