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Theoretical Structure-Activity Studies of β-Carboline Analogs

Requirements for Benzodiazepine Receptor Affinity and Antagonist Activity

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SUMMARY

The techniques of theoretical chemistry have been used to elucidate the molecular properties and modes of receptor binding that modulate receptor affinity and antagonist activity of the β-carbolines, a class of potent benzodiazepine antagonists. Six analogs were chosen in order to investigate the role of the amine (NH) group, the aromatic nitrogen, and the C_3-substituent in determining receptor affinities. Electrostatic potential mapping and characterization of explicit drug-receptor interactions have led to the hypothesis that simultaneous interaction of a model cationic arginine site with the N_2 and C_3-substituents could play a key role in determining receptor affinities. The electron-withdrawing effects of C_3-substituents on the amine nitrogen appear less important, though interactions of these groups with an anionic glutamate or aspartate site could also occur at the receptor. Similarly, stacking interactions with neutral or cationic aromatic residues such as tryptophan or protonated histidine could occur, but do not appear to be determinants of the relative receptor affinity of the β-carbolines.

INTRODUCTION

Ethyl β-carboline-3-carboxylate was discovered (1) in the search for an endogenous ligand that binds with high affinity at the benzodiazepine receptor. Although an apparent artifact of the extraction procedure, β-CCE[1] turned out nevertheless to be the first high affinity, potent benzodiazepine antagonist discovered, antagonizing anxiolytic as well as anticonvulsant, muscle relaxant, and sedative-hypnotic activities of these compounds (2–6).

Since the discovery of this first analog (β-CCE), many such compounds have been synthesized (2–4, 7–15). Most of these focus on modifications of the C_3-ester group (11–14), though very recently, substituents at other positions have also been explored (11, 15).

Together with the more anxioselective analogs, triazolo(1,4*a*)pyridazines, the β-carbolines have been used to probe benzodiazepine receptor heterogeneity. The presence of two independent binding sites (BZ_1 and BZ_2), to which both BZ and β-carbolines bind with high affinity, has been postulated (16–20). Our recent computer-assisted analysis (21) of inhibition of [^{3}H]flunitrazepam and [^{3}H]β-CCE by these three classes of analogs supports this hypothesis. These results also indicate that the higher affinity site for BZ is the lower affinity site for the β-carbolines and the triazolo(1,4*a*)pyridazines. Moreover, other studies report that γ-aminobutyric acid potentiates BZ agonist binding while β-carboline antagonist binding is either unaffected or somewhat diminished (2, 9, 21–24). Furthermore, photoaffinity labeling with flunitrazepam profoundly inhibits agonist binding while only slightly diminishing antagonist binding (25). Thus, although the β-carboline antagonists and BZ agonists are competitive inhibitors, it is possible that they bind to overlapping but somewhat different domains of the receptor. It is of interest, then, to describe key features of agonist and antagonist binding.

In previous theoretical studies, we have investigated the requirements for high affinity and agonist activity in benzodiazepine analogs (26). In these complementary studies, we attempt to elucidate, on a molecular level, the requirements for high benzodiazepine receptor affinity and antagonist activity for the β-carboline class of antagonists. We have not addressed the observed ability of the ester group to modulate subtle differences in intrinsic activity of the β-carbolines (27): i.e., the methyl ester is a convulsant, the ethyl ester is a proconvulsant, the *n*-propyl ester has no intrinsic activity. Specifically, in these studies, we have focused on three themes: the role of the amine (N_9-H) group, the role of the aromatic nitrogen (N_2), and the role of the C_3-substituent in modulating receptor affinity. For this purpose, we have included in this study the six analogs given in Table 1.

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[1] Abbreviations used are: β-CCE, ethyl β-carboline-3-carboxylate; BZ, benzodiazepine; MNDO, modified neglect of differential overlap; MEP, molecular electrostatic potential; INDO, intermediate neglect of differential overlap.

