115266-81-4; **12g**, 115266-82-5; **12h**, 115266-83-6; **12i**, 115266-84-7; **12j**, 115266-85-8; **12k**, 115266-86-9; **12l**, 115227-01-5; **12m**, 115206-11-6; **12n**, 115206-12-7; **12o**, 115266-87-0; **12p**, 115266-88-1; **12q**, 115206-13-8; **12r**, 115266-89-2; **12s**, 115266-90-5; **12t**, 115266-91-6; (±)-**13a**, 115266-92-7; (+)-**13a**, 112966-96-8; (−)-**13a**, 115267-10-2; **13b**, 115266-93-8; **13c**, 115266-94-9; **13d**, 115206-14-9; **13e**, 115266-95-0; **13f**, 115266-96-1; **13g**, 115266-97-2; **13h**, 115266-98-3; **13i**, 115266-99-4; **13j**, 115267-00-0; **13k**, 115267-01-1; **13l**, 115227-02-6; **13m**, 115206-15-0; **13n**, 115206-16-1; **13o**, 115267-02-2; **13p**, 115267-03-3; **13q**, 115267-04-4; **13r**, 115303-09-8; **13s**, 115267-05-5; **13t**, 115267-06-6; (±)-**14a**, 115303-10-1; (+)-**14a**, 115267-11-3; (−)-**14a**, 115303-25-8; **14b**, 115303-11-2; **14c**, 115303-12-3; **14d**, 115267-07-7; **14e**, 115303-13-4; **14f**, 115303-14-5; **14g**, 115303-15-6; **14h**, 115303-16-7; **14i**, 115303-17-8; **14j**, 115303-18-9; **14k**, 115303-19-0; **14l**, 115268-41-2; **14m**, 115267-08-8; **14n**, 115267-09-9; **14o**, 115303-20-3; **14p**, 115303-21-4; **14q**, 115303-22-5; **14r**, 115361-70-1; **14s**, 115303-23-6; **14t**, 115303-24-7; **15**, 115206-17-2; **16**, 115206-18-3.

Synthesis of Novel 3-Substituted β-Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Pharmacophore

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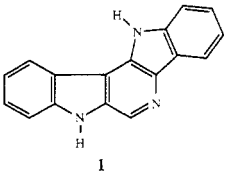
The 3-substituted β-carbolines **2–4** and **5–7** were prepared from 3-amino-β-carboline (**8**) in one step via diazotization, followed by reaction with the appropriate nucleophile in order to determine their binding affinity for benzodiazepine receptors (BzR). All three of the 3-alkoxy-β-carbolines **2** (IC_{50} = 124 nM), **3** (IC_{50} = 24 nM), and **4** (IC_{50} = 11 nM) have high affinities for BzR. The β-carbolines substituted with electron-withdrawing groups including **5** (Cl; IC_{50} = 45 nM), **6** (NO_2; IC_{50} = 125 nM), and **7** (N=C=S; IC_{50} = 8 nM) also had high affinities for BzR. The affinities of **5–8** clearly indicate that a carbonyl moiety at position 3 of a β-carboline is not required for high-affinity binding to BzR. These findings have led to the development of a model for the binding of ligands to an inverse agonist domain at BzR. This model is supported by the recent synthesis of 3-ethoxy-β-carboline (**3**), a potent, long-lived partial inverse agonist, and **7**, an irreversible BzR ligand.

The recent development of models for ligand binding to an inverse agonist site on the (BzR) in these[1a] and other laboratories[2a–d] has prompted the synthesis of a new class of 3-substitued β-carbolines to more rigorously test these models. The β-carbolines possess a broad spectrum of pharmacological actions (inverse agonist,[3–6] antagonist,[7] and agonist[8]) mediated via occupation of benzodiazepine receptors in the central nervous system. Previous studies on the structure–activity relationships (SAR) of β-carbolines had indicated that an ester moiety was required at position 3 in order for the compound to display high affinity for the BzR.[2a–d,4,9] Recently we reported that several β-carboline derivatives lacking a carbonyl group at position 3 possess moderate to high affinities for BzR. Examples of these include the 3,4-disubstituted β-carboline, 7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole (**1**) (IC_{50} = 4 nM),[10]



I

6-(benzylamino)-β-carboline (**12**) (IC_{50} = 106 nM),[11] and the 3-substituted β-carbolines **2–7**, which comprise the subject of the present paper. Consideration of the SAR of the planar, rigid analogue **1**, coupled with other SAR studies,[12–17] has resulted in a model for the requirements of ligand interactions with the inverse agonist binding site on the BzR (Figure 1).[1a] The 3-substituted derivatives of β-carboline were synthesized from 3-amino-β-carboline (**8**) to more rigorously test this model.[18a,b]

Chemistry

The choice of 3-amino-β-carboline (**8**) as the starting material provided facile entry into a variety of 3-substituted β-carbolines, as depicted in Scheme I. Substitution of an electron-withdrawing group (CO_2CH_3,[13] CN,[2a] etc.) at position 3 of a β-carboline is thought to facilitate the interaction of the indole N(9)-H with a hydrogen bond donor[1b] (D_2) site on the receptor.[1a] Thus the chloro **5**, nitro **6**, and isothiocyanate **7** analogues were prepared; these groups exhibit the properties of electron withdrawal in varying degree. Replacement of the diazonium group at position 3 of **8** by a chloride anion has been carried out under classical diazotization conditions ($NaNO_2$, HCl). Moreover the 3-hydroxyl analogue **9** can be prepared by a simple modification. It is important to note that 3-hydroxy-β-carboline (**9**) exists in the pyridone form (Scheme I). This β-carboline was formed from diazotization of the amine **8** with sodium nitrite in 3 N sulfuric acid at 0 °C. The amine functionality on the β-carboline nucleus disposed α (C-3) to the pyridine nitrogen function does not undergo diazotization in the same manner as do other arylamines.[19] In the present case, formation of the diazonium ion does occur. However, because of an apparent lack of stability,[19] it reacts with nucleophiles present in high concentrations such as water or halide in the case of a mineral acid. Chichibabin reported that on treatment of 2-aminopyridine with sodium nitrite and dilute hydrochloric acid, 2-chloropyridine could be isolated in yields of approximately 50%.[20] Under analogous conditions, **8** was diazotized with sodium nitrite in 3 N HCl to furnish the 3-chloro-β-carboline (**5**) in yields of 50–55%, accompanied by the 3-hydroxyl analogue **9**.

