Synthetic and Computer-Assisted Analyses of the Pharmacophore for the Benzodiazepine Receptor Inverse Agonist Site

Michael S. Allen,*,† Yun-C Tan,† Mark L. Trudell,† Kris Narayanan,† Liesl R. Schindler,† Michael J. Martin,† Christopher Schultz,† Timothy J. Hagen,‡ Konrad F. Koehler,*,§ Penelope W. Codding,∥ Phil Skolnick,⊥ and James M. Cook†

Department of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201, Division of Central Nervous System Disease Research and Drug Design Section, Searle Research and Development, Skokie, Illinois 60077, Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 1N4, and Laboratory of Neuroscience, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892. *Received November 6, 1989*

The structural requirements for ligand binding to the benzodiazepine receptor (BzR) inverse agonist site were probed through the synthesis and in vitro evaluation of 3-substituted β-carbolines **6**, **7**, **11**, **12**, γ-carboline **13**, and diindoles **18–21**, **23–25**, **27**, **28**, and **34**. On the basis of the apparent binding affinities of these and other analogues, a hydrogen bond acceptor site (A_2) on the receptor is proposed to interact with the N(9) hydrogen atom of the β-carbolines or the N(7) hydrogen nuclei of the diindoles. Likewise, a proposed hydrogen bond donating site (H_1) interacts with the N(2) nitrogen atom of the β-carbolines or the N(5) nitrogen atom of the diindoles. It appears that interaction with both sites is a prerequisite for high affinity since analogues which have either one or both of these positions blocked exhibit substantial reduction in affinity. Moreover, H_1 appears to be capable of engaging in a three-centered hydrogen bond with appropriately functionalized ligands, which explains the increase in potency observed in the following series of 3-substituted β-carbolines: the *n*-butyl (**12**, IC_{50} = 245 nM), *n*-propoxy (**9**, IC_{50} = 11 nM), and propyl ketone (**11**, IC_{50} = 2.8 nM) congeners. In addition to H_1 and A_2, there appears to be a relatively narrow hydrophobic pocket in the binding cleft that can accommodate substituents at the 3-position of the β-carbolines which have chain lengths ≤ C_5. There is a 1 order of magnitude decrease in affinity between *n*-propoxy analogue **9** (IC_{50} = 11 nM, chain length = 4) and *n*-butoxy derivative **7** (IC_{50} = 98 nM, chain length = 5). Furthermore, α- and γ-branching [e.g. ethoxycarbonyl (**2**), IC_{50} = 5 nM and *tert*-butoxycarbonyl (**31**) IC_{50} = 10 nM] but not β- and δ-branching [e.g. isopropoxy (**6**), IC_{50} = 500 nM and (neopentyloxy)carbonyl (**48**), IC_{50} = 750 nM] at position 3 are tolerated. Occupation of this hydrophobic pocket is clearly important for high affinity as evidenced by the relatively low affinity of **30**, a β-carboline which possesses a hydrogen atom at the 3-position. This same hydrophobic pocket is partially filled by the D and E rings of the diindoles, which accounts for the high affinity of several members of this series. An excluded volume analysis using selected 3-substituted β-carbolines and ring-E substituted pyridodiindoles is consistent with the presence of this hydrophobic pocket (see Figure 1). A model which distinguishes inverse agonists from antagonists is also proposed based in part on experimental findings which demonstrate that 3-*n*-propoxy-β-carboline (**9**) is an antagonist at the BzR with low efficacy and is devoid of proconvulsant activity at the highest dose tested (40 mg/kg). β-Carbolines which possess substituents of shorter length which are constrained to be in the plane of the aromatic ring tend to display inverse agonist activity while β-carbolines with longer substituents which can access regions of space above and below the plane of the aromatic rings are likely to have antagonist activity. Lastly, results from a 3D QSAR analysis of 37 test compounds (cross validated r^2 = 0.59) correlate well with and strongly support the previously proposed model of the pharmacophore for the benzodiazepine inverse agonist receptor site. The 3D QSAR electrostatic map is consistent with the existence of hydrogen bonding sites H_1 and A_2. Moreover, the steric map supports the existence of a hydrophobic binding pocket and is in qualitative agreement with the receptor essential volume obtained from an excluded volume analysis.

Introduction

β-Carbolines evoke a wide range of psychopharmacological actions. Many of these effects are mediated through benzodiazepine receptors,[1,2] which are capable of selectively modulating activity at GABA-gated chloride channels.[3] Several β-carboline-3-carboxylates such as BCCM (**1**), BCCE (**2**), 3-[(methylamino)carbonyl]-β-carboline (FG 7142), and 6,7-dimethoxy-4-ethyl-3-(methoxycarbonyl)-β-carboline (DMCM) have been shown to possess "inverse agonist" actions (anxiogenic, somnolytic, convulsant, and proconvulsant).[4-7] More recently, β-carbolines have been synthesized that possess the full range of agonist (benzodiazepine-like) actions as well as those classified as benzodiazepine receptor antagonists. Analogous to the prototypic Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5*a*][1,4]benzodiazepine-3-carboxylate), they can antagonize the actions of either an agonist or an inverse agonist[8] but exert no remarkable pharmacological actions through a wide dose range. Several congeners of the rigid, planar, pyridodiindole heterocycle **3**[9] also exhibit this type of inverse agonist activity.[10] In addition, there are examples of BzR ligands which do not exhibit the full range of agonist or inverse agonist actions.[3] Such substances may offer significant therapeutic advantages over currently used medications. For example, it would be advantageous to develop a nonsedating anxiolytic. Sim-

† Department of Chemistry, University of Wisconsin—Milwaukee.

‡ Division of Central Nervous System Diseases Research, Searle Research and Development.

§ Drug Design Section, Searle Research and Development; inquiries concerning the computer modeling aspects of this paper should be directed to this author.

∥ Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary.

⊥ National Institute of Diabetes and Digestive and Kidney Diseases.

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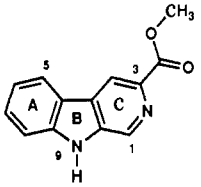
ilarly, an inverse agonist free of convulsant/proconvulsant actions has been proposed as a possible cognitive enhancer.[11,12]



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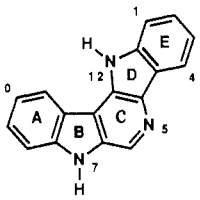
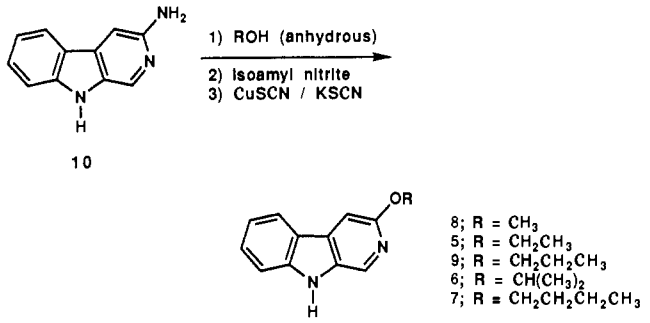


3

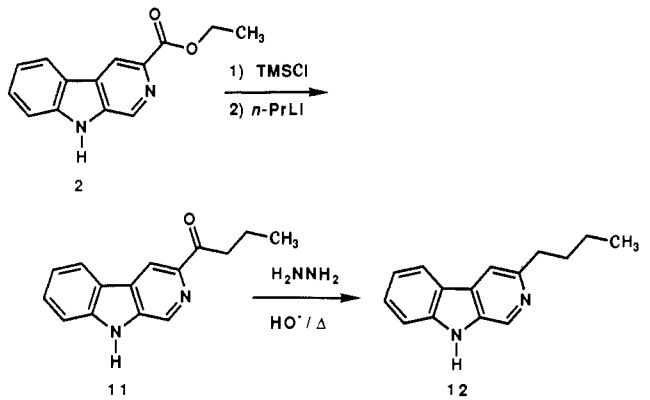
In a recent paper, the synthesis and biological activity of a number of 3-substituted β-carbolines were described.[13] In agreement with the findings of Loew,[14] β-carbolines substituted with electron-withdrawing groups at position 3 displayed high affinity binding for BzR, indicating that a carbonyl group at this position was unnecessary for interaction with this receptor; the synthesis of the irreversible inhibitor, β-carboline 3-isothiocyanate 4, provided an excellent example.[13] This high-affinity acylating agent has been shown to bind irreversibly to BzR, reducing the apparent affinities of [^{3}H]ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5-*a*][1,4]benzodiazepine-3-carboxylate and [^{3}H]BCCE for BzR with no effect on B_{max}.[15] Ligands which carry electron-releasing substituents at position 3 of a β-carboline were also found to bind tightly to BzR. For example, 3-ethoxy-β-carboline (**5**; IC_{50} = 24 nM) was found to be a long-lived inverse agonist at BzR, which may prove useful for in vivo studies since it has a higher affinity for BzR and is more soluble in aqueous solution than the commonly used inverse agonist 3-[(methylamino)carbonyl]-β-carboline.[16] 3-Ethoxy-β-carboline (**5**) exhibits anxiogenic and proconvulsant properties and inhibits stress-induced ulcer formation in mice.[16] This ligand does not induce convulsions when administered alone, suggesting it may be useful in the study of sleep,[17] anxiety,[18] and memory–learning.[11] It has also been suggested that chronic administration of β-carboline inverse agonists induces a prolonged functional deficit at the GABA/Bz/Cl ionophore receptor complex,[19] adding further impetus to this area of research.

Based on the rigid and planar topography of pyridodiindole **3**, a model for ligand binding to an inverse agonist domain at BzR was postulated. With the template approach,[13,20] it has been possible to successfully predict the affinities of ligands to the BzR receptor. Results from these studies support the primary involvement of an indole N(9)–H in a hydrogen-bond interaction of the ligand with a hydrogen bond acceptor site (A_2) on the receptor in contrast to several other pharmacophore models that have been proposed.[21-25] In addition, the proposed model requires the pyridine N(2) nitrogen atom of a β-carboline to exhibit hydrogen bond acceptor (N:) properties with a receptor donating site (H_1) on the protein.[13,20] More recently, our research efforts have led to the design and synthesis of new agents to further test the model and to more clearly define the inverse agonist domain at BzR. Described in this report are the syntheses and structure–activity profiles of new β-carboline and pyridodiindole ligands. Results of these studies indicate that substituents at position 3 of the β-carbolines have a strong influence on both affinity and type of activity displayed by these ligands. An excluded-volume analysis[26,27] of these ligands was also carried out in order to delineate the shape of the

Scheme I



Scheme II



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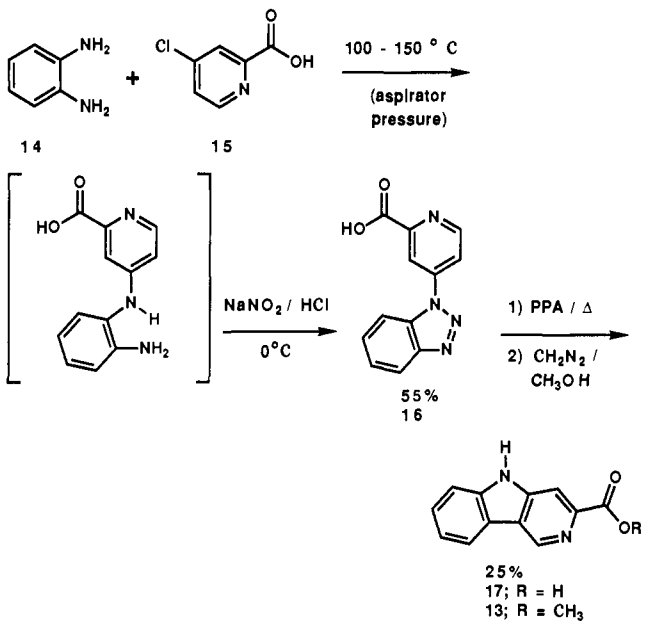
Scheme III



BzR in the vicinity of position 3 of bound β-carbolines. Finally, to more rigorously define and test the proposed model of the pharmacophore for the benzodiazepine receptor inverse agonist site, a 3D quantitative structure–activity relationship (3D QSAR) analysis was performed on 37 test compounds, using the CoMFA (comparative molecular field analysis) approach of Cramer et al.[28]

Chemistry

β-Carbolines **6** and **7** were synthesized to define the structure–activity relationships (SAR) of the 3-substituted alkoxy β-carboline derivatives. These compounds were synthesized in a manner identical with that previously reported for the synthesis of ligands **5**, **8**, and **9**.[13] In brief, the corresponding diazonium salt of 3-amino-β-carboline (**10**) was converted into the desired alkoxy derivatives **5–9** with isoamyl nitrite, a copper(I) salt, and the appropriate anhydrous alcohol (Scheme I).

Ligands **11** and **12** were synthesized and screened to determine the importance of the lipophilic nature of the substituent at the 3-position of a β-carboline. 3-(Ethoxycarbonyl)-β-carboline (**2**) was treated with trimethylsilyl chloride in tetrahydrofuran at −70 °C. The complex which results was then reacted with *n*-propyllithium to give propyl ketone **11**. The propyl ketone was then reduced by utilizing the Huang–Minlon modification[29,30] of the Wolff–Kishner reduction to furnish the 3-*n*-butyl β-carboline **12** (Scheme II).

2-(Methoxycarbonyl)-γ-carboline (**13**), which is essentially a β-carboline with the indole N–H para to the pyridine nitrogen atom, was prepared by utilizing the synthetic sequence depicted in Scheme III. 4-Chloropicolinic acid (**15**) was reacted with *o*-phenylenediamine (**14**) in an Ullman reaction followed by treatment with nitrous acid to produce benzotriazolopyridine **16**, according to the method of Robinson.[31,32] This intermediate was cyclized with polyphosphoric acid via the published procedure[33] and acid **17** which resulted was converted into the desired ester **13** with diazomethane.