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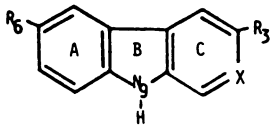
TABLE 1

β-Carboline analogs investigated



Compound	R_3	X	R_6	IC_{50}[a]	AD_{50}[b]
				nM	*μmol/kg*
β-Carboline *1a*	H	N	H	2,500	
β-Carboline *1b*	$CONH_2$	N	H	1,500[c]	189
β-Carboline *1c*	$COOCH_3$	N	H	4.2 (3.5)[d]	(14.5)[d]
β-Carboline *1d*	CN	N	H	3.5	28
β-Carboline *1e*	H	N	NO_2	1,300	
Carbazole *2a*	$COOCH_3$	CH	H	(>10,000)	

[a] IC_{50} values are from competition experiments using [^{3}H]flunitrazepam. Data are from our laboratory (28).

[b] Antagonism of anticonvulsant activity of diazepam (28).

[c] Two IC_{50} values for this compound have been previously reported. In one study, (13), a value of ~617 nM, and in the other (40) a value of 68 nM, has been reported. The two previous authors give no reason for disparities in their data, and we agree with one value (within a factor of 2, which is all the reproducibility of binding studies that can be expected from laboratory to laboratory) and not with the other. In addition, an IC_{50} (nM) *vs.* [^{3}H]β-CCE of 1600 nM obtained by us (28) is consistent with this result as is its lack of antagonist potency.

[d] Values in parentheses are for β-CCE, $R_3 = COOC_2H_5$.

As indicated in Table 1, for these analogs, in previous studies (21) we have determined their competitive inhibition of [^{3}H]flunitrazepam receptor binding to provide a self-consistent measure of receptor affinities. In addition, we have also determined the ability of three of these analogs to antagonize the *in vivo* anticonvulsant activity of diazepam (28). These activities are consistent with their relative receptor affinities. The amide analog (*1b*) had little or no antagonist potency, while the ethyl ester and the cyano derivative (*1d*), both relatively high affinity analogs, had comparable antagonist activities.

While animal testing and receptor affinity exist for many more β-carboline analogs (11, 13, 14), the six chosen illustrate the effect of a variety of modifications of the β-carbolines. Systematic studies of these β-carboline analogs should be helpful in identifying molecular requirements for high affinity and antagonist activity and provide a rationale for the behavior of other analogs not included in this study.

To this end, in the work reported here, the techniques of theoretical chemistry have been used to calculate: 1) conformational properties of C_3-substituents; 2) molecular indicators of the proton-donating ability of the amine nitrogen proton (N_9-H) and proton-accepting ability of the aromatic imine nitrogen (N_2); 3) molecular electrostatic potential contour maps in the vicinity of the ring nitrogens (N_2, N_9) and the C_3-substituents; 4) explicit interactions of β-carbolines with model receptor subsites. These include model drug-receptor complexes involving (a) stacking interactions with a neutral indole ring of a tryptophan residue and a protonated imidazole ring of a histidine residue, (b) interactions of the amine N_9-H group with a model anionic residue such as glutamate or aspartate, and (c) interactions of model protonated lysine and arginine residues with the aromatic ring nitrogen and with the C_3-substituents.

METHODS

Conformational Studies

Initial geometries were constructed for all six analogs studied using a known X-ray structure of carbazole (29) and standard geometries (30) for all substituents. These initial geometries were used as input to a semiempirical all valence electron molecular orbital method called MNDO (31). This method has been parameterized to give reliable geometries for a large number of organic molecules. Totally unconstrained geometries optimization was performed for all analogs except *1e*, the 6-NO_2 compound, where the nitro group was restrained to be planar, as is required to correctly obtain the lowest energy conformation for this substituent. In addition to these minimum energy conformers, a more detailed energy conformation study was made to determine other possible low energy forms of the amide and methyl ester β-carbolines and the methyl ester carbazole analogs *1b*, *1c*, and *2a*, each with C_3-substituents with rotational degrees of freedom. Thus, total geometry optimizations were performed using MNDO for a number of starting conformers involving planar and nonplanar ester and amide groups.

For the C_3-methyl ester of β-carboline analog *1c* (Fig. 1), five geometries involving rotations of the ester plane, with respect to the β-carboline, were investigated: the *cis-* and *trans*-planar geometries ($\tau_1(N_2C_3C_{13}O_{13}) = 0°$, 180°) and three nonplanar geometries ($\tau_1(N_2C_3C_{13}O_{13}) = 45°$, 90°, and 135°.