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In order to prepare an isostere of 3-carbomethoxy-β-carboline (βCCM, **10**, which would not be prone to esterase hydrolysis in vivo, synthesis of 3-nitro-β-carboline was envisaged. As illustrated in Figure 2, the charge delocalization of the nitro group in **6** is similar to that expected for the ester function in **10**. It is known that primary aromatic amines can be oxidized with peracids to furnish the corresponding nitro derivatives.[21,22] In this regard, **8** was oxidized with *m*-chloroperbenzoic acid in chloroform to give 3-nitro-β-carboline (**6**) in moderate yield. 3-Isothiocyanato-β-carboline (**7**) was prepared from thiophosgene and **8** with use of a two-phase system ($CHCl_3$, $NaHCO_3/H_2O$), according to the method of Rice.[23] This procedure gave **7** in good yield.

While substitution of an electron-withdrawing group at position 3 may enhance the ability of the indole N(9)-H to hydrogen bond to the receptor, replacement of a hydrogen atom at position 3 with an electron-releasing group, however, does not destroy affinity to BzR. This has been demonstrated by the synthesis of the potent, anxiogenic inverse agonist 3-ethoxy-β-carboline (**3**) (24 nM). Any diminution in the interaction of the ligand with the receptor on replacement of 3-CO_2CH_3 with 3-OCH_2CH_3 on

(1) (a) Hagen, T. J.; Skolnick, P.; Shannon, H. E.; Cook, J. M. *A New Class of Norharman Derivatives Which Potently Bind to Benzodiazepine Receptors: 6-Substituted Derivatives That Bind at 100 nM*, presented at the 20th Great Lakes Regional Meeting of the American Chemical Society, Marquette University, Milwaukee, WI, June 2–4, 1986; Abstr 262. Hagen, T.; Trudell, M.; Lifer, S.; Tan, Y. C.; Allen, M.; Skolnick, P.; Codding, P.; Cook, J. M. *Synthesis of Pyrido[3,2-b:5,4-b']diindoles and β-Carbolines. The Pharmacophore for the Benzodiazepine Receptor Inverse Agonist Site*, presented at the 43rd Southwest Regional Meeting of the American Chemical Society, Little Rock, AR, Dec 2–4, 1987; Abstr 214. (b) The terms "donor" and "acceptor" indicate the function involved in the process of charge transfer. Donor refers to an electron-pair donor for a hydrogen bond. Hence a hydrogen bond donor (X:) donates its pair of electrons that goes to the formation of hydrogen hydrgen bond. A hydrogen bond acceptor (–H) receives the pair of electrons from the donor forming the hydrogen bond. This format of nomenclature (in the article) has been adopted from the following two books: Baker, B. R. *Design of Active-Site-Directed Irreversible Enzyme Inhibitors*; Wiley: New York, 1967; Chapter 2, pp 23–47. Gutmann, V. *The Donor–Acceptor Approach to Molecular Interactions*; Plenum: New York, 1978; Chapter 1. (c) Controversy exists regarding the molecular interactions responsible for the efficacy at the benzodiazepine receptor. Several groups have independently proposed a three-state model that explains this phenomenon (see below). This model assumes that the benzodiazepine receptor exists in three energy states that affect coupling with the GABA receptor and chloride ionophore. The three different energy states proposed correspond to agonist, antagonist, and inverse agonist activities, respectively. In the current model, the similarities between inverse agonists (β-carbolines and pyridodiindoles) are treated independently from agonists and antagonists. [See Polc, P.; Möhler, H.; Schaffner, R.; Haefely, W. "A three state model of the benzodiazepine receptor explains the interactions between the benzodiazepine tranquilizers, β-carbolines and phenobarbitone". Polc, P.; Bonetti, E. P.; Schaffner, R.; Haefely, W. *Nauyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *321*, 260. Nutt, D. J.; Cowen, P. J.; Little, H. J. *Nature (London)* **1982**, *436*, 295. Jensen, L. H.; Peterson, E. N.; Braestrup, C. *Life Sci.* **1983**, *33*, 393. Prado de Carvalho, L.; Grecksch, G.; Chapouthier, G.; Rossier, J. *Nature (London)* **1983**, *301*, 64.] There is evidence that suggests that β-carboline inverse agonists bind to different regions (domains) on the BzR from benzodiazepine agonists (see: Skolnick, P.; Paul, S. *J. Clin. Psychiatry* **1983**, *12*, 44). The agonist site model generated in these laboratories (see ref 1a) consists of three important hydrogen bond acceptor sites, while the inverse agonist site contains one hydrogen bond donating and one acceptor site. It is possible that one of the hydrogen bond donating sites of an agonist overlaps with a similar site on inverse agonists (see ref 1 and 2). Additional studies at the molecular level with irreversible inhibitors such as **7** will be necessary to confirm this. While this model is useful for determining the requirements for high-affinity binding of inverse agonists to the benzodiazepine receptor, it cannot account for differences in pharmacological properties. Thus, while the high affinities of compounds such as DMCM (3-carbomethoxy-6,7-dimethoxy-4-ethyl-β-carboline), βCCE, and **3** would be predicted, in vivo DMCM is a potent convulsant ("full" inverse agonist), and the latter compounds are referred to as partial inverse agonists since they are not convulsant under most circumstances. While pharmacokinetic factors may contribute to the partial inverse agonist profile of βCCE (Schweri, M.; Martin, J.; Mendelson, W.; Barrett, J.; Paul, S.; Skolnick, P. *Life Sci.* **1983**, *1505*, 3), the partial inverse agonist qualities of the long-lived **3** (Table II) suggest that pharmacodynamic factors distinguish full from partial inverse agonists at BzR.