In order to determine the relative importance of steric and electronic factors on the interaction of ring E substituted pyridodiindoles with BzR and to complete a structure–activity profile for the pyridodiindoles, four methyl-substituted pyridodiindoles (**18–21**) were synthesized.[34] These compounds **18–21**, which contain a methyl group on the E ring of **3**, were prepared by the Fischer indole cyclization of 2-benzoyl-4-oxotetrahydro-β-carboline (**22**) with the corresponding tolylhydrazines (Scheme IV). The monomethylation of the indole N–H at positions 7 (**23**) and 12 (**24**) of **3**, as well as the methylation of the pyridine nitrogen atom N(5) of **3**, has also been accomplished, regioselectively (Scheme V). Moreover, the 7,12-dimethylpyridodiindole **25** was prepared. 7-Methyl analogue **23** was synthesized via a Fischer indole cyclization using N_B-methyl-2-benzoyl-4-oxotetrahydro-β-carboline (**26**) and phenylhydrazine. The 12-methyl and 7,12-dimethyl compounds were synthesized by reaction of **3** with sodium/liquid ammonia and methyl iodide at −70 °C to provide the 12-methyl analogue **24** as the major material (Scheme V). Indolocarbazole **27** was synthesized by utilizing a Fischer indole cyclization according to the method of Mann,[35] and 6-ethyl-7,12-dihydropyridodiindole **28** was prepared by heating 1-ethyl-2-(trichloroacetyl)-4-oxo-1,2,3,4-tetrahydro-β-carboline (**29**)[36] in phenylhydrazine at 150 °C for 12 h (Scheme VI).

Excluded Volume and 3D QSAR Methods

The structures of BzR inverse agonists used in the excluded volume and 3D QSAR analysis were constructed by using version 2.7.7 of CONCORD,[37] adjusting the N(2)–C(3)–C=O torsional angle in the β-carbolines to 0° and optimizing with MNDO.[38] Since MNDO exaggerates steric effects,[39] certain torsional angles were fixed at 0° or 180° to more closely correlate with X-ray crystal structures of the ligands.[20,40–42] These torsional angles include all aromatic atoms, hydrogens directly attached to these aromatic atoms, and functional groups in direct conjugation with the aromatic rings (i.e. ester, ketone, and nitro groups). All other internal coordinates were fully optimized. Atomic charges were obtained from a Mulliken population analysis of a single-point PRDDO calculation.[43]

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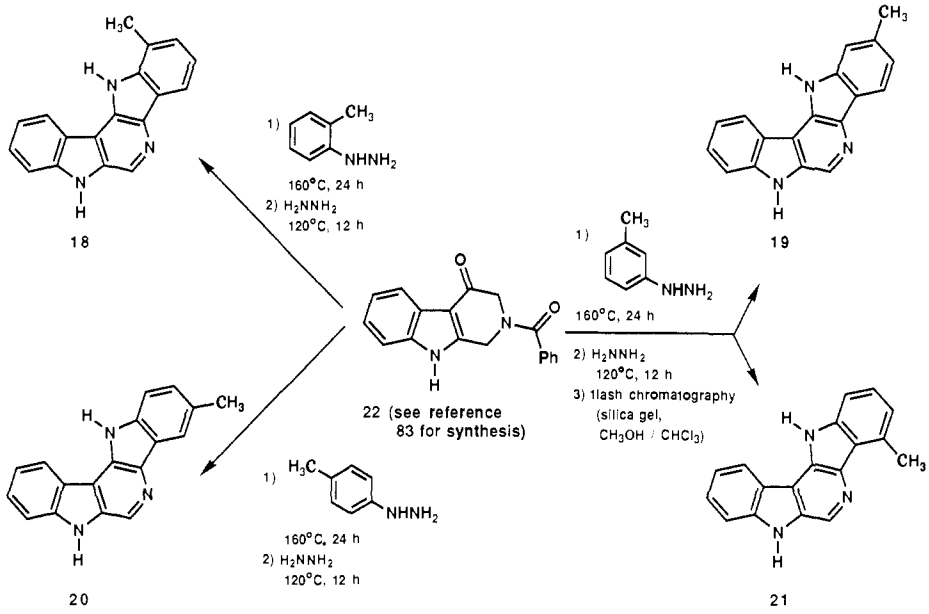
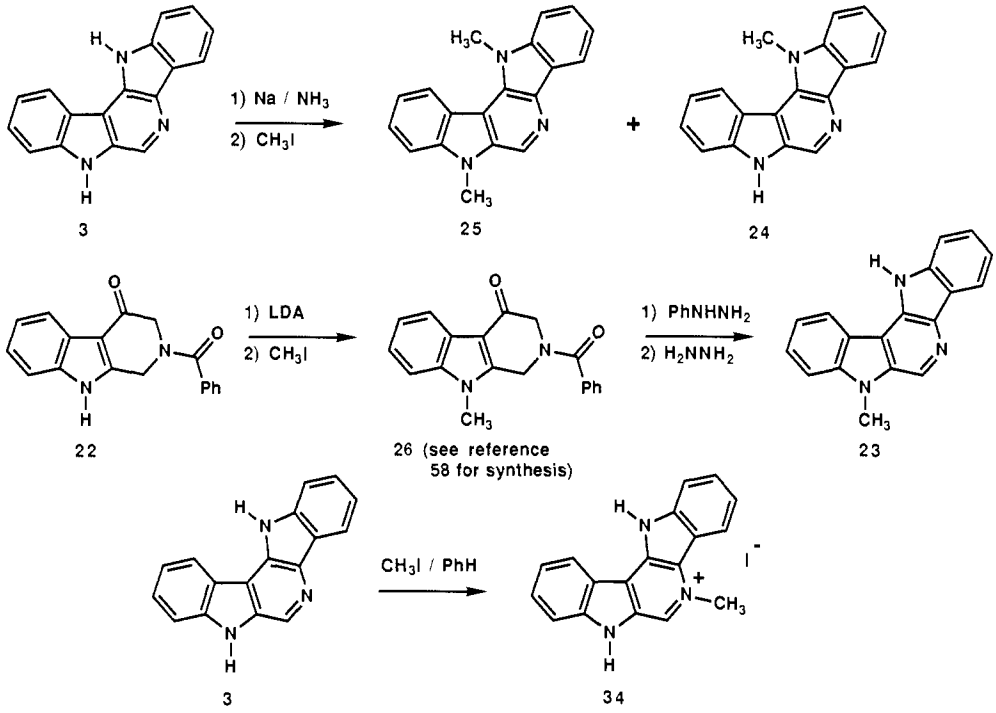
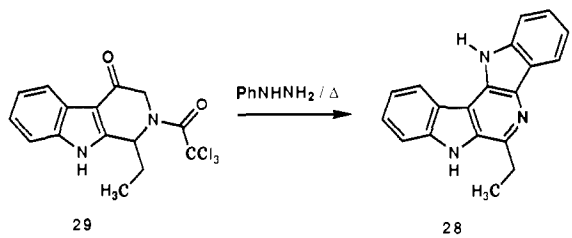
Scheme IV



Scheme V



Scheme VI

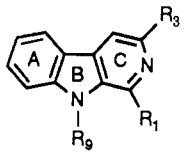


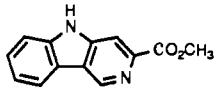
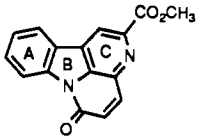
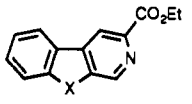
The centroid of BCCM (1) was placed at the origin and the molecule was rotated to bring the aromatic ring parallel with the X-Y plane. The remaining molecules were least-squares fit to BCCM by using the FIT option of SYBYL version 5.22.[37] Nine atoms were used in the fit. These included the six aromatic carbon atoms of the A ring, the indole nitrogen moiety, and the attached hydrogen atom (B ring), as well as the oxygen atom of the C ring (CGS compounds) or the nitrogen atom (C ring) of the β-carbolines and diindoles. The root mean square (RMS) deviation of the β-carbolines and diindoles, as compared to that of BCCM (1), were less than 0.05 Å. The RMS deviation of the fit between BCCM (1) and the CGS compounds was 0.24 Å. Octanol/water partition coefficients and molar refractivity values were estimated by using the CLOGP and CMR algorithms respectively in version 3.54 of the MedChem software.[44]

Of the 44 compounds listed in Tables I–III, 37 (**1–3, 5–9, 11, 12, 18–21, 23–25, 27, 30, 31, 35–38, 41–53**) were included in the CoMFA analysis. Compounds 4 and **39** were excluded from the analysis due to chemical instability.

(44) Available from Daylight Chemical Information Systems, Inc., Irvine, CA 92714.

Table I. In Vitro Binding of Selected β-Carboline Ligands to BzR



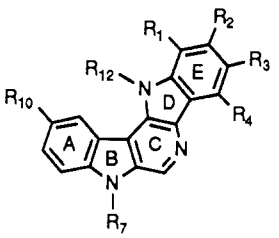
no.	R_3	R_9	R_1	IC_{50},[a] nM
1	CO_2CH_3	H	H	5
2	$CO_2CH_2CH_3$	H	H	5
4	N=C=S	H	H	4–12[b]
5	OCH_2CH_3	H	H	24[b]
6	$OCH(CH_3)_2$	H	H	500 ± 23
7	$OCH_2CH_2CH_2CH_3$	H	H	98 ± 18
8	OCH_3	H	H	124[b]
9	$OCH_2CH_2CH_3$	H	H	11
11	$COCH_2CH_2CH_3$	H	H	2.8 ± 0.8
12	$CH_2CH_2CH_2CH_3$	H	H	245 ± 15
30	H	H	H	1620[c]
31	$CO_2C(CH_3)_3$	H	H	10
33	CO_2CH_3	CH_3	H	>50000[c]
39	CO_2CH_3	$COCH_3$	H	9[e]
46	Cl	H	H	45[b]
47	NO_2	H	H	125[b]
48	$CO_2CH_2C(CH_3)_3$	H	H	750
49	CO_2CH_3	H	CH_2CH_3	7540[c]
50	H	H	CH_2CH_3	250000[c]
51	H	H	CH_3	12400[c]
13				653
32				100
				
35		X = C(=O)		26000[d]
36		X = C(=NOH)		5000[d]
37		X = O		9200[d]
38		$X = CH_2$		680[d]
52		X = C(=O)N(H)		2400[d]
53		X = S		1700[d]

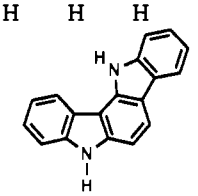
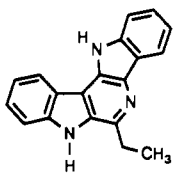
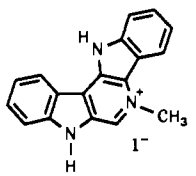
[a] Values for new (previously unpublished) compounds are listed with statistical limits and represent $\bar{X}$ of three or more experiments. Compound 4 is an irreversible ligand. Unlike competitive, reversible ligands, the apparent IC_{50} of this compound is highly dependent upon the receptor (tissue) concentration. See ref 54 for details. [b] See ref 13 for details. [c] See ref 62 for details. [d] See ref 63 for details. [e] See ref 65 for details; unreliable data due to instability (this paper).

Partition coefficient estimates for the pyridinium salt **34** are unavailable; therefore, this compound was not considered in the CoMFA analysis. Finally compounds **13** and **32** have structures which differ substantially from the others in the study, making their alignment to BCCM uncertain, hence **13** and **32** were also dropped from the 3D QSAR analysis.

The CoMFA[28] analysis was carried out by using the QSAR option of SYBYL version 5.30. Unless specifically stated otherwise, default settings were used throughout. The molecular coordinates were taken directly from the least-squares fitting procedure described above and used as is. The steric and electrostatic potentials were generated by using an sp^3 carbon probe with a +1 charge. The grid used in the CoMFA analysis had a resolution of 2.0 Å and the grid dimensions ran from −11.0 to +11.0 Å along the

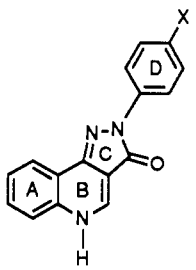
Table II. In Vitro Binding of Selected Pyridodiindole Ligands to BzR



no.	R_7	R_{12}	R_1	R_2	R_3	R_4	IC_{50},[a] nM
3	H	H	H	H	H	H	4
18	H	H	CH_3	H	H	H	83 ± 13
19	H	H	H	CH_3	H	H	10 ± 1.3
20	H	H	H	H	CH_3	H	224 ± 35
21	H	H	H	H	H	CH_3	6860 ± 800
23	CH_3	H	H	H	H	H	1163 ± 317
24	H	CH_3	H	H	H	H	157 ± 45
25	CH_3	CH_3	H	H	H	H	1917 ± 520
44	H	H	H	H	H	OCH_3	250 (n = 1)[b]
45	H	H	H	H	H	Cl	715 ± 160[b]
27							1970 ± 301
28							285 ± 35
34							4660 ± 650

[a] Values of $\bar{X}$ represent three or more experiments unless otherwise stated. [b] Trudell, M. L. Ph.D. Thesis, University of Wisconsin—Milwaukee, 1989.

Table III. Benzodiazepine Receptor in Vitro Binding of Selected CGS Compounds[a]



compound	X	IC_{50}, nM
CGS-8216 (**41**)	H	0.4
CGS-9896 (**42**)	Cl	0.6
CGS-9895 (**43**)	OCH_3	0.1

[a] Yokayama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.