For the C_3-methyl ester of the carbazole analog *2a* (Fig. 2), three rotational conformers of the ester group were investigated: *cis-* and *trans*-planar geometries ($\tau_1(N_2C_3C_{13}O_{13}) = 0°$, 180°) and one nonplanar geometry, with the ester group perpendicular to the β-carboline ($\tau(N_2C_3C_{13}O_{13}) = 90°$).

For *1b*, the 3-amido-β-carboline (Fig. 3), two types of *cis-* and *trans*-planar conformers were considered, with the lone pair on the amide nitrogen perpendicular to and in the plane of the molecule ($\tau_2(O_{13}C_{13}N_{13}HN_1) = 151.4°, 61.4°$).

Electronic structures in terms of electron densities and bond overlap densities were obtained using MNDO-optimized structures.

Calculation of the Energies of the Cationic, Anionic, and Tautomeric Forms of the β-Carbolines

The role of N_2 and N_9 as hydrogen acceptor and donor, respectively, was investigated by calculating: (a) the ease of protonation of the imine N_2, (b) the ease of proton loss from N_9, and (c) the ease of transfer of the proton from N_9 to N_2. To this end, heats of formation for the anionic species (loss of the hydrogen at N_9), cationic species (protonation at N_2), and the tautomeric form (transfer of the hydrogen from N_9 to N_2) were calculated and compared with the heats of formation of the parent analogs. Initial geometries of the cationic and tautomeric forms, created from those of the MNDO-optimized parents, were used to obtain optimized geometries for these species using MNDO (see Table 2).

Calculation and Plotting of a Molecule's Electrostatic Potential Contours

The molecular electrostatic potential generated by each atom and electron at specific points in selected regions around five β-carbolines (*1a–1d*, *2a*) were calculated, focusing primarily on regions around the aromatic ring nitrogen and the C_3-substituent. The value of the electrostatic potential, plotted as equipotential contours in a given plane, is the energy of interaction of the entire molecule with a point positive charge placed anywhere on that contour. Such MEP contour maps serve as a guide to select specific positions of anionic and cationic receptor sites for optimum drug-receptor electrostatic interactions.

The MEP program used (32) was formulated for maximum efficiency when used with the orthogonalized molecular orbital functions from a semiempirical molecular orbital program called INDO (31, 33). Thus,