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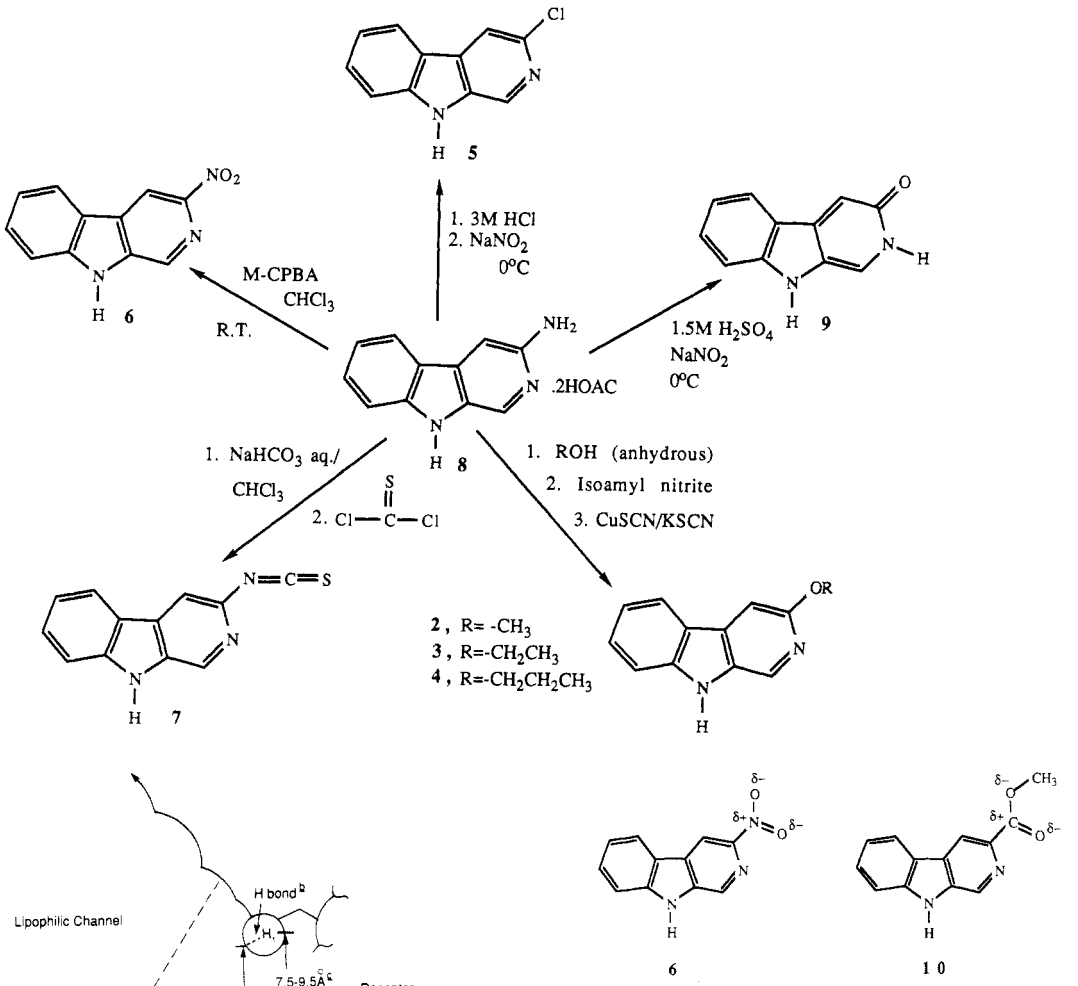
Scheme I



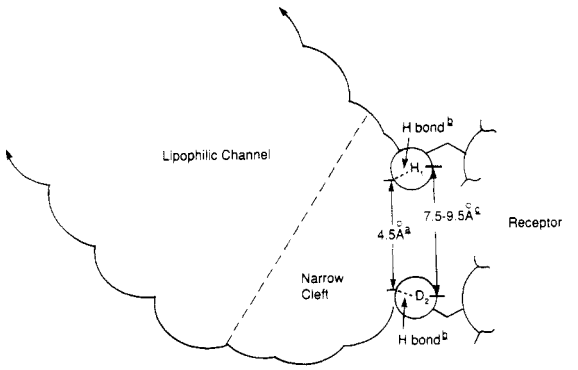


Figure 1. Proposed pharmacophore for the benzodiazepine receptor inverse agonist site.[1] (a) Average interatomic distance between binding sites on ligands. (b) Intermolecular hydrogen bond lengths obtained from crystal structures of ligands. (c) Distance between binding-site residues on receptor dependent on hydrogen bond lengths (b).

the β-carboline nucleus is compensated for by the increased electron density on the pyridine nitrogen atom and the lipophilicity of the ethyl substituent in **3** (Figure 3). The importance of the alkyl function in **3** in regard to BzR affinity can be seen on comparison of the structures of **1** and **3**. The shaded portions of the drawings represent areas of lipophilic overlap at position 3. The requirement for electron density on the pyridine nitrogen atom of β-carboline has been amply demonstrated previously by Loew et al.[2a] and in our laboratories.[1a,13]

The 3-ethoxy-β-carboline (**3**) was prepared by conversion of **8**, into its corresponding diazonium salt with an alkyl nitrite, anhydrous ethanol, and a copper(I) salt. Diazotization reactions of α-aminopyridines occur slowly since they are π deficient.[24] When generated, however, they are very reactive toward nucleophiles or hydrolyze rapidly under traditional diazotization conditions.[19] Doyle has demonstrated that addition of a copper salt is critical for the substitutive deamination reactions of arylamines to aryl halides effected by alkyl nitrites.[25] In fact, it is known that the Sandmeyer reaction is catalyzed by low concentrations of copper(I) halide.[26,27,29a,b] In agreement with this, the reaction of **8** with isoamyl nitrite in ethanol in the absence of a copper(I) salt returned only starting material. For the above reasons a copper(I) salt was chosen to catalyze the reaction whose anion counterpart (SCN) demonstrates weak nucleophilicity to retard reaction of it with the diazonium group. When **8**, isoamyl nitrite, and copper(I) thiocyanate were stirred in ethanol at −20 °C, a 52% yield of the desired **3** was realized (Scheme I). Even when the concentration of thiocyanate anion was increased to 100 equiv by addition of potassium thiocyanate, the formation of 3-ethoxy-β-carboline was not retarded. The syntheses of 3-methoxy-β-carboline (**2**) and the corresponding 3-propoxy-β-carboline (**4**) were executed under

Figure 2.

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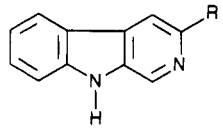
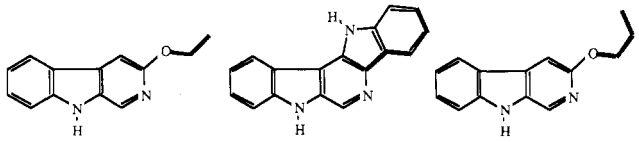
Table I. In Vitro Binding of 3-Substituted β-Carbolines to BzR



β-carboline	R	in vitro IC_{50},[a] nM
5	Cl	45
6	NO_2	125
7	N=C=S	8
10	CO_2CH_3	5
11	H	1620
2	OCH_3	124
3	OCH_2CH_3	24
4	$OCH_2CH_2CH_3$	11
8	NH_2	25000[b]
9	OH	4000
diazepam		6

[a] See: Trudell, M. L.; Basile, A.; Shannon, H. E.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1987**, *30*, 456 and references cited therein. Also see text for biological protocols. Values represent $\bar{X}$ of three or more experiments. SEM were usually <10%. [b] See ref 18 for details.



3 1 4

Figure 3.

analogous conditions to those employed for **3** by choice of the appropriate solvent.