X and Y axes and from −6.0 to +6.0 along the Z axis. These dimensions insured that the grid extended beyond the molecular dimensions by 4.0 Å in all directions and that the Z coordinates of the aromatic rings (0.0 Å) matched exactly the Z coordinate of one plane of the potential grid.

Log P, $(\log P)^2$, and molar refractivity were included as additional correlates in the regression and the MUM_SIGMA value was set to 1.00.

On the basis of the apparent affinities for the BzR, β-carbolines 1 and **31**, pyridodiindole **19**, and pyrazoloquinoline **41** were considered as "active" and β-carbolines **6** and **48** and diindoles **20** and **21** were considered as "inactive" in the excluded volume analysis[26,27] of the BzR receptor. The conformations of the "inactive" 3-substituted β-carbolines were generated and minimized by using the MULTIC (30° torsional resolution) and MM2 BatchMin options of MacroModel[45] version 2.1, respectively. All conformations which were within 3 kcal/mol of the global energy minimum were included in the excluded-volume analysis since presumably none of the low-energy conformations fit the receptor well. In the minimization process, all atoms except those of the 3-substituents were held fixed. The excluded receptor volume was generated by using the MVOL option of SYBYL 5.22 by subtracting the union of the volume of the "actives" from the union of volume of the "inactives".

Results and Discussion

The potencies of several substituted β-carbolines and γ-carboline **13** to inhibit radioligand binding to BzR are presented in Table I. It was previously suggested that electron-releasing substituents at the 3-position of the β-carboline enhance the hydrogen-bonding strength of the pyridine N(2) nitrogen atom through resonance.[13,20,46-48] In order to examine possible steric effects of substituents in the 3-position, several additional analogues were examined. One of these, 3-*n*-butyl-β-carboline (**12**) is approximately 7 times as potent as β-carboline (**30**). Furthermore, in the 3-substituted alkoxy series, there is a steady increase in potency as chain length is increased from methoxy (**8**), to ethoxy (**5**), and finally to *n*-propoxy (**9**). These observations suggest that the binding of β-carbolines to BzR is stabilized by favorable interactions between hydrophobic substituents at the 3-position and a hydrophobic pocket in the receptor. Additional support for the existence of a hydrophobic receptor pocket may be obtained from the examination of data from bulkier substituents at the 3-position. When the alkyl side chain of 3-propoxy-β-carboline **9** is increased in length by one methylene unit to afford 3-*n*-butoxy derivative **7**, potency is decreased by 1 order of magnitude. Furthermore, side chains with β or δ-branching (β-carbolines **6** and **48**, respectively) display a marked decrease in potency, while a β-carboline with γ-branching (**31**) displays high affinity. These data suggest a hydrophobic pocket with a definite length and width. In order to assess the relative importance of resonance and hydrophobic contributions to ligand potency, it is instructive to compare the affinity of the 3-*n*-butyl-β-carboline (**12**) vs the *n*-propoxy analogue **9**. These two β-carbolines have isosteric side chains, yet the latter is more than 1 order of magnitude more potent. This observation indicates that resonance effects are at least as important as steric effects in determining potency in the 3-alkoxy series. 3-Substituted propyl ketone derivative **11** (IC_{50} = 2.8 nM) has the same approximate side chain length as *n*-propoxy derivative **9** (IC_{50} = 11 nM), yet it is significantly more potent. The propyl ketone function is electron withdrawing; therefore, it should strengthen the hydrogen bond involving the N(9) indole hydrogen and weaken the hydrogen-bonding ability of N(2) of the β-carboline ring. Since these two effects may cancel to some extent, the increased potency displayed by propyl ketone **11** may be primarily due to the formation of a strong three-centered hydrogen bond involving the pyridine nitrogen and carbonyl oxygen of **11** and a hydrogen atom from the receptor.[13,20,47]

Modifications of the alkyl group in the β-carboline-3-carboxylate series not only affect potency but also strongly influence the pharmacological profiles of these compounds.[49,50] A transition from inverse agonist to antagonist is seen as the chain length is increased from methyl to ethyl, and finally to *n*-propyl carboxylate. An antagonist response is also elicited by the presence of large, bulky alkyl groups at position 3. For example, *tert*-butyl ester **31** has been shown to antagonize the anticonvulsant and antipunishment effects of diazepam.[51] These data indicate that in vivo biological activity is related to the molecular volume of the alkyl groups in the case of the β-carboline-3-carboxylates.[52,53] Likewise the 3-substituted alkoxy-β-carbolines follow a similar trend. 3-Ethoxy-β-carboline (**5**) displays inverse agonist properties,[16] while 3-propoxy-β-carboline (**9**) elicits an antagonist response in mice.

The interaction of a ligand at both H_1 and A_2 of the BzR enhances ligand affinity and appears obligatory for inverse agonist activity.[13,20,47] In the case of β-carbolines, this would require interaction of the receptor with both the indole N(9)–H and the pyridine N(2) nitrogen. In order to further test this hypothesis, γ-carboline **13** was synthesized. This compound was found to have a low affinity (IC_{50} = 653 nM, Table I), consistent with the hypothesis that a simultaneous interaction with both sites is required for potent affinity. The indole hydrogen and the pyridine nitrogen atom are on opposite sides of the molecule in **13**, limiting the ligand to only one hydrogen-bond interaction with the BzR (Figure 2). Interestingly, the 2-(methoxycarbonyl)canthin-6-one derivative **32** has the indole N–H function blocked, yet has an affinity of 100 nM.[54] This compound may bind in an inverted manner with respect to the 3-substituted carboxylate esters of β-carboline[52,54] (Figure 3). As previously suggested, a ligand must be able to hydrogen bond at both sites (H_1, A_2) in order to elicit

(45) Available from the Department of Chemistry, Columbia University, New York, NY 10027.
(46) Allen, M. S.; Hagen, T. J.; Skolnick, P.; Cook, J. M. Synthesis of 3-Substituted-β-Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Inverse Agonist site via the Template Approach. Presented at the 20th Central Regional Meeting of the American Chemical Society, Morgantown, WV, June 1–3, 1988; Abstract 168.
(47) 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 B-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, December 2–4, 1987; Abstract 214.
(48) In order to appear more consistent with the computational community (drug design), the term hydrogen-bond donor and acceptor has been reversed from what has been reported earlier from these laboratories. Therefore the term hydrogen-bond donor donates a hydrogen atom to an acceptor (X:). Likewise a hydrogen-bond acceptor (X:) accepts a hydrogen atom (H) capable of undergoing a hydrogen-bonding interaction.
(49) Skerritt, J. H.; Johnston, G. A. R.; Braestrup, C. *Eur. J. Pharmacol.* **1983**, *86*, 299.
(50) Braestrup, C. *J. Neurochem.* **1981**, *37*, 333.
(51) Shannon, H. E.; Guzman, F.; Cook, J. M. *Life Sci.* **1984**, *35*, 2227.
(52) Codding, P. W.; Szkaradzinska, M. B.; Roszak, A. W.; Aha, L. J.; Hagen, T. J.; Cook, J. M. *Can. J. Chem.* **1988**, *66*, 2981.
(53) Allen, M. S.; Skolnick, P.; Cook, J. M., unpublished results.
(54) Guzman, F.; Cain, M.; Larscheid, P.; Hagen, T.; Cook, J. M.; Schweri, M.; Skolnick, P.; Paul, S. M. *J. Med. Chem.* **1984**, *27*, 564.

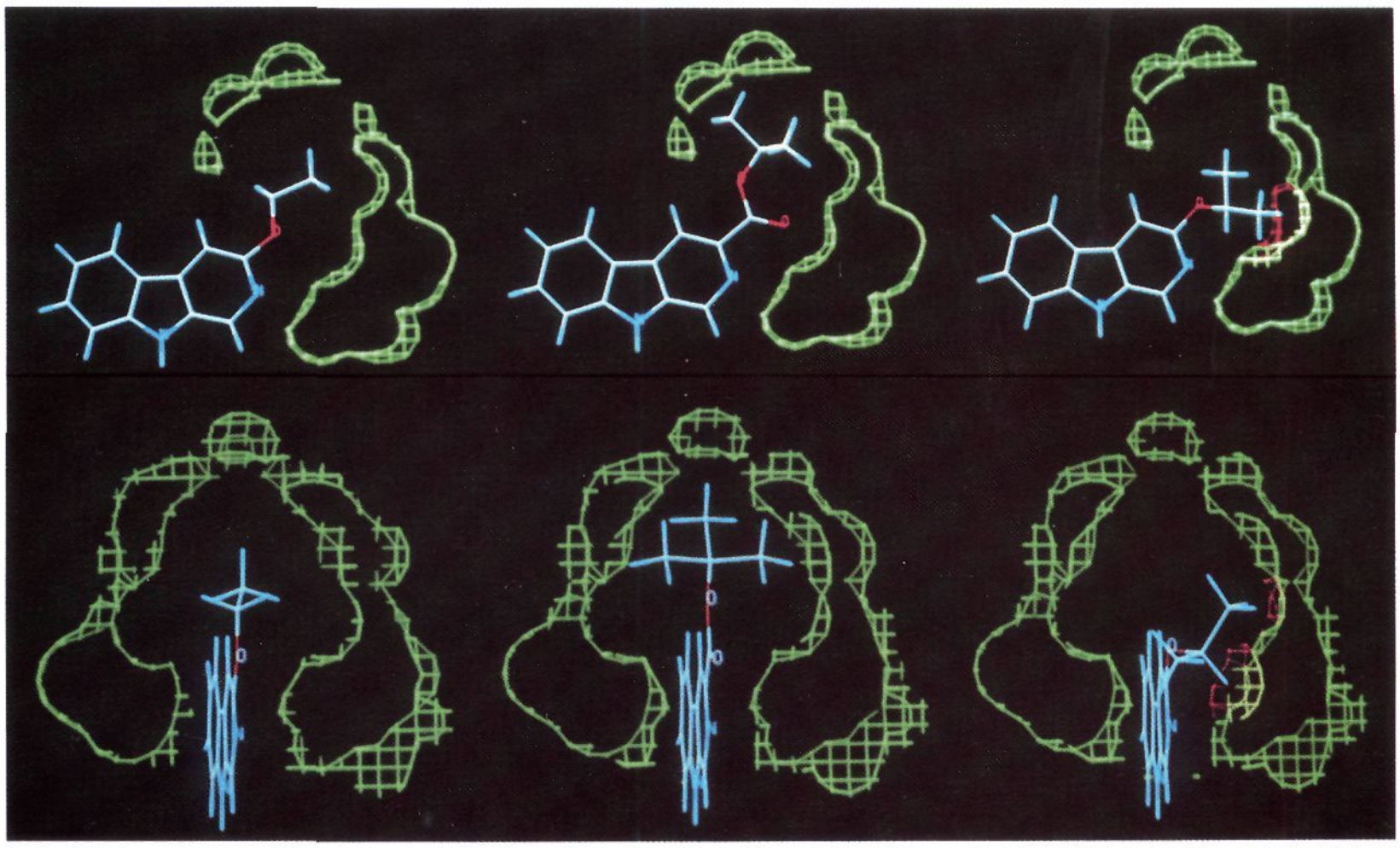


Figure 1. Orthogonal views of 3-ethoxy-β-carboline (**5**; left, IC_{50} = 24 nM), BCCT (**3**; middle, IC_{50} = 10 nM), and 3-isopropoxy-β-carboline (**6**; right, IC_{50} = 500 nM) in the proposed receptor (green) of the benzodiazepine receptor inverse agonist site. Receptor volume is Z-clipped for the sake of clarity. This figure represents a map of the lipophilic pocket in the region of position 3 of the β-carboline ligands depicted above.