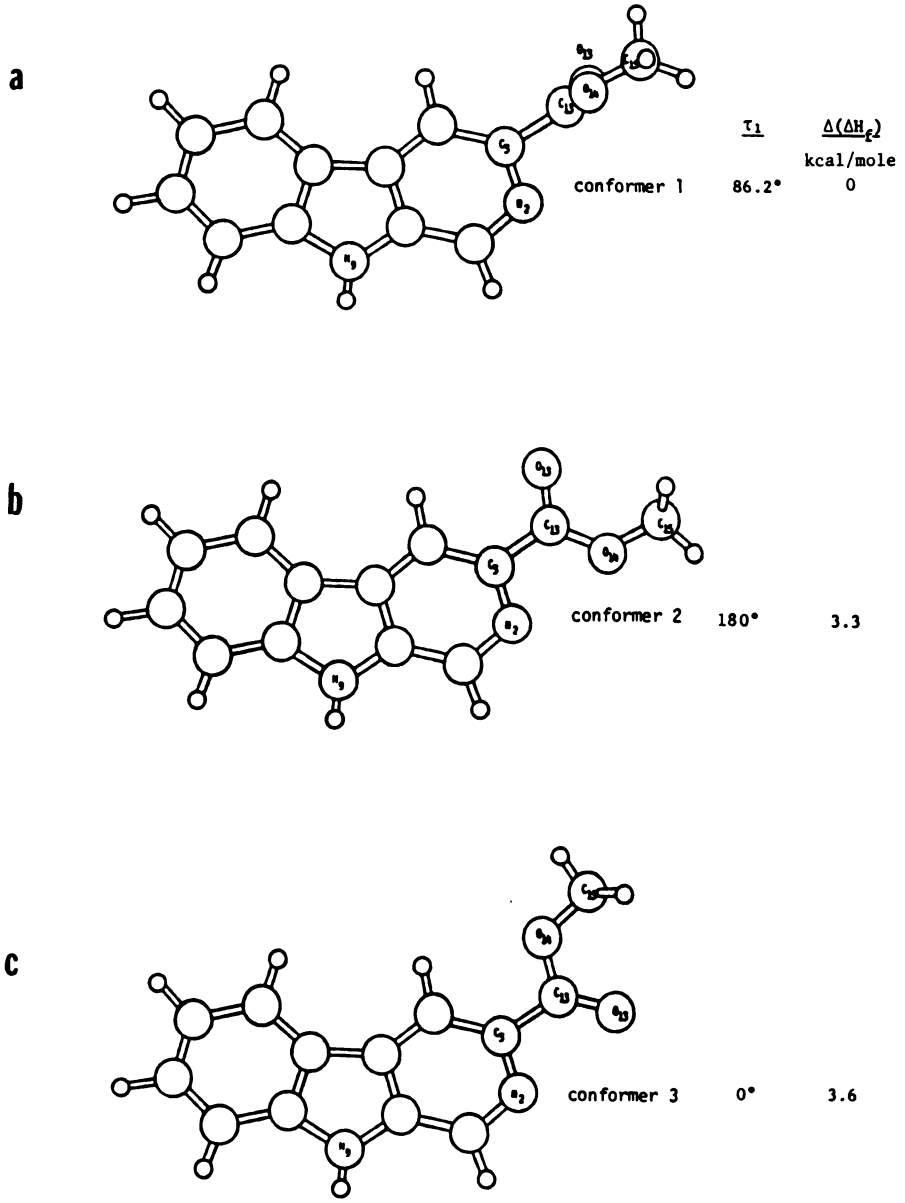


FIG. 1. *Three low energy conformers of β-CCM, 1c, calculated by total optimization from different initial geometries using the MNDO method* $\tau_1 = \tau(N_2\text{-}C_3\text{-}C_{13}\text{-}O_{14})$, and has indicated values for conformers 1, 2, and 3. $\tau_2 = \tau(O_{13}\text{-}C_{13}\text{-}O_{14}\text{-}C_{15}) = 0°$, for conformers 1, 2, and 3. $\tau_3 = \tau(C_{13}\text{-}O_4\text{-}C_{15}\text{-}H_{15}) = 180°$ for conformers 1, 2, and 3.

MNDO-geometry-optimized structures were used to calculate INDO (33) electron distributions. These INDO wave functions were subsequently used as input to the separate program (32) which calculates values of molecular electrostatic potentials at specified points. We are currently in the process of developing a more general MEP program. However, if limited to results in the plane of aromatic and unsaturated systems, the current program yields reliable relative behavior among related analogs (32, 34). Equipotential contour maps in these planes were created from the calculated single points values using programs supplied by the National Center for Atmospheric Research (see Figs. 4 and 5).

Model Drug-Receptor Subsite Interactions

Intermolecular interactions can be calculated using a variety of formulations and approximations (35). In the study described here, we have used an empirical energy formulation to calculate energies of interaction. The particular empirical energy program used is based on one called ECEPP (36), which is used extensively for peptide conformation studies. We have expanded the capabilities of this latter program to include intermolecular interactions (37), particularly hydrogen bonding and stacking interactions using known systems to determine parameters in each term.

In this program, which we call MOLECULE, the total energy is expressed as the sum of a Lennard-Jones 6-12 potential energy term for dispersion and repulsion, an electrostatic term, and a hydrogen-bonding term:

$$E_{tot} = E_{nb} + E_{hb} + E_{es}$$

where

$$E_{nb} = \sum_{ij} \frac{A^{ij}}{r_{ij}^{12}} - \frac{B^{ij}}{r_{ij}^{6}}$$

$$E_{hb} = \sum_{ij} \frac{A^{ij}}{r_{ij}^{12}} - \frac{B^{ij}}{r_{ij}^{10}}$$

$$E_{es} = \sum_{ij} \frac{q_i q_j}{D r_{ij}}$$

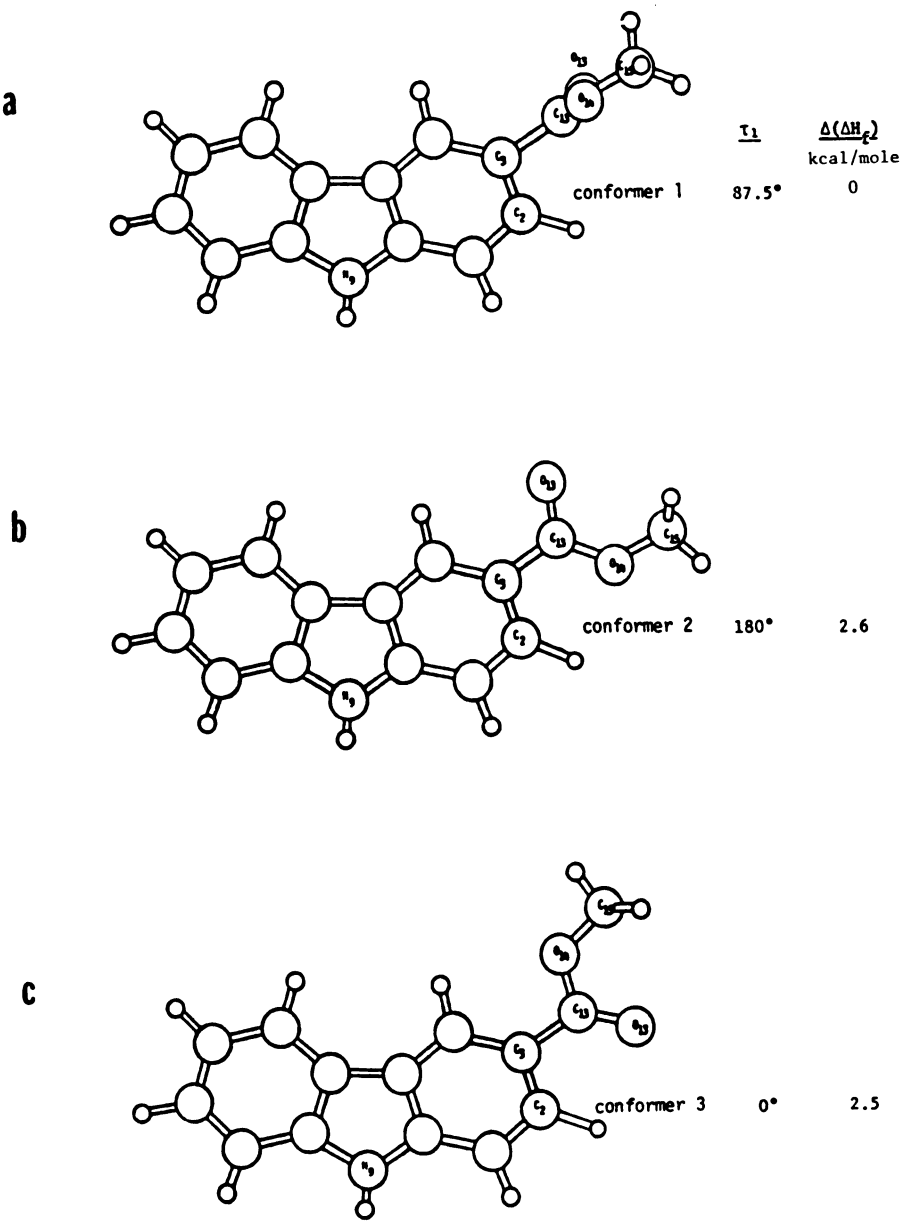


FIG. 2. *Three low energy conformers of the carbazole ethyl ester 2a, calculated by total optimization from different initial geometries using the MNDO method*

$\tau_1 = \tau(C_2\text{-}C_{13}\text{-}O_4)$, and has indicated values for conformers 1, 2, and 3. $\tau_2 = \tau(O_{13}\text{-}C_{13}\text{-}O_{14}\text{-}C_{15}) = 0°$, for conformers 1, 2, and 3. $\tau_3 = \tau(C_{13}\text{-}O_{14}\text{-}C_{15}\text{-}H_{15}) = 180°$ for conformers 1, 2, and 3.

A description of the parameters used can be found in the literature (36, 37).

MOLECULE is coupled to an efficient structure-generating program with graphics display capabilities and includes the option of intra- and intermolecular geometry optimization. We have used these combined capabilities in the past to characterize adduct formation between carcinogens and nucleic acid bases (38) and to model opiate-receptor interactions (39).