Results and Discussion

Summarized in Table I are the relative potencies for various substituted β-carbolines for BzR in vitro. The chloro **5** (σ_p = 0.24), nitro **6** (σ_p = 0.81), isothiocyanate **7** (σ_p = 0.48), and methoxycarbonyl **10**[13] (σ_p = 0.44[28]) derivatives were prepared and screened to examine the importance of substitution of an electron-withdrawing moiety at position 3 of a β-carboline. These analogues are more potent inhibitors of [^{3}H]diazepam binding to BzR than the parent β-carboline **11** (1620 nM).[11] The decrease in potency of the nitro analogue **6** (IC_{50} = 125 nM) with respect to βCCM (**10**; IC_{50} = 5 nM) is believed to be due to the increased hydrophilic character of the nitro group when compared to the methyl ester in **10**. The potency of **6** confirms that the nitro group serves as an isostere for the labile ester group in this series, as earlier illustrated in Figure 2. Controversy exists as to whether the indole N(9)-H is necessary in the ligand–receptor interaction at BzR;[1a,2a,b] however, the effect of electron-withdrawing groups on binding affinity of β-carbolines to BzR appears to be consistent since the potency of **6** is in close agreement with the potency of 3-cyano-β-carboline (σ_p = 0.70[28]) reported by Loew et al.[2a]. An electron-withdrawing group at position 3 of a β-carboline could enhance the ability of the indole N(9)-H to interact with a hydrogen bond donor site ($-D_2$, Figure 1) on the receptor via polarization of the indole N–H bond. If this is the case, it suggests that the indole N(9)-H of β-carboline plays a significant role in the interaction of ligands with the receptor. It has been recently shown by X-ray crystallography that the indole N(9)-H of βCCM (**10**) does undergo hydrogen bonding in the crystal lattice.[2e] However, the possibility also exists that secondary interactions of the 3-substituent of β-carbolines **5**–**7** stabilize the ligand–receptor hydrogen bond

Table II. Proconvulsant Effect of 3-Ethoxy-β-carboline (**3**) in Comparison with That of βCCE

	dose,[a] mg/kg	% convulsion
		20
	15-Minute Interval	
3	20	100
	10	84
	5	50
	2.5	40
	1.25	30
βCCE	25	96
	10	33
	120-Minute Interval	
3	10	60[b]
βCCE	25	30

[a] The protocol used to examine proconvulsant activity was as follows: Mice received an intraperitoneal injection of vehicle (0.1 mL of 20% diluted Emulphor in saline) or drug at the dose indicated. Pentylenetetrazole (40 mg/kg, ip) was injected 15 or 120 min later and the number of animals that had clonic–tonic convulsions was recorded. At least 10 animals were used at every dose range. [b] Significantly different from βCCE at 120 min, χ^2 analysis.

(Figure 1) at N(2), resulting in enhanced activity. Further studies are needed to confirm these hypotheses. Replacement of the ester function of βCCM (**10**; 5 nM) with an amino **8** (25 000 nM) or hydroxyl **9** (4000 nM) substituent resulted in large reductions in potency, while the ethoxy analogue **3** retained a high affinity for BzR. The high affinity of **3** for BzR may depend on three factors, the most important of which is related to the lipophilicity of the alkyl group since the potency of β-carbolines for BzR increase as the lipophilicity of the 3-substituent increases (for example **6** vs **10**, Table I). Additional evidence for the importance of lipophilicity is seen by comparison of the IC_{50} values for the 3-ethoxy analogue **3** to the 3-methoxy **2** (IC_{50} = 124 nM) and 3-propoxy **4** (IC_{50} = 11 nM) derivatives. Clearly, 3-propoxy-β-carboline bears the most lipophilic substituent and most closely resembles the lipophilicity of the pyridodiindole **1** (4 nM) upon comparison of octanol/water partition coefficients (see Figure 3).[30] A second contributing factor is that the 3-ethoxy group and its congeners release electron density (σ = 0.14) to the pyridine ring, enhancing the basicity of the pyridine nitrogen atom, which results in an increased ligand–receptor interaction (Figure 1). Finally, the oxygen atom of **3** may stabilize the hydrogen bond interaction between N(2) and the receptor although this effect may be of secondary importance.

In vivo, **3** is a partial inverse agonist that has a longer duration of action than the isosteric ester, βCCE (Table II). Thus, **3** did not produce convulsions in mice at doses of up to 40 mg/kg, ip, but, like other BzR partial inverse agonists (e.g. βCCE), it potentiated the convulsant effect of PTZ (Table II). The ether function is not amenable to esterase attack as are many β-carboline esters with high affinities for benzodiazepine receptors.[31] Thus, ip injection of βCCE, which has an affinity of ~1 nM for benzodiazepine receptors in vitro, required 25 mg/kg to produce the same proconvulsant effect observed with 10 mg/kg of **3**. Furthermore, if a 120-min interval elapses between drug and PTZ challenge, the βCCE effects are reduced to a much greater extent than those of **3**.

The low potencies of **8** and **9** may be attributed to the lack of the necessary lipophilicity at C-3 of the β-carboline

(30) log $P_{oct/water}$ = 3.87 (**1**), 3.58 (**4**), 2.84 (**3**). Trudell, M. L.; Martin, M. J.; Cook, J. M., unpublished results.

(31) Schweri, M.; Martin, T. V.; Mendelson, W. B.; Barrett, J. E.; Paul, S. M.; Skolnick, P. *Life Sci.* **1983**, *33*, 1505.

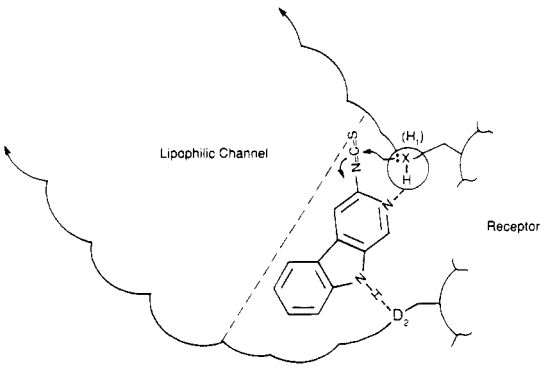


Figure 4. The irreversible binding of 3-isothiocyanato-β-carboline (**7**) to the proposed pharmacophore for the benzodiazepine receptor inverse agonist site.