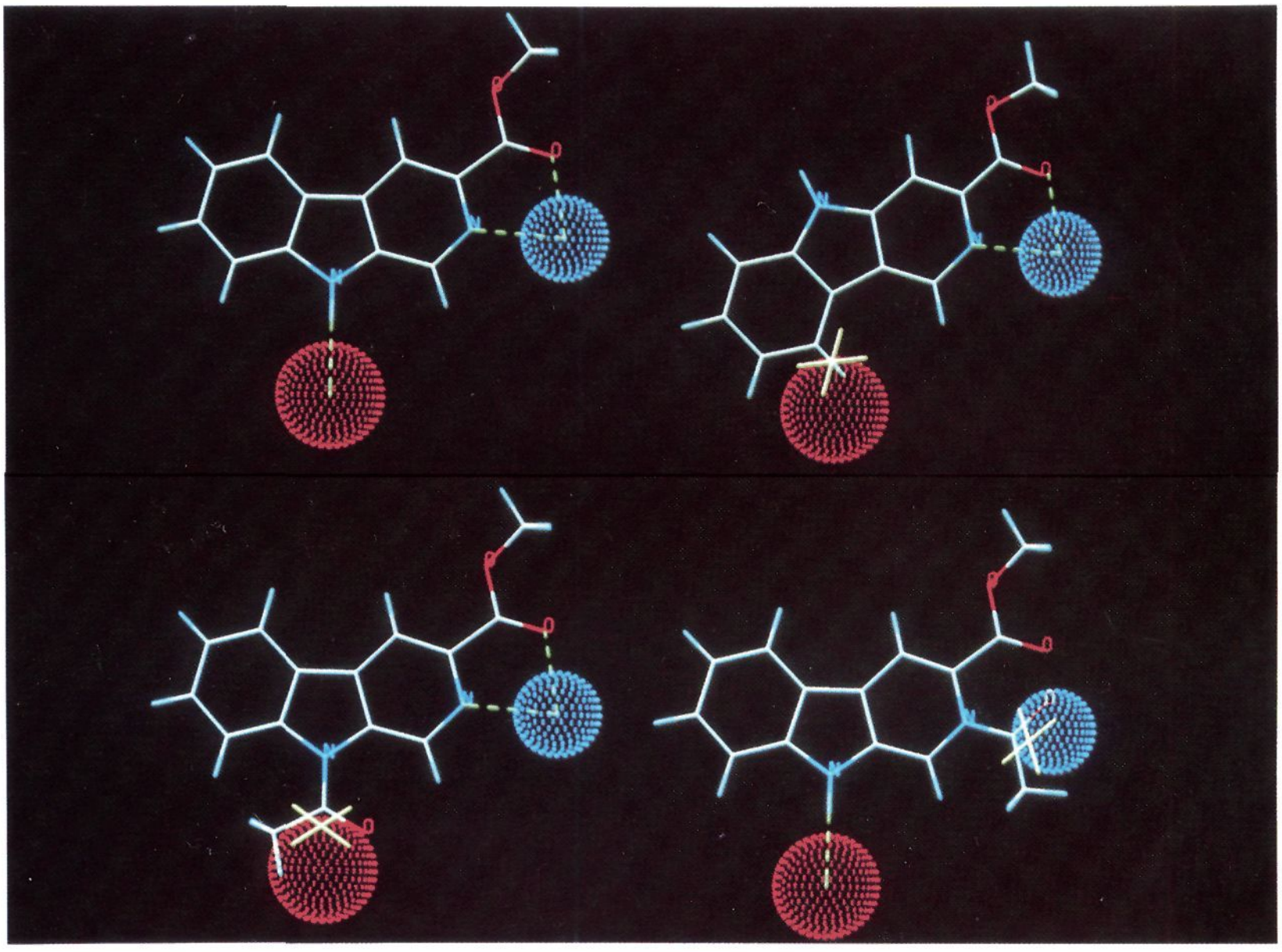


Figure 2. Interactions of selected β-carboline and γ-carboline ligands with the proposed hydrogen bond donor H_1 (blue sphere) and acceptor A_2 (red sphere) sites on the BzR: BCCM (**1**; top left, IC_{50} = 5 nM), 2-(methoxycarbonyl)-γ-carboline (**13**; top right, IC_{50} = 653 nM), N_a-acetyl-BCCM (**39**; bottom left) and N_b-acetyl-3-(methoxycarbonyl)-1,2-dihydro-β-carboline (**40**; bottom right).

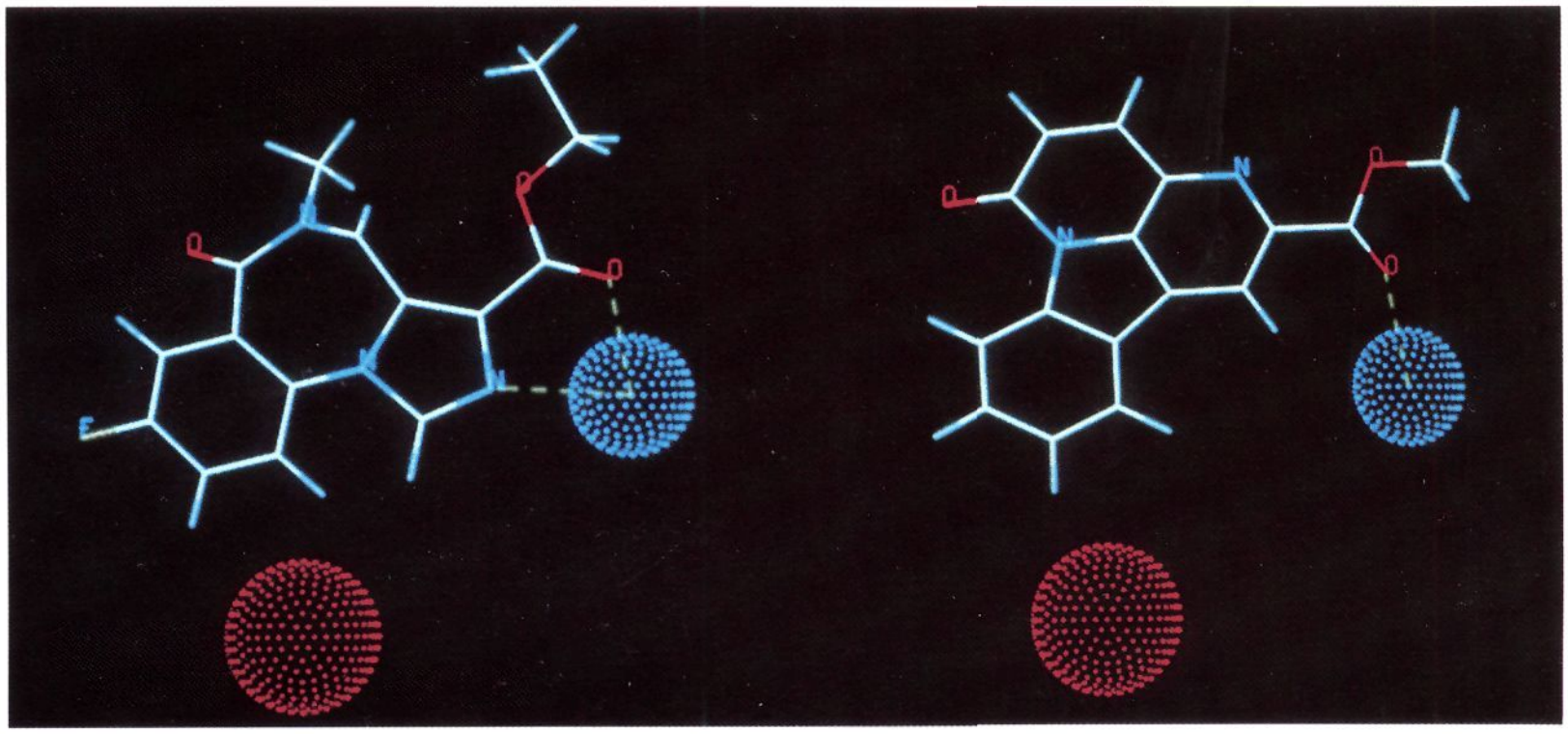


Figure 3. Interactions of the antagonist ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (left) and 2-(methoxycarbonyl)canthin-6-one (**32**; right, IC_{50} = 100 nM) with the proposed hydrogen bond donor H_1 (blue sphere) and acceptor A_2 (red sphere) sites on the BzR.

an inverse agonist profile.[13,20,47] Consistent with this hypothesis, neither ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate nor canthin-6-one **32** exhibit potent inverse agonist activity. An increase in the interaction of the above 1,4-benzodiazepine antagonist with the hydrogen bond donor site H_1 via formation of a three-centered hydrogen bond could account for the increase in binding affinity of this antagonist over **32** (Figure 3).

Since the discovery of the rigid, planar high affinity BzR ligand 7,12-dihydropyridodiindole **3**,[55] several different functional groups were substituted on rings A and E as well as positions R_1, R_2, R_3, R_4, and R_{10} of this molecule.[10,56–59] The effects of substitution on ring E on in vitro affinity indicate that the pyridine nitrogen atom at N(5) and indole N(7)–H are important sites for ligand–benzodiazepine receptor site interactions. In order to more precisely define the extent of the hydrophobic pocket, the four methyl derivatives **18–21** were synthesized and examined for their affinities in vitro (Table II). 2-Methyl derivative **19** (IC_{50} = 8 nM) displays the highest affinity.[58] An interesting comparison can then be made between **19** and the highly active 3-(ethoxycarbonyl)-β-carboline (**2**) and 3-*n*-propoxy-β-carboline (**9**). If the A and B rings of the pyridodiindoles and β-carbolines are superimposed and if the 3-substituents of **5** and **9** are placed in their extended all-anti conformations, the terminal methyl groups of the latter two compounds are in close proximity to the methyl substituent of **19**. The intermolecular distance between the terminal methyl group carbon atoms of the overlayed structures of **2** and **19** is 1.70 Å and the corresponding distance between **5** and **19** is 0.79 Å. This observation suggests that the torsional angles of the 3-substituents of **2** and **9** are all anti when bound to BzR.

In contrast, 1-methyl (**18**; IC_{50} = 83 nM) and 3-methyl (**20**; IC_{50} = 224 nM) pyridodiindoles are substantially less potent than the 2-methylpyridodiindole analogue. This observation is consistent with the 1 order of magnitude decrease in affinity observed when the side chain of 3-*n*-propoxy-β-carboline (**9**) is lengthened by one carbon atom to produce 3-*n*-butoxy-β-carboline (**7**). If the A and B rings of 3-*n*-butoxy derivative **7** are superimposed on the corresponding rings of 2-methyl- and 3-methylpyridodiindole and if the side chain is again placed in the all-anti conformation, the terminal methyl group of **7** is then situated between the 2- and 3-methyl groups of **19** and **20**, respectively. These observations, when taken together, suggest that the presence of a 3-methyl substituent on the pyridodiindole nucleus or β-carbolines which carry substituents at position 3 with a chain length of greater than four interfere with binding in a hydrophobic pocket in the receptor.

4-Methyl congener **21** (IC_{50} = 6860 nM) displays the lowest affinity of the four methyl derivatives **18–21**. The substituent at the 4-position should be able to affect the indole N(7)–H hydrogen atom, and the pyridine N(5) nitrogen atom in a similar manner as substituents at the 2 position. A similar affinity between the 2- and 4-substituted derivatives would be predicted solely on electronic considerations. Since this is not the case, it is probable that steric constraints are present. The 4-methyl substituent of **21** presumably interferes with the proposed hydrogen-bond interaction between the pyridine nitrogen atom N(5) and the hydrogen bond donor site (H_1) of the protein, resulting in a dramatic decrease in affinity (Figure 4). Since alkyl groups release only a small amount of electron density into the π-system of the heterocycle, the data above suggest that the effect of these substituents in this region of the interacting ligand with the receptor site is primarily steric in nature rather than electronic.

If steric interactions strongly influence ligand affinity as described for the methyl congeners **18–21**, investigation by a receptor essential volume analysis[26,27] should result in a map of the shape of the receptor in the vicinity of the

(55) Trudell, M. L.; Craig, R.; Skolnick, P.; Cook, J. M. Simple Synthesis of 3,4-Indolosubstituted β-Carbolines. A New Class of Benzodiazepine Antagonists. Presented at the 19th Great Lakes Regional Meeting of the American Chemical Society, Purdue University, West Lafayette, IN, June 10–12, 1985; Abstract 239.

(56) Trudell, M. L.; Tan, Y. C.; Lifer, S.; Cook, J. M., manuscript in preparation.

(57) Trudell, M. L.; Fukada, N.; Cook, J. M. *J. Org. Chem.* **1987**, *52*, 4293.

(58) Trudell, M. L.; Tan, Y. C.; Cook, J. M. *J. Org. Chem.* **1988**, *53*, 4873.

(59) Lifer, S. L. M.S. Thesis, University of Wisconsin—Milwaukee, 1987.

bound ligand. The union of volumes of the active ligands was subtracted from the union of the volumes of inactive ligands to yield forbidden regions that are likely occupied by the receptor (see Methods section for details). This essential receptor volume is displayed in Figure 1. The receptor pocket also appears to have a hole in the vicinity of the 2-methyl substituent of pyridodiindole **19**. Full occupancy of the receptor pocket (see Figure 1) leads to high-affinity antagonists (e.g. β-carboline **31**) while partial occupancy of the region leads to high-affinity inverse agonists. Ligands which occupy this pocket with "flat" substituents [e.g. BCCM (**1**)] or rings (e.g. pyridodiindole **3**) tend to exhibit inverse agonist properties.[60] If the volume of a ligand occupies common space with the receptor, as is the case with β-carboline **48**, affinity is greatly diminished (see Figure 1).

Since the N(9)–H functionality of the β-carboline ligands is required for high affinity at BzR, it was of interest to determine the role of the indole N–H functionalities at N(7) and N(12) in the pyridodiindole series. It was proposed that methylation of the indole nitrogen atom of **3** at N(7) would inhibit interaction with the hydrogen bond accepting site A_2 on the receptor and result in a substantial decrease in binding affinity. Recently a regioselective synthesis of monomethyl derivatives **23** and **24** was achieved that has permitted the discrimination of the nearly identical indole nitrogen atoms at N(7) and N(12) of **3**.[58] The synthesis of 7,12-dimethyl analogue **25** was also of interest as a positive control in regard to in vitro binding assays. According to the model, if one of the two necessary hydrogen bonds between the ligand and receptor is unable to form, then the apparent affinity of the ligand should dramatically decrease and inverse agonist properties would not be expected. The in vitro potencies of the N-methylated pyridodiindoles are listed in Table II, and the proposed ligand–receptor interactions are depicted in Figure 4. The designation X as in Figure 4 is included to illustrate the absence of a hydrogen-bonding interaction postulated as necessary for high-affinity binding to BzR.[13,47,58] Replacement of the indole N(7)-hydrogen of **3** (IC_{50} = 4 nM) with methyl (**23**) results in a compound that is more than 2 orders of magnitude less potent (IC_{50} = 1163 nM) than **3**. This finding is consistent with the difference in affinity between BCCM (**1**; IC_{50} = 5 nM) and 9-methyl-BCCM (**33**; IC_{50} > 50 000 nM).[14,61,62] Moreover, replacement of the indole N(9)–H moiety of BCCE (**2**) with an oxygen atom (**37**; IC_{50} = 9200 nM) or a methylene group (**38**; IC_{50} = 680 nM) resulted in ligands with dramatically reduced affinities for BzR as reported by Huth et al.[63] These data strongly suggest the involvement of the indole N(7)–H with a hydrogen bond accepting site (A_2) on the receptor. Further support for this involvement can be found in the reduced affinity of 6-ethyl derivative **28** (IC_{50} = 285 nM), which is probably a result of unfavorable steric interactions between the ethyl group of **28** and the receptor sites A_2 and H_1. However, the affinity of **28** does not decrease to the same extent as that of the N(7) methylated analogue **23**, where the hydrogen-bonding capability of the N(7)–H is completely eliminated. According to the model of the pharmacophore, methylation of the indole N–H function at N(12) should have only a minimal effect on the apparent affinity at BzR,[13,47,63] since no hydrogen-bond interaction between the indole N(12)–H and the receptor site is predicted. The N(12) methyl functional group of **24** should neither lie in the lipophilic pocket nor interfere with the affinity of the ligand to BzR. Although the binding affinity of **24** (IC_{50} = 157 nM) is reduced in comparison to that of pyridodiindole **3** (IC_{50} = 4 nM), it is much more potent than the corresponding N(7)-methyl regioisomer **23** (IC_{50} = 1163 nM). This supports the hypothesis that the N(12)–H of the diindoles does not play a major role in the interaction with the benzodiazepine receptor. The apparent affinity of 7,12-dimethylpyridodiindole **25** (IC_{50} = 1917 nM), as expected, is far lower than that of **24** and is consistent with the value obtained for 7-methyl congener **23**.