In the studies made here, we have chosen to investigate a number of model drug-receptor subsite interactions using this empirical energy program coupled to structure-generating and computer graphics capabilities. Specifically, we have characterized interactions between β-carbolines and models for three types of amino acid residues: (a) stacking interactions with aromatic residues, (b) in-plane interactions between hydrogen-donating groups and anionic residues, and (c) in-plane interactions between hydrogen-accepting groups and cationic residues. To consistently model the electrostatic component of these interactions, electronic charges calculated using the semiempirical CNDO (31) method were used as input to the MOLECULE program, since the other terms in the empirical energy expression were parameterized using CNDO-calculated charges in the electrostatic term (36). For all these model drug-receptor interactions, this interactive system allowed the following protocol for characterizing intermolecular interactions to be used. 1) For each model receptor-drug interaction studied, an intermolecular geometry was generated using the structure-generating program. 2) This complex geometry was displayed on a graphics terminal and changes were made to optimize the initial choice of intermolecular geometry. 3) The initial geometry was used as input to the program MOLECULE, which allows total optimization of the intermolecular geometry. 4) The final complex geometry was again displayed graphically and key intermolecular distances, bond angles, and torsion angles were determined. A more specific description of each type of interaction is given below.

Stacking interactions. Since all of the β-carboline analogs investi-

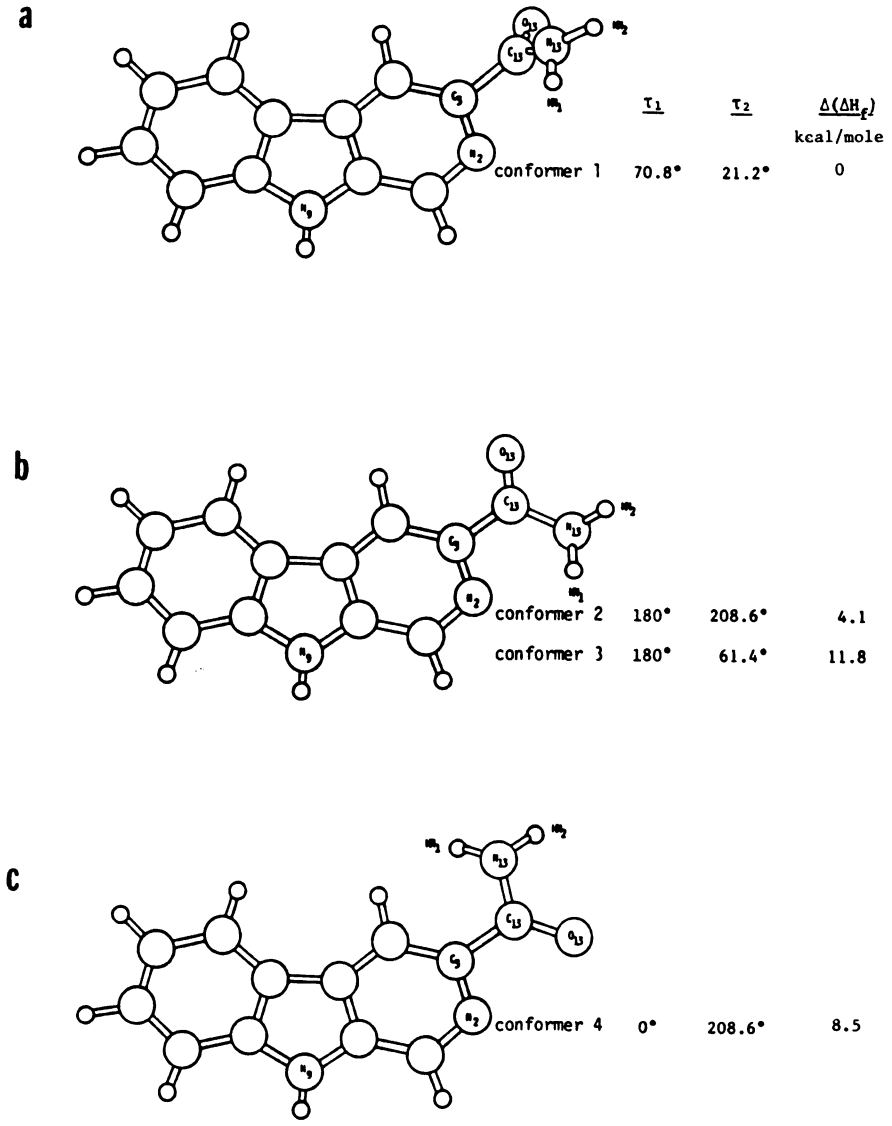


FIG. 3. *Conformation structures*

a, the only minimum energy conformation obtained for the 3-amido-β-carboline analog *1b*. This minimum (conformer 1) was obtained from all initial geometries tried. b, conformation and relative enthalpy of formation of two initial *trans*-planar conformers which optimized to conformer 1. c, conformation and energy of initial *cis*-planar conformers which optimized to conformer 1.