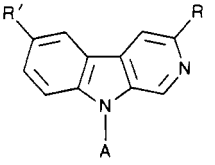
[compare **2** vs **4** (Table I); **8** (IC_{50} = 25 000 nM) vs 3-(ethylamino)-β-carboline (IC_{50} = 460 nM)[18a]] at BzR. Moreover, the aliphatic N–H functionality of the amine **8** may interact unfavorably at the receptor site as the imino–pyridine tautomer[20b] of the amino-β-carboline. In this manner, the favorable and necessary interaction between the pyridine nitrogen N(2) on the ligand (see **1**, **3**, or **10**) and the hydrogen bond acceptor (Figure 1) portion of the receptor protein is decreased. The tautomeric effect is more clearly seen in 3-hydroxy-β-carboline (**9**), which exists in the pyridone form[20a] and also exhibits low affinity for the BzR. The pyridine ring of **9** has been altered (pyridone, IR C=O, 1680 cm^{-1}). As a result, the pyridine nitrogen N(2) atom now functions as a hydrogen bond acceptor (N–H) rather than donor. This interferes with the hydrogen bond between the donor portion of the ligand (N:) and the acceptor site (H_1) on the receptor. In the model proposed earlier (Figure 1),[1a] the requirement for N(2) to exhibit hydrogen bond donor (N:) properties (rather than behaving as an acceptor) was illustrated. Moreover, the lone pair of electrons (N:) in **3** is in the plane of the aromatic ring directed toward the receptor, whereas the lone pair on nitrogen in **9** is directed away from the plane and is delocalized through the amide π-system. The hydrogen bond acceptor interaction of BzR may be in the same plane as the β-carboline ring, and the loss of affinity of **9** can, in part, be attributed to the existence of this derivative as the pyridone tautomer.

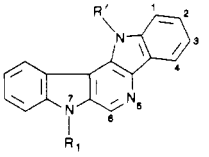
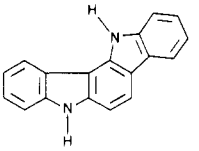
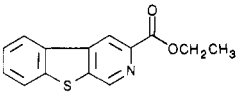
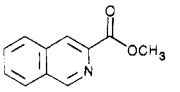
If the pyridine N(2) lone pair of electrons on the ligand is important for the formation of a strong hydrogen bond with the receptor acceptor site (H–X) on the protein, the synthesis of a compound to confirm the existence of this acceptor site should be of prime importance. This has now been achieved with the synthesis of the irreversible inhibitor, 3-isothiocyanato-β-carboline (**7**; Figure 4). This alkylating agent demonstrates high affinity (IC_{50} = 8 nM) at BzR and has been shown to bind irreversibly to BzR, reducing the apparent affinities of [^{3}H]Ro 15-1788 and [^{3}H]βCCE for BzR with no effect on B_{max}.[32] This irreversible ligand(**7**)-receptor interaction provides strong evidence that the hydrogen bond acceptor (H–X) portion of the BzR inverse agonist site is interacting with N(2) on the β-carboline ligands **2**–**10**.

The model depicted in Figure 1 was developed by utilizing the template approach.[1a] The rigid, planar pyridodiindole **1** (IC_{50} = 4 nM) was employed as the foundation, and the sites of electron density common to **1** and other inverse agonists/antagonists were defined.[1a] A valid model of a receptor pharmacophore successfully predicts the

(32) Lueddens, H.; Skolnick, P.; Allen, M.; Hagen, T. J.; Cook, J. M., manuscript in preparation.

Table III. In Vitro Binding of Selected Ligands to BzR



compd	R	R′	A	in vitro IC_{50},[a] nM
10	CO_2CH_3	H	H	5
12	H	$NHCH_2Ph$	H	106
13	$NHCH_2Ph$	H	H	1280[b]
15	CO_2CH_3	H	CH_3	>50000
1 (R_1 = R′ = H)				4
14 (R_1 = CH_3, R′ = H)				1163
17,				1920
18,				980[c]
16,				13200

[a] See ref 16 for details. [b] 1280 nM at 30% inhibition. [c] Private communication, G. Adelstein, Searle Laboratories.

design of new agents that bind to the receptor with enhanced affinity relative to previous congeners. Norharmane (**11**) was found to have relatively low affinity (IC_{50} = 1620 nM)[13] for BzR and it exerts its tremorgenic actions via other mechanisms[33,34] However, by consideration of the topography of **1** and the lipophilic interactions at the receptor site (see Figure 3) based on previous SAR,[1a,10,11,13,14,16,17] the hydrogen atom at position 3 of **11** has been replaced with OCH_2CH_3 or $OCH_2CH_2CH_3$. The result is a 150-fold increase in affinity from 1620 nM (**11**) to 11 nM (**4**). Moreover, the 3-ethoxy derivative is a potent partial inverse agonist in vivo and the irreversible inhibitor **7** (8 nM) has been synthesized on the basis of the model depicted in Figure 1.

In contrast, 3-(*N*-benzylamino)-β-carboline (**13**; IC_{30} = 1280 nM) exhibits only weak affinity for BzR (see Table III). Although the *N*-benzyl substituent at C(3) is lipophilic, the size of this group prevents maximum interaction of the ligand **13** to the receptor site at region H_1 (Figure 1) on the protein.

A series of rigid, planar pyridodiindoles[10] has recently been synthesized in order to rigorously test the model proposed in Figure 1. These molecules, as well as their respective IC_{50} values, are outlined in Table III. Replacement of an indole N(7)-hydrogen atom on **1** with a methyl function results in a decrease in affinity from 4 nM

(33) Schweri, M.; Cain, M.; Cook, J. M.; Paul, S.; Skolnick, P. *Pharmacol. Biochem. Behav.* **1982**, *17*, 457.
(34) Haefely, W. *Psychopharmacology (Amsterdam)* **1985**, *2*, 92.

(1) to 1163 nM (14) for BzR.[35] This decrease in affinity parallels the decrease in potency in going from βCCM (10; 5 nM) to 9-methyl-βCCM (15; >50 μM).[13] This result supports the involvement of an indole N(9)-H in a hydrogen bond interaction of the ligand with a hydrogen bond donor site (:D–R) on the receptor. Although Loew[2a] and Gilli[2b] have reported that the interaction of an indole N(9)-H at the inverse agonist site is a secondary one, data here suggest it is an important interaction. As mentioned earlier, the ability of indole N–H functions to undergo H-bonding interactions is well documented in the literature.[2e,36,37] Perhaps more importantly, when the indole ring of a β-carboline such as βCCM (10) is replaced by a benzene ring (see isoquinoline 16, Table III), the potency is reduced to 13 200 nM. This is perhaps the strongest evidence to date documenting the importance of the indole N(9)-H in compounds such as 10 in the interaction of ligands (3-substituted β-carbolines) with the BzR. The relatively low potency of the 9-thio analogue 18 (980 nM) also supports this hypothesis.