The ability of the pyridine nitrogen atom contained in ring C of β-carbolines [N(2)] and pyridodiindoles [N(5)] to exhibit hydrogen bond acceptor characteristics is of equal importance to high affinity binding to the BzR. To test this hypothesis in the pyridodiindole series, the N-(5)-methyl derivative **34** was synthesized. The IC_{50} of this methiodide salt increased to 4660 nM in comparison to parent diindole **3** (IC_{50} = 4 nM), which could be attributed to either the loss of a free lone pair of electrons on N(5) or to the ionic nature of the ligand. Either a hydrogen bond cannot form between N(5) and H_1 on the receptor or the ionic nature of the ligand affects the approach of the ligand at the binding site and results in an incorrect orientation of the ligand at the receptor site. The data from Figure 4, in combination with the binding affinity of the ligands in Table II, indicate that the indole N(7)H and the pyridine nitrogen atom N(5) of the pyridodiindole ligands are required for high affinity to the BzR. To further examine this possibility, indolocarbazole **27** was prepared. This base lacks the pyridine nitrogen function at N(5) and has a markedly reduced affinity (IC_{50} = 1970 nM), presumably due to its inability to interact with the hydrogen bond donating site (H_1) on the BzR inverse agonist site (Figure 4). This observation is in agreement with the work from our laboratories[54,62] and of Loew et al.[14,61]

Additional support for the present model of the pharmacophore of the inverse agonist binding site has been demonstrated by the recent synthesis and reported binding affinities of ligands **35–38** by Huth et al.[63] These ligands are devoid of the indole N–H group and carry substituents that are incapable of interacting with the hydrogen bond acceptor site (A_2) on the binding site. The oxime derivative **36** could interact at A_2 via the hydroxyl hydrogen atom, but steric constraints limit this interaction. Moreover, ligands which lack substituents other than N–H (ligands

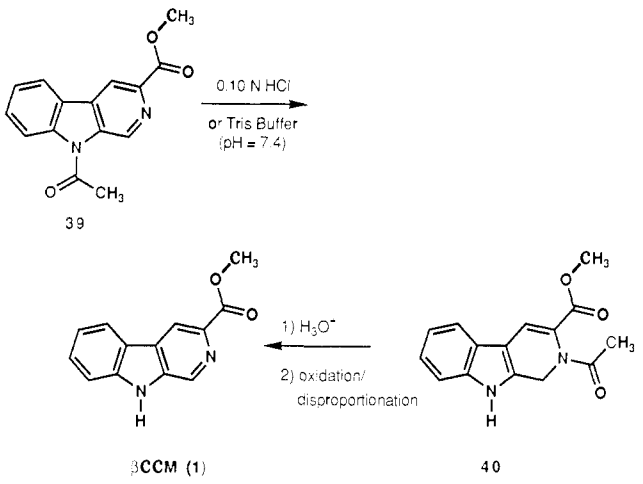
(60) An alternate explanation for the different activity displayed by the inverse agonists and antagonists was offered by one of the reviewers who suggested that the *s*-cis and *s*-trans conformations about the N(5)–C–C=O torsional angle are nearly isoenergetic. It was further proposed that the inverse agonist pocket could accommodate the smaller *s*-cis β-carboline esters while a larger antagonist site is able to accommodate the *s*-trans conformation of bulkier β-carboline esters. The reviewer's and our hypothesis may be equivalent however if the larger receptor pocket is above the plane containing the aromatic rings. Longer alkoxy chains from either rotamer could then easily be oriented so as to occupy a common volume. It is unlikely that the larger antagonist binding pocket is in the plane containing the aromatic rings since this would place the pocket in close proximity to the 3- and 4-methyl groups of the relatively inactive pyridodiindoles **19** and **20**. This proximity in turn would not be consistent with the existence of high-affinity antagonists.

(61) Loew, G.; Nienow, J.; Lawson, J.; Toll, L.; Uyeno, E. T. *Mol. Pharmacol.* **1985**, *28*, 17.

(62) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081.

(63) Huth, A.; Schmiechen, R.; Motoc, I.; Beetz, I.; Breitkopf, A.; Frost, E.; Schumann, I.; Thielert, K. *Arch. Pharm. (Weinheim)* **1988**, *321*, 297.

Scheme VII



35–38) exhibit low affinities. This evidence supports the hypothesis that the indole N(9)–H function is important for high affinity binding to BzR.

Recently, several optically active *N*-(indol-3-ylglyoxylyl) amino acid derivatives were synthesized by Primofiore et al. and examined for their potencies to displace [^{3}H]flunitrazepam.[64] Results indicate that derivatives with electron-withdrawing substituents (Cl, Br, NO_2) at the indole 5-position exhibited the highest affinities, whereas hydrogen or methoxy substitution decreased the inhibition of [^{3}H]flunitrazepam binding. It is believed that substitution of electron-withdrawing substituents para to the indole N(9)H function enhances the ability of this group to interact with a hydrogen bond acceptor site (A_2) on the receptor via polarization of the indole N–H bond. This work supports involvement of the indole N(9)–H function with a hydrogen bond acceptor site (A_2) on the receptor.

In contrast to these and other findings, 9-acetyl-3-(methoxycarbonyl)-β-carboline (**39**) (Scheme VII) was reported to exhibit an affinity for BzR equal to that of the parent BCCM (1).[65] This finding apparently contradicts the hypothesis that an indole N(9)–H of a β-carboline is necessary for potent inverse agonist activity. However, it has been reported that 1-acyl indole derivatives are especially prone to hydrolysis in aqueous acidic and alkaline media.[66] As a result, the stability and the reported affinity of 9-acetyl-BCCM (**39**) are suspect. In order to determine if **39** had been hydrolyzed prior to the in vitro and in vivo experiments, the stability of **39** was determined. Solutions of 9-acetyl-BCCM (**39**) were prepared according to the original procedures[67] and monitored by TLC and UV spectroscopy (see the Experimental Section for details). Preparation of a 0.10 N HCl solution of **39** for in vitro assay resulted in complete hydrolysis at 25 °C within 4 h to provide BCCM (1) (Scheme VII). It was also determined that samples of 9-acetyl-BCCM (**39**), prepared in TRIS buffer (pH 7.4) were hydrolyzed to the parent BCCM (1). Hydrolysis was nearly 50% complete after 2 h at 25 °C (Scheme VII). These results suggest that the acetyl group of **39** can be hydrolyzed under in vitro and perhaps in vivo assay conditions. In fact, *N*-acetyl derivatives have been used as prodrugs in order to improve solubility and bioavailability of the intrinsically more potent parent drugs.[68–72] In addition, these acylated prodrugs can exhibit enhanced membrane-transport properties resulting from an increase in lipophilic character.[69,71,72] Use of the acetyl group has been popular in in vivo studies due to its relative ease of chemical and enzymatic hydrolysis. Collectively, this information strongly suggests that in vivo the effects of BCCM (1) were measured and not those of the 9-acylated congener **39**.[73]

In order to better define the model of the pharmacophore, the SAR has been subjected to a 3D QSAR analysis.

Table IV. Statistics for CoMFA Analysis of IC_{50} Data for 37 Benzodiazepine Receptor Ligands

number of cross validation groups	r^2	*F* value	standard error estimate	probability $r^2 = 0$
37	0.59	7.21	1.018	0.000
0	0.83	39.51	0.623	0.000

Table V. Relative Contributions of log *P*, Molar Refractivity, and Steric and Electrostatic Potentials to the CoMFA Regression Equation[a] for Binding Affinity (IC_{50}) to BzR

regressor	normalized coefficient	relative contribution
CLOGP[b]	0.000	0.000
CLOGP2[b]	0.002	0.001
CMR[c]	0.000	0.000
steric[d]	2.263	0.837
electrostatic[e]	0.439	0.162
total	N/A[g]	0.999[f]

[a] $pIC_{50} = 6.024 - (0.000)(CLOGP) - (0.009)(CLOGP)^2 - (0.000)(CMR) + \sum_{xyz}(\text{steric coefficient})_{xyz}(\text{steric potential})_{xyz} + \sum_{xyz}(\text{electrostatic coefficient})_{xyz}(\text{electrostatic potential})_{xyz}$ [b] CLOGP: MedChem version 3.54 estimated log *P*. [c] CMR: MedChem version 3.54 estimated molar refractivity. [d] Combined contribution of 7 × 11 × 11 = 1008 steric potentials. [e] Combined contribution of 1008 electrostatic potentials. [f] Does not add up to 1.000 due to rounding errors. [g] N/A = not applicable.

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(65) Mele, L.; Massotti, M.; Gatta, F. *Pharmacol. Biochem. Behav.* **1988**, *30*, 5. It was reported that 2-acetyl-3-(methoxycarbonyl)-1,2-dihydro-β-carboline (**40**, IC_{50} = 9.1–12.9 nM) and 3-(methoxycarbonyl)-β-carboline (BCCM, 1, IC_{50} = 6.4–15.8 nM) exhibited similar binding affinity to BzR. On the basis of the stability studies performed on the 9-acetyl derivative **39** in these laboratories, we feel that the in vitro binding data of the 2-acetyl-1,2-dihydro derivative **40** does not truly represent the nature of this species in solution. The 1,2-dihydro-β-carbolines are extremely unstable and would be expected to quickly oxidize to the fully aromatic β-carboline, afterwhich hydrolysis of the acetyl function would be very rapid. Moreover, in the case of **40**, loss of acetaldehyde across the 1-2 carbon–nitrogen bond would also facilitate the formation of the fully aromatic β-carboline. The binding affinity of these acetylated β-carboline derivatives **39** and **40** is in direct conflict with the present pharmacophore model (see Figure 5) and in both cases it is believed that observable effects are due to the formation of BCCM (1) and not from the original acetylated derivatives **39** and **40**.

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(73) An alternative explanation for the high affinity of **39** was advanced by one of the reviewers who suggested that this *N*-acyl derivative may acylate the receptor releasing free BCCM (1) and that acylated BzR has as high or higher affinity for BCCM than does nonacylated BzR. More importantly, the hydrolysis of **39** may be accelerated by the attack of a nucleophile (H_2O) in the highly lipophilic environment of the BzR binding cleft.