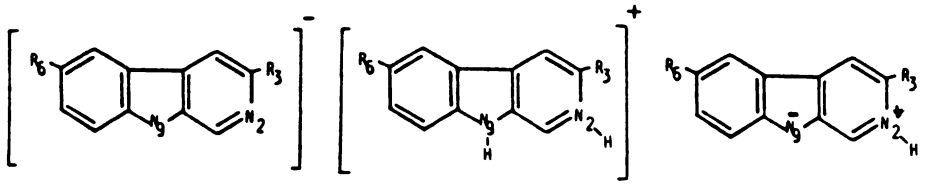
gated are essentially planar, stacking interactions with either neutral or cationic aromatic amino acid residues could play a possibly discriminating role in receptor-binding. As shown in Fig. 6, four possible stacking geometries of a model tryptophan residue with the β-carbolines were used as starting configurations. In two (a and c) the five-membered ring and the amine nitrogen in the indole ring of the model tryptophan is exactly above the five-membered ring and the amine nitrogen in the β-carboline analog. In one of these (a), the six-membered indole ring overlaps the A-ring in the β-carboline, and in the other (c) it had overlap with the C ring. The other two initial stacking geometries chosen (b and d) are the exact "inverse" of these with the model tryptophan rotated 180° about an axis that passes through the plane of both indole rings. These studies were made with analogs *1a* and *1c* as examples of an inactive and active analog. An initial stacking distance of 3.2 Å was chosen for the starting conformations used in the stacking interactions (Fig. 6). For each optimized β-carboline-model tryptophan complex, the analog other than the one used in the optimization was substituted into the complex, e.g., *1a* for *1c* and *1c* for *1a*, and single point energies were calculated. This last step was done to investigate whether there is a preferred stacking orientation involving a high affinity analog, such as *1c*, that would not be energetically favorable with a low affinity analog such as *1a*.

As shown in Fig. 7, stacking interactions with a model for a protonated histidine residue, 1-methylimidazolium, were also investigated. In these studies, two analogs with different aromatic systems, the inactive carbazole (*2a*) and the potent β-CCM (*1c*), were chosen with three initial stacking geometries shown in Fig. 7, a, b, and c. In one (a), the N_1 of imidazole was placed over N_2 of β-CCM; in the second (b), the N_2 of imidazole was placed over the N_2 of β-CCM; and in a third (c), the C_2 of the imidazole was placed over the N_2 of β-CCM. Corresponding initial geometries were chosen for the carbazole with N_1, N_2, and C_2 of the imidazole stacked over the C_2 of the carbazole. Stacking interactions were optimized as a function of interplane distance.

N_9-H-anionic site interactions. To simulate the interaction of the amine (N_9-H) group with a postulated anionic receptor site, an acetate anion was used as a model for an aspartate or glutamate residue. The acetate anion was created using standard geometry and optimized using MNDO. Electron distributions to use in the empirical energy program were obtained from CNDO. Complexes were then formed between this anion and the N_9-H group by creating a linear hydrogen bond at a

TABLE 2

Heats of formation of parent β-carboline analogs and the relative heats of formation of their anionic (A), cationic (C), and tautomeric (T) forms



A C T

Parent compound	ΔH_f (parent)	$\Delta(\Delta H_f)_A$[a]	$\Delta(\Delta H_f)_C$[b]	$\Delta(\Delta H_f)_T$[c]
1a	62.7	−38.6	150.1	21.0
1b	−17.5	−47.0	155.0	20.9
1c	−17.2	−47.0	154.6	20.2
1d	92.9	−48.2	158.8	22.4
1e	78.4	−55.7	159.5	18.2
2a	−25.9	−41.8		

[a] $\Delta(\Delta H_f)_A$ is defined as the difference between the heat of formation of the anionic form of the compound, and the heat of formation of the parent $\Delta(\Delta H_f)_A = \Delta H_f$ anion $- \Delta H_f$ parent.