In the proposed model of the pharmacophore (Figure 1)[1a] for inverse agonists, an important requirement is that N(2) in the β-carboline nucleus have high electron density (N:). In this regard, the benzodiindole 17 has recently been synthesized by Schultz et al.[38] and tested for in vitro affinity. This compound is devoid of the pyridine nitrogen atom postulated by Loew,[2a] Gilli,[2b] Fryer,[2c] Martin,[2d] Codding,[36] and ourselves[1a,10,13,37] as an important site of interaction. The relatively low affinity of 17 (1920 nM)[38] compared to the parent pyridodiindole 1 (4 nM) is in agreement with the report of Loew on the importance of the pyridine N(2) nitrogen atom, on 10 in the ligand–receptor interaction.[2a]

Finally, if the indole N(9)-H function and the pyridine N(2) nitrogen atom are critical for high-affinity binding of β-carboline ligands to the BzR, a 3-substituent (OCH_2CH_3) that increases the electron density at the N(2) function should enhance inverse agonist activity. Thus, the substituent in 3 donates electron density to the nitrogen N(2) atom [compare the pK_a of pyridine (5.25) to that of 2-methoxy pyridine (6.47)],[39] which should result in a stronger pyridine N(2)–H_1 interaction.

In summary, it appears that interactions of a ligand at both H_1 and D_2 of the BzR inverse agonist site are required for high-affinity binding of inverse agonists, as illustrated in Figure 1. In the case of β-carbolines, this suggests that both the indole N(9)-H and the pyridine N(2) nitrogen functions are required for high affinity. The distance of 4.5 Å in Figure 1 refers to the interatomic distance between probable binding sites on the ligand. Therefore, the distance between the center of H_1 and D_2 is in the range of 7.5–9.5 Å depending on the ligand and the symmetry of the three-center hydrogen bond.[36,37,40a,b] Any large perturbation from these distances results in a decreased potency or an antagonist profile of activity. The BzR binding cleft into which ligands must pass is narrow in agreement with the work of Borea and Gilli and co-workers[2b] and is also consistent with the planar or psuedoplanar hypothesis put forth earlier from these laboratories.[13,17] Support for the proposed model originates from the potent affinity of the rigid, planar pyridodiindoles 1[1a] for BzR coupled with the lack of affinity for ligands such as the β-carboline 15, the isoquinoline 16, the pyridodiindole 14, the benzodiindole 17, and the 9-thio analogue 18. The in vitro SAR of the other ligands depicted in Tables I and III is entirely consistent with the present model.

Substitution of an electron-withdrawing group (CO_2CH_3, CN, Cl, NO_2, N=C=S) at position 3 of a β-carboline results in high affinity at BzR, which suggests that this substituent facilitates the interaction of the indole N(9)-H with a hydrogen bond donor site D_2 on the receptor, although additional studies are necessary to confirm this hypothesis. Substitution of an electron-releasing group at position 3 as in 3 does not destroy affinity for the BzR. If the electron density on the pyridine nitrogen atom N(2) is enhanced, the affinity is increased even though the interaction of the indole N(9)-H with D_2 would be decreased slightly. These effects balance each other in the case of the potent partial inverse agonist 3 (24 nM). Studies are under way to examine the hypothesis of a common binding domain for both agonists and inverse agonists at BzR.[1a,1c,2b,2c,41,42]

Experimental Section

Receptor Binding. [^{3}H]Diazepam binding to rat cerebral cortical membranes was accomplished by using a modification of the method previously described.[10] 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 (15 s, setting 6-7, Brinkmann 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 resuspended in 50 volumes of buffer. 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 mM; specific activity, 76 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 from Hill plots.

Proconvulsant Actions. To assess whether 3-ethoxy-β-carboline (3) was proconvulsant, adult, male mice (~25 g, Veterinary Resource Branch, NIH, Bethesda, MD) were injected (0.1 mL) with the drug solution and challenged 15 or 120 min later with pentylenetetrazole (PTZ) (40 mg/kg, 0.1 mL). PTZ (0.1 mL) was dissolved in water; 3 and βCCE were suspended in 20% diluted Emulphor/80% saline (diluted Emulphor is Emulphor (GAF Corp., Wayne, NJ) diluted 1:1 (w/w) with ethanol). The

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(41) Skolnick, P.; Schweri, M.; Kutter, E.; Williams, E.; Paul, S. *J. Neurochem.* **1982**, *39*, 1142. Williams, E. F.; Rice, K. C.; Mattson, M.; Paul, S. M.; Skolnick, P. *Pharmacol. Biochem. Behav.* **1981**, *14*, 487.
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animals were observed for 15 min after PTZ for the presence of clonic/tonic convulsions.

Irreversible Binding of 7. Brain membranes preincubated with nM concentrations of **7** followed by extensive washing (resuspension and recentrifugation) demonstrated a persistent inhibition of radioligand binding to BzR compared with either control tissues or membranes preincubated with comparable concentrations of compounds such as βCCM or βCCE. Furthermore, the IC_{50} of **7** is strongly dependent on the tissue (i.e. receptor) concentration, a relationship not observed with reversible ligands. These data strongly suggest that **7** irreversibly inhibits radioligand binding to BzR.

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker 250-MHz NMR 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 employed were UV-active silica gel on plastic.

3-Ethoxy-β-carboline Hydrochloride (3).[25] To a stirred solution of 3-amino-β-carboline diacetate (**8**; 1.0 g, 3.3 mmol) in anhydrous ethanol (250 mL) at −20 °C was added isoamyl nitrite (7.4 mL, 5.4×10^{-2} mol). The solution was allowed to stir for 2 min, followed by the addition of potassium thiocyanate (10.4 g, 0.11 mol) and copper(I) thiocyanate (6.7 g, 0.55 mol) in 150 mL of anhydrous ethanol at −20 °C. After stirring for 4 h at this temperature, the reaction mixture was allowed to warm up to room temperature. When the presence of starting material was no longer detected by TLC (silica gel, 15% methanol–ethyl acetate, eluent), the reaction mixture was filtered and the solvent removed under reduced pressure. The resulting solid residue was taken up in 150 mL of a 0.5 N sodium bicarbonate solution and extracted with ethyl acetate (4 × 125 mL). The combined organic portions were then dried over sodium sulfate, and the volume was then reduced to 10 mL. This compound was then purified by flash chromatography (SiO_2) with ethyl acetate as the eluent. Upon the addition of a saturated solution of ethanol–hydrogen chloride, a precipitate formed which was filtered and washed with cold ether (3 × 10 mL) to provide pure **3**[18b] as the hydrochloride salt (0.423 g, 52%): mp 221–223 °C; IR (KBr) 1655, 1610, 1420, 1245, 1020 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.4 (t, 3 H, J = 7 Hz), 4.45 (q, 2 H, J = 7 Hz), 7.3 (m, 1 H), 7.64 (m, 2 H), 8.11 (s, 1 H), 8.36 (d, 1 H, J = 8.0 Hz), 8.66 (s, 1 H), 11.88 (s, indole 1 H); MS (CI, CH_4), 213 (M + 1); high-resolution MS, m/e 212.0937 ($C_{13}H_{12}N_2O$ requires 212.0950). Anal. ($C_{13}H_{12}N_2O$·HCl) C, H, N.