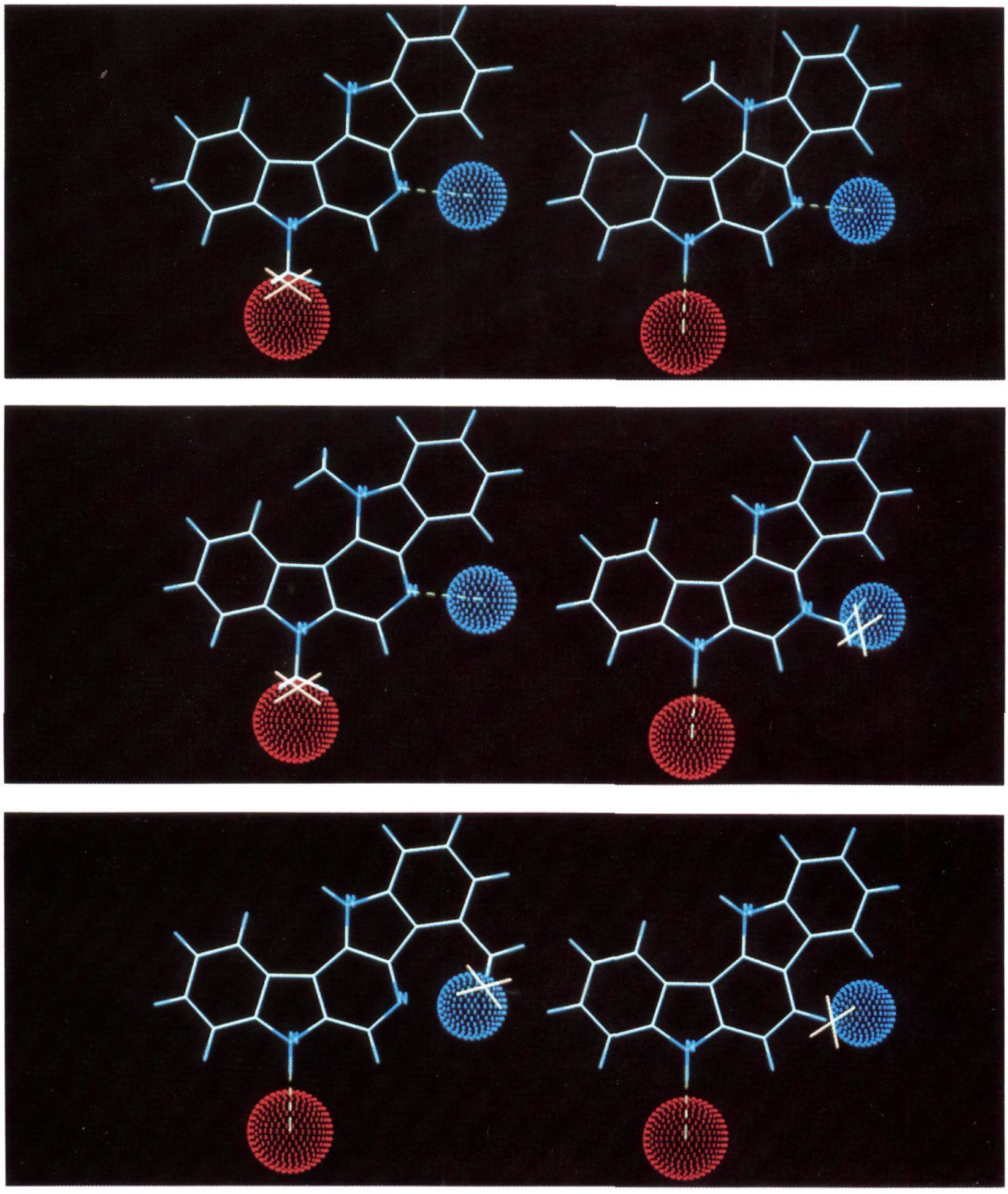

Figure 4. Interactions of selected pyridodiindole ligands with the proposed hydrogen bond donor H_1 (blue sphere) and acceptor A_2 (red sphere) sites on the BzR: **23** (top left, IC_{50} = 1163 nM), **24** (top right, IC_{50} = 157 nM), **25** (middle left, IC_{50} = 1917 nM), **34** (middle right, IC_{50} = 4660 nM), **21** (bottom left, IC_{50} = 6860 nM), **27** (bottom right, IC_{50} = 1970 nM).

Initial regression equations from the 3D QSAR analysis were derived by using cross validation.[28] The number of optimal components was found to be four. The analysis was repeated without cross validation using four components. The statistics are summarized in Table IV and the fitted predictions for each of the compounds in the study are included in the supplementary material. An overlay of BCCM (1), diindole **3**, and pyrazoloquinoline **41** is depicted in Figure 5, while the CoMFA coefficient contour maps of steric and electrostatic potentials are displayed in Figures 6 and 7, respectively. The steric maps show a very strong favorable interaction in the vicinity of the E ring of the diindole series, the phenyl ring of the CGS series, and the alkyl groups of the β-carboline series. This favorable region is flanked by unfavorable regions that correlate with the decrease in affinity observed for β-carbolines possessing bulky ester substituents in the three position and is qualitatively similar to the excluded-volume map (Figure 1). There is also an unfavorable region below the β-carboline indole nitrogen atom which correlates with a marked decrease in activity observed with the N_a-methyl indole analogues **23**, **25**, and **33**. Even though the electrostatic contribution to the overall regression is smaller than the steric contribution (16% and 84%, respectively; see Table V), the electrostatic potential maps are readily interpretable. As expected, the electrostatic map indicates

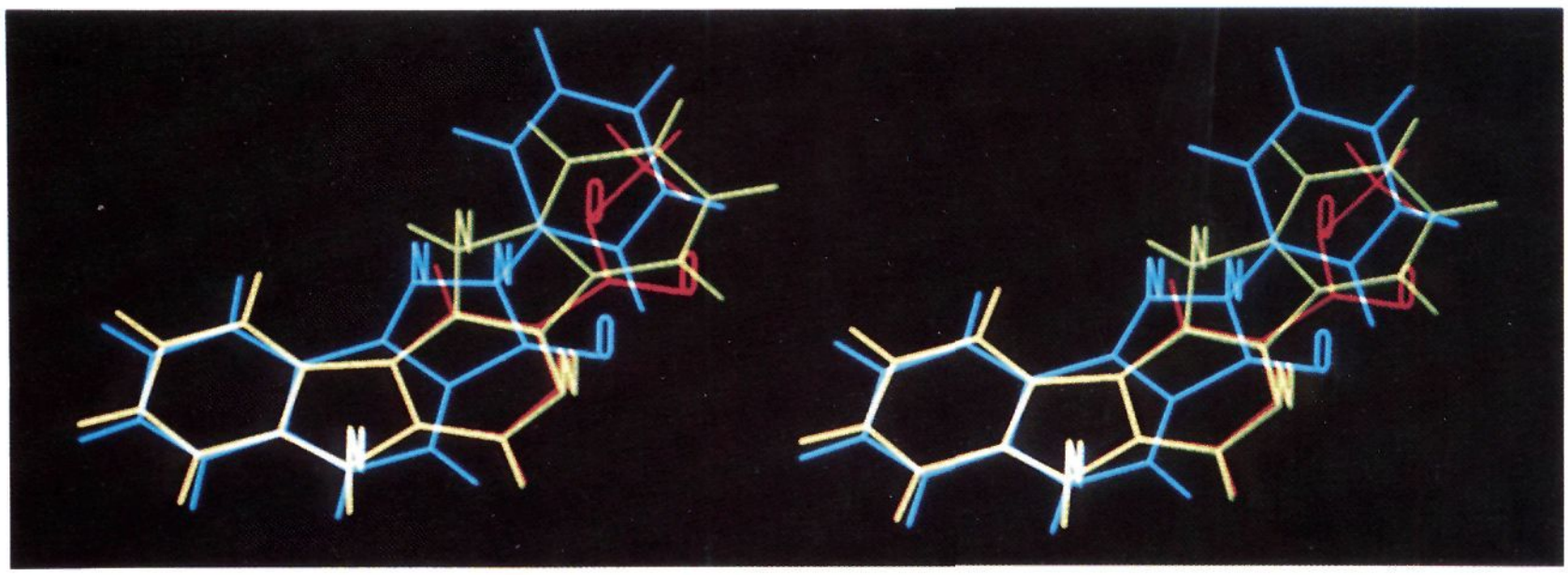

Figure 5. Stereo view of the alignment of BCCM (**1**) (red), pyridodiindole **3** (green), and 3*H*-2-phenyl-2,5-dihydropyrazolo[4,3-*c*]-quinolin-3-one (**41**) used in the CoMFA analysis.

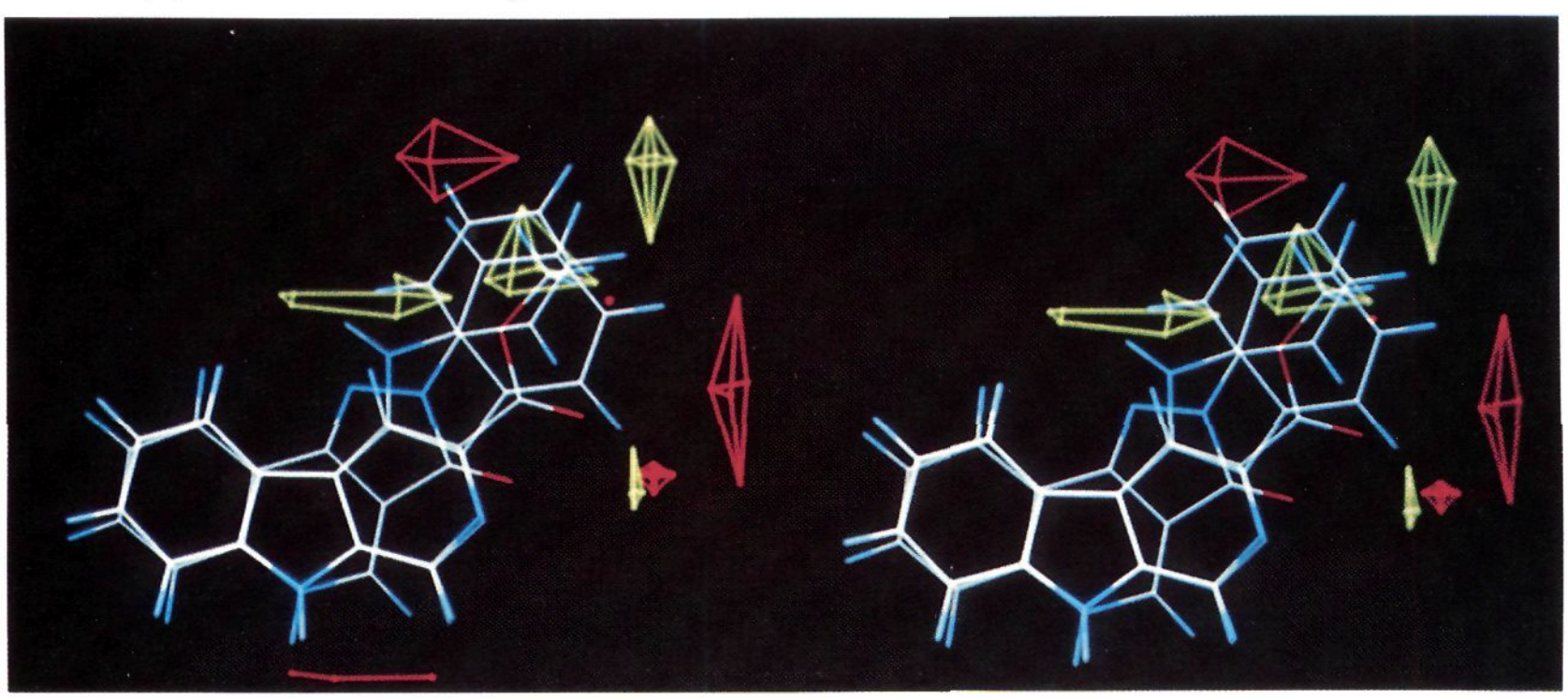

Figure 6. Stereo view of steric CoMFA map. Green contours surround regions where a higher steric interaction would increase binding (the QSAR coefficients times the standard deviation of the corresponding columns are greater than 0.08). Red contours surround regions where a lower steric interaction would increase binding (less than −0.10). Molecules displayed are an overlay of BCCM (**1**), pyridodiindole **3**, and 3*H*-2-phenyl-2,5-dihydropyrazolo[4,3-*c*]quinolin-3-one (**41**).

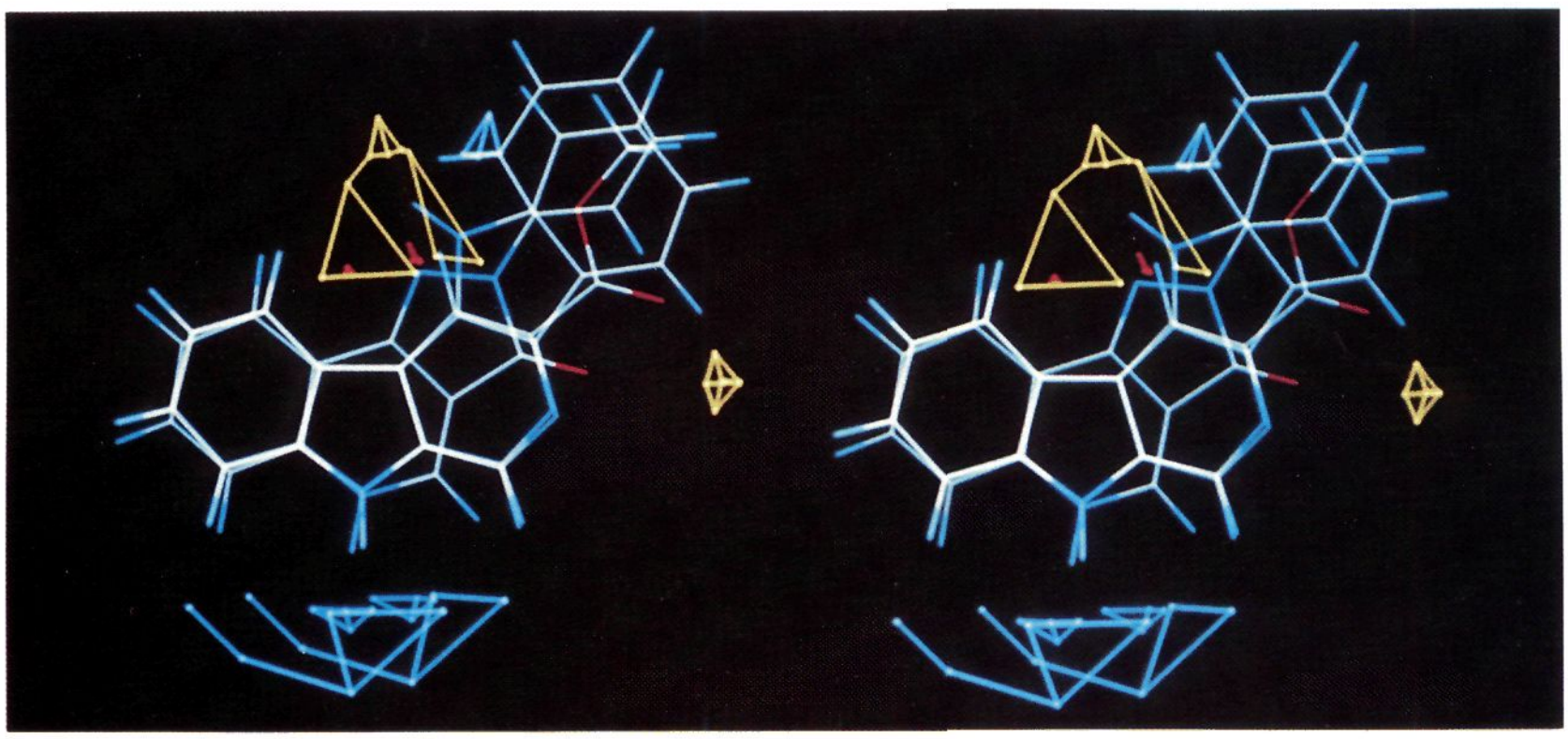

Figure 7. Stereo views of electrostatic CoMFA map. Red and yellow contours surround regions where a more negative electrostatic interaction would increase binding (the QSAR coefficients times the standard deviation of the corresponding column are less than −0.05 and 0.01 respectively). Cyan and blue contours surround regions where a more positive electrostatic interaction would increase binding (greater than 0.02 and 0.05, respectively). Molecules displayed are an overlay of BCCM (**1**), pyridodiindole **3**, and 3*H*-2-phenyl-2,5-dihydropyrazolo[4,3-*c*]quinolin-3-one (**41**).