3-Methoxy-β-carboline Hydrochloride (2). 3-Amino-β-carboline (**8**), anhydrous methanol, isoamyl nitrite, potassium thiocyanate, and copper(I) thiocyanate were reacted under analogous conditions to those employed for the preparation of **3** above to provide **2** (0.205 g, 41%) crude product, (0.103 g, 21%) pure product after chromatography. No attempts to maximize this yield have been made: mp 215–217 °C; IR (KBr) 1655, 1615, 1540, 1430, 1245, 1020 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.09 (s, 3 H), 7.28 (m, 1 H), 7.63 (m, 2 H), 8.10 (s, 1 H), 8.35 (d, 1 H, J = 8.0 Hz), 8.66 (s, 1 H), 11.96 (s, indole 1 H); MS (CI, CH_4), m/e 199 (M + 1); high-resolution MS, m/e 198.0795 ($C_{12}H_{10}N_2O$ requires 198.0793). Anal. ($C_{12}H_{10}N_2O$·HCl) C, H, N.

3-Propoxy-β-carboline Hydrochloride (4). 3-Amino-β-carboline (**8**), anhydrous propanol, isoamyl nitrite, potassium thiocyanate, and copper(I) thiocyanate were reacted under the analogous conditions employed for preparation of **3** above to provide **4** (0.553 g, 65%) crude product. Chromatography provided **4** in 38% yield (0.326 g): mp 194–195 °C; IR (KBr) 1655, 1610, 1420, 1245, 1020 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.04 (t, 3 H, J = 7 Hz), 1.84 (m, 2 H), 4.36 (t, 2 H, J = 7 Hz), 7.28 (m, 1 H), 7.64 (m, 2 H), 8.14 (s, 1 H), 8.35 (d, 1 H, J = 8.0 Hz), 8.66 (s, 1 H), 11.94 (s, indole 1 H); MS (CI, CH_4) 227 (M + 1); high-resolution MS, m/e 226.1101 ($C_{14}H_{14}N_2O$ requires 226.1106). Anal. ($C_{14}H_{14}N_2O$·HCl) C, H, N.

3-Chloro-β-carboline (5). To a stirred solution of 3 N HCl (600 mL) at 5 °C was added 3-amino-β-carboline diacetate (**8**; 0.375 g, 124 mmol). To the homogeneous solution that resulted was added sodium nitrite (910 mg, 13.2 mmol), and the solution was allowed to stir for 2 h. The mixture was then brought to pH 8 (concentrated NH_4OH). The aqueous layer was extracted with ethyl acetate (3 × 200 mL), and the organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield **5** (140 mg, 56%), which was homogeneous by TLC (R_f = 0.44, silica gel, 85% ethyl acetate–15% CH_3OH): mp 278–281 °C dec; IR (KBr) 3150, 1645, 1450, 1405, 745 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.24 (m, 1 H), 7.58 (m, 2 H), 8.27 (m, 2 H), 8.67 (s, 1 H), 11.80 (s, indole 1 H); MS (CI, CH_4), m/e 203 (M + 1); high-resolution MS, m/e 202.0288 ($C_{11}H_7N_2Cl$ requires 202.0298).

3-Nitro-β-carboline Hydrochloride (6). To a stirred solution of the starting amine **8** (0.32 g, 1.75 mmol as free base) in chloroform (500 mL) was added *m*-chloroperbenzoic acid (432 mg, 2.5 mmol) at room temperature. The reaction mixture that resulted was allowed to stir for 12 h, after which the organic layer was washed with 0.1 N sodium bicarbonate (2 × 150 mL) and dried (K_2CO_3). The volume of the solvent was then reduced under vacuum and a saturated solution of ethanolic hydrogen chloride (50 mL) was added. The organic solvent that remained was removed under reduced pressure to yield an oil. This gave a fine, tan colored precipitate of **6** (0.125 g, 25%) upon the addition of ethyl acetate: mp >300 °C; IR (KBr) 3130, 1550, 1500, 1350, 1310 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.37 (m, 1 H), 7.69 (m, 2 H), 8.51 (d, 1 H, J = 8 Hz), 8.86 (s, 1 H), 9.29 (s, 1 H), 12.42 (s, indole 1 H); MS (CI, CH_4), m/e 214 (M + 1); high-resolution MS, m/e 213.0545 ($C_{11}H_7N_3O_2$ requires 213.0538). The free base of **6** was homogeneous by TLC (R_f 0.25, silica gel, 85% ethyl acetate–15% methanol).

3-Isothiocyanato-β-carboline (7). To a stirred solution of the starting amine **8** (1.0 g, 3.91 mmol), as the dihydrochloride in 100 mL of (H_2O/$NaHCO_3$), was added 100 mL of chloroform. After 15 min of vigorous stirring at room temperature, thiophosgene (0.305 mL, 4.0 mmol) was syringed into the reaction mixture. After the reaction mixture was allowed to stir for 1 h, the chloroform layer was removed and the aqueous layer was extracted with an additional portion of chloroform (100 mL). The combined organic layers were dried (Na_2SO_4) and reduced to 5 mL under reduced pressure. Upon dropwise addition of hexane to the solution, a precipitate formed that was filtered and dried to yield **7** (450 mg, 51%). Compound **7** was homogeneous by TLC (R_f 0.64, silica gel, 85% ethyl acetate–15% CH_3OH): mp 171–173 °C; IR (KBr) 3130, 2020, 1630, 1500, 1455 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.28 (m, 1 H), 7.58 (m, 2 H), 8.24 (d, 1 H, J = 8 Hz), 8.28 (s, 1 H), 8.75 (s, 1 H), 11.93 (s, indole 1 H); MS (CI, CH_4), m/e 226 (M + 1); high-resolution MS, m/e 225.0379 ($C_{12}H_7N_3S$ requires 225.0361).

3-Hydroxy-β-carboline (9). To a stirred solution of 3 N H_2SO_4 (400 mL) at 5 °C was added 3-amino-β-carboline dihydrochloride (**8**; 0.5 g, 1.98 mmol). To the homogeneous solution that resulted was added sodium nitrite (150 mg, 2.17 mmol), and the mixture was stirred for 2 h. The solution was then brought to pH 8 (concentrated NH_4OH) and the aqueous layer was extracted with ethyl acetate (3 × 125 mL). The organic extracts were combined and dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield **9** (80 mg, 22%). This compound was homogeneous by TLC (R_f 0.42, silica gel, 85% ethyl acetate, 15% methanol): mp >300 °C; IR (KBr) 3300–3000, 1680, 1630, 1470 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.10 (m, 2 H), 7.61 (m, 1 H), 7.95 (s, 1 H), 8.25 (m, 1 H), 8.65 (s, 1 H), 10.80 (s, indole 1 H); MS (CI, CH_4), m/e 185 (M + 1); high-resolution MS, m/e 184.0635 ($C_{11}H_8N_2O$ requires 184.0637).

3-(Benzylamino)-β-carboline Hydrochloride (13). Glacial acetic acid (0.5 mL) was added to a stirred suspension of 3-amino-β-carboline diacetate (**8**) (0.150 g, 0.495 mmol) in dry methanol (50 mL). To the resulting solution was added benzaldehyde (157 mg, 1.48 mmol) in methanol (5 mL). After the mixture was stirred for 30 min, sodium cyanoborohydride (265 mg) was added and the solution that resulted was stirred for 18 h at room temperature. The reaction solution was then washed with HCl (6 N, 200 mL). The acidic solution was cooled to 0 °C after which the pH was adjusted to 10 (concentrated NH_4OH). The aqueous layer was extracted with chloroform (4 × 100 mL). The combined organic extracts were washed with brine (150 mL) and dried (Na_2SO_4), and the solvent was removed to provide an oil. Hot methanolic HCl (50 mL) was then added and the solvent volume was reduced to 25 mL. The precipitate that resulted was collected by vacuum filtration to yield **13** (61 mg, 45%): mp

238–240 °C; (IR) (KBr) 1660, 1625, 1500, 1450, 1425 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.65 (s, 2 H), 7.18–7.66 (m, 8 H),, 7.78 (s, 1 H), 8.07 (s, br, NH), 8.23 (d, J = 8.0 Hz, 1 H), 8.41 (s, 1 H), 11.56 (s, indole 1 H); MS (CI, CH_4), 274 (M + 1). Anal. ($C_{18}H_{15}N_3 \cdot HCl \cdot {}^1/_8EtOH$) C, H, N.

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Registry No. **2**, 114819-74-8; **2** (free base), 91985-82-9; **3**, 114819-73-7; **3** (free base), 91985-81-8; **4**, 114819-75-9; **4** (free base), 91985-83-0; **5**, 91985-80-7; **6**, 114819-76-0; **6** (free base), 114819-77-1; **7**, 114819-79-3; **8**, 114819-72-6; **8** (free base), 73834-77-2; **8** (dihydrochloride), 114819-78-2; **9**, 91985-78-3; **13**, 114819-81-7; **13** (free base), 114819-80-6; **18**, 106613-33-6; benzaldehyde, 100-52-7.

Structure–Activity Relationships among Benextramine-Related Tetraamine Disulfides. Chain Length Effect on α-Adrenoreceptor Blocking Activity[1]

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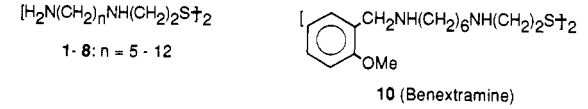
Several N′-substituted N,N''-(dithiodi-2,1-ethanediyl)bis(1,ω-alkanediamines) were prepared and evaluated for their blocking activity on α-adrenoreceptors in the isolated rat vas deferens and human blood platelets. The results were compared with those obtained for benextramine (N,N''-(dithiodi-2,1-ethanediyl)bis[N'-[(2-methoxyphenyl)-methyl]-1,6-hexanediamine], **10**). Bendotramine (N,N''-(dithiodi-2,1-ethanediyl)bis[N'-[(2-methoxyphenyl)-methyl]-1,12-dodecanediamine], **16**) proved to be as active as **10** on α_1-adrenoreceptors, showing that optimum activity is associated with two carbon chain lengths separating inner from outer nitrogens of tetraamine disulfides. On the other hand, **16** had no activity up to 20 μM at α_2-adrenoreceptors. The optimum activity at this receptor subtype was associated with a six to eight carbon chain (**10–12**). Furthermore, **10** proved to be more selective toward α_2-adrenoreceptors whereas **16** was a selective α_1-antagonist. The tetraamine disulfides were shown also to be potent inhibitors of human platelet aggregation induced by ADP or epinephrine. The potency increased with the carbon chain length. However, the results on platelets did not parallel those found in the rat vas deferens, indicating that differences exist between the α-adrenoreceptor subtypes investigated. In conclusion, **10** may be a useful tool in characterizing α_2-adrenoreceptors whereas **16** might help in investigating α_1-adrenoreceptors.

The development of polymethylene tetraamines, whose main feature is a cystamine moiety carrying aminoalkyl substituents on the nitrogens, as α-antagonists started from the observation that the linear tetraamine disulfide **1** (n = 5), originally developed as a radioprotective agent,[2] showed a relatively weak but apparently selective and irreversible α-blocking activity. Extensive structure–activity relationships were carried out with the aim of improving the potency of **1** and elucidating the active-site topography in the region of the target thiol.[3,4] These studies led to the discovery of compounds such as benextramine (**10**),[4a] pyrextramine,[4g] or **4** (n = 8)[4a] displaying an α_1-blocking activity about 2 orders of magnitude higher than that of the parent compound **1**.



10 (Benextramine)

It was shown that α_1-adrenoreceptor inhibition by tetraamine disulfides is the result of covalent bond formation between a receptor target thiol and the disulfide bridge of the antagonist through a disulfide–thiol interchange reaction.[3,4a] It was also found that optimum activity is associated with two different carbon chain lengths separating the inner from the outer nitrogens and depends on the type of substituents on the terminal nitrogens. Thus, optimum α_1-blocking activity in the series with benzyl-type substituents on the terminal nitrogens is associated with a six-carbon chain, as in **10**, on rat vas deferens[4a,g] and rabbit aorta[4d] and left atrium[5] whereas in the unsubstituted series it is associated with an eight-carbon chain, as in **4**, on rat vas deferens[4a] and a seven-carbon chain, as in **3**, on rabbit aorta.[4d]

The prototype of tetraamine disulfides **10** has been fairly well investigated in both functional studies and binding experiments.[4a,g,6–27] Compound **10** irreversibly blocked

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