that affinity is enhanced by increasing negative electrostatic potential about the ligand in the vicinity of the hydrogen bond acceptor site A_2. Likewise, increasing positive electrostatic potential about the hydrogen bond donor site H_1 enhances affinity. An unexpected prediction obtained from the electrostatic map is the enhancement of affinity by increasing the negative electrostatic potential about the imine C-ring nitrogen of the CGS series. This result suggests the possible existence of an additional hydrogen bond donating site H_3 in the binding cleft and would account for the exceptionally high affinity displayed by CGS derivative **41** (IC_{50} = 0.4 nm), but is not required for inverse agonist activity.[74]

In summary, the present study correlates well with previous work on the development of a pharmacophore for the inverse agonist site at BzR.[13,20,47] The lipophilic requirement of substituents at the 3-position of a β-carboline has been demonstrated with the potent activity of 3-*n*-propoxy derivative **9** (IC_{50} = 11 nM), 3-substituted *n*-propyl ketone **11** (IC_{50} = 2.8 nM), and 3-*n*-butyl derivative **12** (IC_{50} = 245 nM). The fact that an ester or carbonyl group at position 3 is not required for BzR affinity can be demonstrated by comparison of the activity of **9** with that of **11**. The absence of the oxygen atom of **9**, however, did prove detrimental to BzR affinity by decreasing the interaction at H_1 [compare **9** (IC_{50} = 11 nM) to **12** (IC_{50} = 245 nM)]. All three ligands (**9**, **11**, **12**) have lipophilic substituents at the 3-position with a chain length of four; however, only ketone **11** is capable of forming a three-centered hydrogen bond with the receptor site H_1. It also appears that the ether oxygen atoms in the 3-alkoxy-β-carboline series **5–9** are responsible for facilitating the interaction of the pyridine N(2) nitrogen atom with the receptor site H_1 through resonance release of electron density.

As mentioned earlier in the Discussion section, the excluded-volume analysis strongly suggests a hydrophobic pocket in the vicinity of position 3 of β-carbolines. The ability of a ligand to interact favorably with this hydrophobic pocket is, however, not the only criteria for high-affinity binding to this region. From the moderate binding affinity of the 3-*n*-butyl derivative **12** (IC_{50} = 245 nM), it is apparent that an oxygen heteroatom or a carbonyl group adjacent to the β-carboline ring can enhance affinity by increasing the interaction at H_1 through resonance or the formation of a three-centered hydrogen bond, respectively.[13,20,47] The relatively low affinities of 2-(methoxycarbonyl)-γ-carboline (**13**) and the 9-substituted β-carbolines **35–38** confirm the importance of interactions between the indole N(9)–H and A_2 on the binding site.

From the examination of the pyridodiindole ligands **3**, **18–21**, **23–25**, **27**, **28**, and **34**, it is evident that the indole N(7)–H and the pyridine N(5) nitrogen atoms are required for high-affinity binding at BzR. Addition of certain substituents to the parent pyridodiindole[56–58] profoundly affects affinity and/or efficacy. For example, addition of a methyl group to position 4 (**21**) and to a lesser extent positions 3 and 1 (**20** and **18**) diminishes binding affinity. In contrast, addition of substituents to position 2 (e.g. **19**) has little effect on potency. The addition of a methyl substituent at C-4 may interfere with the hydrogen-bond interaction between N(5) and the hydrogen bond donor site H_1 (see Figure 4), while the addition of substituents at position 1 and especially position 3 is hypothesized to interact unfavorably with the hydrophobic receptor pocket (see Figure 1). As shown above, the importance of the pyridine N(5) nitrogen atom is demonstrated by the lack of affinity of indolocarbazole **27** (IC_{50} = 1970 nM) and the N(5)-methyl derivative **34** (IC_{50} = 4660 nM). Methylation of the indole N(7)–H of **3** resulted in the formation of the 7-methyldiindole **23** (IC_{50} = 1163 nM). This ligand **23** exhibited less affinity for BzR than the 12-methyldiindole **24** (IC_{50} = 157 nM), suggesting that the BzR preferentially recognizes the indole N(7)–H rather than the indole N-(12)–H.

The pyridodiindole ligand is a special case when considering optimum substituent length for inverse agonist or antagonist activity. Because of its planar topography, this ligand is able to penetrate the receptor cleft and interact favorably at the hydrogen bonding acceptor (A_2) and donor (H_1) sites. Substituents on the E ring at the 2-position lie primarily in a lipophilic pocket of the receptor; a pocket which can accommodate various groups without affecting in vitro binding affinity.[10,57,58] It appears that electronic characteristics of the substituents at the 2-position of the diindoles correlate with biological activity. For example, pyridodiindole **3**, which is unsubstituted at the 2-position, the 2-methoxy analogues, and 2-hydroxy derivatives[75] are inverse agonists. In contrast, the 2-chloro derivative [10] and the smaller 2-fluoro derivative[76] are both antagonists. It has also recently been reported that variation of the size of the alkyl substituent on a series of thienylpyrazoloquinolines results in a shift from inverse agonist to antagonist properties.[77] A somewhat similar trend has been reported in the ZK series.[78–80] Collectively, the N(2) nitrogen and the indole N(9)–H of β- carbolines and the N(5) nitrogen and the N(7)–H of the diindoles are necessarily important structural features in determining whether these ligands will demonstrate a high affinity to BzR and indicates that these ligands bind to the same region of the receptor. Lastly, the data generated from this 3D QSAR CoMFA analysis correlate well with results derived from the use of the template approach. This method provides a reasonable correlation with a cross validated r^2 value of 0.59. The steric and electrostatic contour maps (Figures 6 and 7) are entirely consistent with the SAR of these compounds and provide guidelines for designing more potent ligands. Work is in progress to expand the 3D QSAR analysis to diverse BzR ligands and

(74) The existence of H_3 is not consistent with the relatively high affinities displayed by the parent pyridodiindole **3** (IC_{50} = 4 nM) and it's 12-methyl analogue **24** (IC_{50} = 157 nM). However, the hydrogen bond donating site H_3 could be converted into an accepting site A_3 through a torsional rotation. For example if H_3 were a serine side chain hydroxyl hydrogen atom, rotation about the C–C–O–H torsional angle of 120° would replace the hydrogen with a lone pair of electrons. This rotation would also minimize unfavorable steric interactions with **24**. Since inverse agonists such as **1–3** do not interact with H_3, it is possible that this hydrogen bonding site is necessary for agonist activity. (See Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1990**, *33*, 1062).

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to develop a model which would distinguish between inverse agonists and agonists.

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 homogenizer (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 was 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 nM; 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 using at least six concentrations of inhibitor.

Stability Studies. A standing solution of 9-acetyl-BCCM (**39**; 2.73 mM, 18.3 mg in 25.00 mL of 0.10 N HCl) at 25 °C was monitored periodically by TLC (SiO_2, ethyl acetate/ethanol 85:15). After 15 min, the presence of BCCM (**1**; R_f = 0.55) could be detected which resulted from the hydrolysis of 9-acetyl derivative **39** (R_f = 0.76). After 4 h, 9-acetyl-BCCM (**39**) was completely hydrolyzed (no longer observable on TLC with use of a hand-held UV lamp) to BCCM (**1**).

A solution of 9-acetyl-BCCM (**39**; 0.74 mM) was prepared by dissolving 9-acetyl-BCCM (**39**; 1.0 mg, 3.7×10^{-6} mol) in 2 drops of ethanol and diluted with TRIS buffer (5.00 mL, 50 mM, pH = 7.4). The solution was monitored by UV spectroscopy (Beckman DU-8 spectrometer). The decrease of a maximum absorption at 325 nm was observed. After 2 h at 25 °C 9-acetyl derivative **39** was 50% hydrolyzed to BCCM (**1**).

Biological Evaluation of 3-Propoxy-β-carboline (9). The proconvulsant actions of 3-propoxy-β-carboline were assessed based on similar tests performed on 3-ethoxy-β-carboline (**5**).[12] Male NIH mice (Veterinary Resources Branch, Bethesda, MD, 25–30 g) were injected (ip) with 0.1 mL of 3-propoxy-β-carboline hydrochloride (**9**; 40 mg/kg). Fifteen minutes later, the mice received pentylenetetrazole (PTZ, 36 mg/kg, 0.1 mL). PTZ (0.1 mL) was dissolved in water; **9** was suspended in 20% diluted Emulphor/80% saline [diluted Emulphor is Emulphor (GAF Corp., Wayne, NJ) diluted 1:1 (w/w) with ethanol]. The animals were observed for 15 min after injection of **9** for the presence of clonic/tonic convulsions. The blocking action of 3-propoxy-β-carboline **9** was tested to determine if **9** could antagonize the anticonvulsant effects of diazepam as previously described.[62] Mice were injected with diazepam (2.5 mg/kg ip) and 15 min later injected (ip) with 3-propoxy-β-carboline (40 mg/kg). Fifteen minutes later, the animals were challenged with pentylenetetrazole (PTZ, 80 mg/kg).

Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal Model IA8100 digital melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker 250-MHz NMR spectrometer or on a GE 500-MHz instrument. Infrared spectra were recorded with a Beckman Acculab-1 or a Mattson Polaris IR-10400 spectrometer. Mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5855 GC-mass spectrometer, while high-resolution mass spectral data were obtained from a Finnigan HR mass spectrometer. Microanalyses were performed on an F and M Scientific Corp. Model 185 carbon, hydrogen, and nitrogen analyzer. Analytical TLC plates employed were E. Merck Brinkmann UV active silica gel (Kieselgel 60 F254) on plastic. The preparation of ligands 18–21 and 28 are described in ref 75.

2-Carboxy-4-(1-benzo[1,2,3]triazolyl)pyridine (16). *o*-Phenylenediamine (14, 1.08 g, 10 mmol) and 4-chloropicolinic acid (**15**, 1.57 g, 10 mmol) were mixed and heated initially at 100 °C under aspirator pressure. The temperature was gradually increased to 150 °C and maintained at this temperature for 4 h. The melt was allowed to cool to room temperture and dissolved in 10% hydrochloric acid (25 mL), filtered, and further cooled to 0 °C. Sodium nitrite (1.24 g) in water (12 mL) was gradually introduced into the solution. The precipitate obtained was filtered after 2 h and dried to give 1.46 g (55%) of the desired 2-carboxy-4-(1-benzo[1,2,3]triazolyl)pyridine (**16**): mp 249–250 °C; ^{1}H NMR (Me_2SO-d_6) δ 7.50–7.60 (t, 1 H, J = 5 Hz), 7.70–7.80 (t, 1 H, J = 5 Hz), 8.10–8.30 (m, 3 H), 8.55 (s, 1 H), 8.95 (d, 1 H, J = 2 Hz); IR (KBr), 3600–2400, 1630, 1350 cm^{-1}; MS (EI, 15 eV) m/e 240 (M^+). Anal. ($C_{12}H_8N_4O_2$·HCl) C, H, N.

γ-Carboline-3-carboxylic Acid (17). This compound was prepared according to the literature procedure.[33] Acid **16** (1.20 g, 5 mmol) was suspended in polyphosphoric acid (30 mL) and heated to 150–160 °C. Heating was continued until nitrogen evolution ceased. The reaction mixture was then cooled to room temperature, poured onto ice, and neutralized with 10% aqueous ammonia (pH 5). The precipitate obtained was filtered and dried to give the title compound **17** (285 mg, 25%): mp 258–260 °C (lit.[33] mp 258–259 °C); ^{1}H NMR (Me_2SO-d_6) δ 7.30–7.40 (t, 1 H, J = 5.0 Hz), 7.50–7.60 (t, 1 H, J = 5.0 Hz), 7.70 (d, 1 H, J = 5.0 Hz), 8.20 (s, 1 H), 8.40 (d, 1 H, J = 5.0 Hz), 9.45 (s, 1 H); IR (KBr) 3100, 1660 cm^{-1}; MS (EI, 15 eV) m/e 212 (M^+).

3-(Methoxycarbonyl)-γ-carboline (13). γ-Carboline-3-carboxylic acid (**17**; 212 mg, 1 mmol) was dissolved in hot methanol and then cooled to 0 °C. Excess diazomethane in ether was added dropwise and the solution was allowed to warm to room temperature. Stirring was continued for a few hours, after which time the solution was filtered and concentrated in vacuo. The resulting residue was chromatographed on silica gel using 5% ethanol/ethyl acetate as eluent to provide **13** (50 mg): mp 258–260 °C (hydrochloride salt); ^{1}H NMR (Me_2SO-d_6) δ 4.00 (s, 3 H), 7.50 (t, 1 H, J = 6 Hz), 7.7 (t, 1 H, J = 6 Hz), 7.85 (d, 1 H, J = 6 Hz), 8.50 (s, 1 H), 8.60 (d, 1 H, J = 6 Hz), 9.80 (s, 1 H), 11.50 (s, indole 1 H); IR (KBr) 1680 cm^{-1}; MS (EI, 15 eV) 226 (M^+); High resolution MS, m/e 226.0734 ($C_{13}H_{10}N_2O_2$ requires 226.0742).

3-(1,1-Dimethylethoxy)-9*H*-pyrido[3,4-*b*]indole Hydrochloride (6). To a stirred solution of 3-amino-β-carboline diacetate[81] (500 mg, 1.65 mmol) in anhydrous 2-propanol (250 mL) at −20 °C was added isoamyl nitrite (4 mL, 30 mmol). The solution was allowed to stir for 2 min, followed by the addition of potassium thiocyanate (5.52 g, 0.057 mol) and copper(I) thiocyanate (3.45 g, 0.028 mol) in 150 mL of anhydrous 2-propanol at −20 °C. After 4 h, the reaction mixture was warmed to room temperature. The reaction mixture was filtered and the solvent was removed under reduced pressure. The resulting solid residue was taken up in 100 mL of 0.5 N sodium bicarbonate solution and extracted with ethyl acetate (3 × 100 mL). The combined organic portions were then dried over sodium sulfate, and the volume was then reduced to 10 mL. The compound was then purified by flash chromatography (SiO_2) with ethyl acetate as the eluent. Upon the addition of a cold saturated methanol–hydrogen chloride solution, a precipitate formed which was filtered and washed with cold ether (3 × 10 mL) to provide pure **6**[82] as the hydrochloride salt (217 mg, 50%): mp 168–172 °C; IR (KBr) 1655, 1610, 1420, 1245, 1020 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.39 (s, 6 H) 5.16 (s, 1 H), 7.21 (m, 1 H), 7.62 (m, 2 H), 8.12 (s, 1 H), 8.35 (d, 1 H, J = 8.0 Hz), 8.64 (s, 1 H), 11.93 (s, indole 1 H); MS (CI, CH_4) m/e 227 (M + 1); high-resolution MS m/e 226.1109 ($C_{14}H_{14}N_2O$ requires 226.1106). Anal. ($C_{14}H_{14}N_2O$·HCl) C, H, N.

3-*n*-Butoxy-9*H*-pyrido[3,4-*b*]indole Hydrochloride (7). 3-Amino-β-carboline,[81] anhydrous 1-butanol, isoamyl nitrite, potassium thiocyanate, and copper(I) thiocyanate were reacted under the analogous conditions employed for the preparation of 6 above to provide **7**. Chromatographic separation provided **7**

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in 46% yield (251 mg): mp 178–181 °C; IR (KBr) 1655, 1610, 1420, 1245, 1020 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.97 (t, 3 H, J = 7.0 Hz), 1.50 (m, 2 H), 1.81 (m, 2 H), 4.40 (t, 2 H, J = 6 Hz), 7.29 (m, 1 H), 7.64 (m, 2 H), 8.16 (s, 1 H), 8.36 (d, 1 H, J = 7.7 Hz), 8.67 (s, 1 H), 12.03 (s, indole 1 H); MS (CI, CH_4) 241 (M + 1); high-resolution MS m/e 240.1269 ($C_{15}H_{16}N_2O$ requires 240.1262). Anal. ($C_{15}H_{16}N_2O$·HCl) C, H, N.

1-(9*H*-Pyrido[3,4-*b*]indol-3-yl)-1-butanone (11). To a stirred solution of 3-(ethoxycarbonyl)-β-carboline (**2**; 1.0 g, 4.17 mmol) in anhydrous tetrahydrofuran (250 mL) under nitrogen at −70 °C was added via syringe neat chlorotrimethylsilane (2.64 mL, 33.7 mmol). n-Propyllithium (14.6 mL, 1.1 M, 3.8 equiv) was then slowly syringed into the stirring reaction mixture. After the reaction mixture was allowed to stir for 3 h, it was warmed to room temperature. The solution was then quenched with the addition of ethanol (2 mL) followed by water (2 mL) and 4 N HCl (4 mL). The solvent was then removed in vacuo and the resulting residue was brought to pH 8 with a 1 N sodium bicarbonate solution. The aqueous solution was extracted with ethyl acetate (3 × 100 mL), followed by washing of the combined organic portions with a saturated aqueous sodium chloride solution and dried over sodium sulfate. The solvent was then evaporated to dryness and the resulting solid was taken up in ethyl acetate (15 mL), at which time a precipitate formed that was filtered and dried to yield **11** (415 mg, 42%): mp 209–211 °C; IR (KBr) 3275, 1670, 1590, 1380, 1340, 1250, 1180, 1020, 745 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.96 (t, 3 H, J = 7.5 Hz), 1.70 (m, 2 H), 3.22 (t, 2 H, J = 7.25 Hz), 7.28 (m, 1 H), 7.60 (m, 2 H), 8.39 (d, 1 H, J = 8.0 Hz), 8.81 (s, 1 H), 8.96 (s, 1 H), 12.07 (s, indole 1 H); MS (CI, CH_4), 239 (M + 1); high-resolution MS m/e 238.1114 ($C_{15}H_{14}N_2O$ requires 238.1106). Anal. ($C_{15}H_{14}N_2O$) C, H, N.

3-*n*-Butyl-9*H*-pyrido[3,4-*b*]indole Hydrochloride (12). To a stirred solution of hydrazine hydrate (2.2 mL), sodium hydroxide (300 mg), and diethylene glycol (12 mL) was added propyl ketone **11** (200 mg, 0.84 mmol). The resulting reaction mixture was then brought to a reflux temperature of 190 °C for 20 h before being cooled to room temperature. The hydrazine hydrate and the diethylene glycol were then removed via Kugelrohr distillation. The residue was then taken up in water (10 mL) and adjusted to pH 8.5 with 1 N HCl, followed by extraction with chloroform (4 × 50 mL). The combined organic portions were washed with water and dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting residue was taken up in ethyl acetate. This compound was then purified by flash chromatography (SiO_2) with ethyl acetate as the eluent. Upon the addition of a cold saturated solution of methanol–hydrogen chloride, a precipitate formed which was collected and dried to provide pure **12** as the hydrochloride salt (62 mg, 33%): mp 202–204 °C; IR (KBr) 1645, 1615, 1505, 1455, 1340, 1255, 755 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.93 (t, 3 H, J = 7.5 Hz), 1.36 (m, 2 H), 2.48 (m, 2 H), 3.11 (t, 2 H, J = 7.75 Hz), 7.40 (m, 1 H), 7.76 (m, 2 H), 8.44 (d, 1 H, J = 8.0 Hz), 8.66 (s, 1 H), 9.10 (s, 1 H), 10.25 (s, indole 1 H), MS (CI, CH_4) m/e 225 (M + 1); high-resolution MS m/e 224.1321 ($C_{15}H_{16}N_2$ requires 224.1313). Anal. ($C_{15}H_{16}N_2$·HCl). C, H, N.

9-Acetyl-3-(methoxycarbonyl)-β-carboline (39).[67] β-Carboline-3-carboxylate methyl ester (**1**; 0.226 g, 1.00 mmol) was dissolved in acetic anhydride (5 mL). The resulting solution was stirred at 60 °C for 12 h, after which time the acetic anhydride was removed under reduced pressure. The solid residue was then taken up in ethyl acetate and washed with aqueous sodium bicarbonate. The organic extract was dried over Na_2SO_4 and evaporated to provide **39** (0.190 g, 71%): mp 191 °C (lit.[67] mp 201–203 °C); ^1H NMR ($CDCl_3$) δ 2.95 (s, 3 H), 4.10 (s, 3 H), 7.50 (m, 1 H), 7.65 (m, 1 H), 8.10 (d, 1 H, J = 8.0 Hz), 8.20 (d, 1 H, J = 8.0 Hz), 8.75 (s, 1 H), 9.70 (s, 1 H); MS (EI, 15 eV) m/e 268 (M^+).

7-Methyl-7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole Hydrochloride (23). N_a-Methyl-2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (**26**,[58] 100 mg, 0.33 mmol) was dissolved in phenylhydrazine (5 mL) and stirred at 160 °C for 7 h. The mixture was then cooled to 120 °C and anhydrous hydrazine (4 mL) was added and the mixture heated to reflux for 16 h. The mixture was cooled to room temperature and the precipitate which resulted was filtered and washed with ethanol (1 × 1 mL) and ethyl ether (2 × 5 mL). The yellow solid which resulted was dissolved in a solution of methanol–hydrogen chloride to afford hydrochloride salt **23** (68.3 mg, 77%): mp >300 °C; IR (KBr) 3300, 1500, 1310, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.20 (s, 3 H), 7.43 (t, 1 H, J = 7.2 Hz) 7.55 (t, 1 H, J = 7.02 Hz) 7.65 (t, 1 H, J = 7.23 Hz), 7.85 (t, 1 H, J = 8.13 Hz), 7.93 (d, 1 H, J = 8.4 Hz), 7.96 (d, 1 H, J = 8.4 Hz), 8.55 (d, 1 H, J = 7.9 Hz), 8.97 (d, 1 H, J = 7.9 Hz), 9.45 (s, 1 H), 13.10 (s, indole 1 H); MS (CI, CH_4) m/e 272 (M + 1). Anal. ($C_{18}H_{13}N_3$·HCl) C, H, N.

7,12-Dihydro-12-methylpyrido[3,2-*b*:5,4-*b*']diindole (24) and 7,12-Dihydro-7,12-dimethylpyrido[3,2-*b*:5,4-*b*']diindole (25). A 250-mL three-neck flask equipped with a mechanical stirrer and dry ice condenser was cooled in a dry ice/acetone bath and filled with liquid ammonia. Metallic sodium (70 mg, 3 mmol) and $Fe(NO_3)_2$·$9H_2O$ (0.4 g) were added with stirring. After 1 h, dihydropyridodiindole **3** (257 mg, 1 mmol) was added, followed by dropwise addition of methyl iodide (454.2 mg, 3.2 mmol). Stirring was continued for 6 h, followed by removal of the cooling bath, and the ammonia was allowed to evaporate in a hood overnight. Warm water was added to the residue and the precipitate which resulted was filtered and recrystallized from methanol/acetonitrile to give 12-methylpyridodiindole **24** (76 mg, 32%) and 7,12-dimethylpyridodiindole **25** (91 mg, 36%) in pure form. 12-Methylpyridodiindole **24**: mp >300 °C; IR (KBr) 3300, 1500, 1300, 700 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.50 (s, 3 H), 7.43 (t, 1 H, J = 7.25 Hz), 7.49 (t, 1 H, J = 7.0 Hz), 7.69 (t, 1 H, J = 7.25 Hz), 7.75 (t, 1 H, J = 8.0 Hz), 7.86 (d, 1 H, J = 8.25 Hz), 7.96 (d, 1 H, J = 8.25 Hz), 8.62 (d, 1 H, J = 8.0 Hz), 8.83 (d, 1 H, J = 8.25 Hz), 9.14 (s, 1 H), 12.96 (s, indole 1 H); MS (CI, CH_4) m/e 272 (M + 1). Anal. ($C_{18}H_{13}N_3$·HCl·$^1/_4H_2O$) C, H, N. 7,12-Dimethylpyridodiindole **25**: mp >300 °C; IR (KBr) 3300, 1450, 1300, 750 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.25 (s, 3 H) 4.66 (s, 3 H), 7.47 (t, 1 H, J = 7.5 Hz), 7.52 (t, 1 H, J = 7.5 Hz), 7.72 (t, 1 H, J = 7.5 Hz), 7.88 (t, 1 H, J = 7.5 Hz), 8.00 (d, 1 H, J = 8.25 Hz), 8.05 (d, 1 H, J = 8.25 Hz), 8.57 (d, 1 H, J = 7.5 Hz), 8.89 (d, 1 H, J = 7.5 Hz), 9.43 (s, 1 H); MS (EI, 15 eV) m/e 285 (M^+). Anal. ($C_{19}H_{15}N_3$·HCl) C, H, N.

7,12-Dihydropyrido[3,2-*b*:5,4-*b*']diindole 5-Methiodide Salt (34). 7,12-Dihydropyridodiindole **3** (92.2 mg, 0.36 mmol) was dissolved in dry tetrahydrofuran (20 mL), to which toluene (30 mL) was added, and the resulting solution was heated to reflux for 5 min. Methyl iodide (169 mg, 1.2 mmol) was added and the reaction mixture was heated to reflux for 2 h. The mixture was cooled to room temperature. The resulting yellow precipitate was filtered, washed with ethanol (1 × 10 mL) and tetrahydrofuran (2 × 15 mL), and dried to yield the desired methiodide salt **34** (100.8 mg, 70.5%): mp >300 °C; IR (KBr) 3200, 1620, 1400, 720 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.00 (s, 3 H), 7.50 (t, 1 H, J = 7.5 Hz), 7.55 (t, 1 H, J = 7.5 Hz), 7.85 (m, 4 H), 8.50 (d, 1 H, J = 7.5 Hz), 8.95 (d, 1 H, J = 7.5 Hz), 9.33 (s, 1 H), 13.10 (s, 2 H); MS (CI, CH_4) m/e 286 (M + 1 + 15), 258 (M + 1 − 15). Anal. ($C_{18}H_{14}N_3I$·$^1/_4H_2O$) C, H, N.

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Supplementary Material Available: Predicted IC_{50} values for BzR receptor binding, estimated log P and molar refractivity values for all structures used in the 3D QSAR analysis as well as coordinates, connection tables, and fractional charges for these compounds (17 pages). Ordering information is given on any current masthead page.