[b] $\Delta(\Delta H_f)_C = \Delta H_f$ cation $- \Delta H_f$ parent.

[c] $\Delta(\Delta H_f)_T = \Delta H_f$ tautomer $- \Delta H_f$ parent.

distance of 1.86 Å (Table 3). These drug-anionic complexes were then optimized using MOLECULE.

Cationic receptor interactions with the aromatic nitrogen and the C_3-substituent. These studies focus on three different types of drug-cation interactions: 1) a model for a lysine cation [$(CH_3NH_3)^+$] was allowed to interact directly with N_2 (Table 4); 2) a model for an arginine residue [$(CH_2NCN_2H_4)^+$], a larger cation, was allowed to interact simultaneously with both the C_3-substituent and N_2 (Table 5); 3) two model lysine residues were allowed to interact with N_2 and the C_3-substituent independently (Table 6). Each model receptor subsite was created from standard geometries and optimized with MNDO, and the electronic charges were calculated using CNDO. Initial linear H-bonds were constructed between a cationic residue and N_2, and between a residue and the C_3-substituent, at a heavy atom-hydrogen distance of 1.86 Å. Optimizations using MOLECULE were then performed.

In all of these calculations, structure-building and construction of plausible initial complex geometries were facilitated using a flexible structure-generating program coupled to both graphics capabilities and energy-calculating capabilities in MOLECULE.

RESULTS AND DISCUSSION

Conformational Characteristics of the β-Carbolines

The MNDO-optimized geometries for all analogs, *1a–1e* and *2a*, have essentially identical β-carboline ring structures. The main conformational flexibility lies in the C_3-substituent. Thus, analogs with no C_3-substituents and the linear C_3-CN compounds, *1a*, *1d*, and *1e*, have only one stable form. However, for the methyl ester analog of β-carboline (Fig. 1, a, b, and c) and of carbazole (Fig. 2, a, b, and c), three local minima were obtained. These correspond to *cis*- and *trans*-planar geometries of nearly equal energy, and a nonplanar structure to which all nonplanar initial geometries optimized and which was the lowest energy conformer obtained. All three conformers, however, are close enough in energy to be considered candidates for receptor interactions. For the β-carboline ester, planar ester groups have both carbonyl and ester oxygens in the plane of the aromatic nitrogen ring, a conformation particularly fortuitous for simultaneous interaction of the nitrogen and oxygen moieties with cationic receptor sites.

By contrast, for the 3-amido group of analog *1b* shown in Fig. 3, only one minimum energy conformation (conformer 1) was found. In this conformation, the amide group is not co-planar with the β-carboline; the carbonyl group is rotated ~70° with respect to the β-carboline, and the amide hydrogens are located pyramidally such that the lone pair of electrons on the amide nitrogen (N_{13}) is perpendicular to the plane of the amide itself. Both *cis*- and *trans*-planar initial geometries (conformers 2 and 4) optimized to this single minimum energy conformer (1). Rotation of the amide hydrogens, placing the lone pair of electrons in the plane of either the *cis* (conformer 3) or *trans* (conformer 4) amide groups, a potentially more favorable conformer for receptor interaction, does not lead to any local minima, nor are such conformers low energy forms. The high energy of these *cis*- and *trans*-planar forms (3 and 4) is perhaps due to the pyramidization of the amide nitrogen to avoid hydrogen eclipsing. Conformers 2, 3, and 4 correspond then to "induced" conformers, not stable in the isolated molecule, but which could occur at the receptor during complex formation if a net gain of energy is achieved.

Taken together, these results suggest that conformational differences alone do not clearly account for differences in affinity among the six analogs studied.

Effect of Substituents on Electronic Structures

Similar to the results discussed above for conformational properties, there is very little correlation between the effects of various substituents on net charges in different regions of these antagonists and their measured receptor affinity. Although the C_3-substituent is *para* to the amine nitrogen (N_9), its presence had no significant effect on the net atomic (Mulliken) charge on N_9, on the hydrogen on N_9, or on the bond overlap density of the N_9-H bond. While the net charge on the aromatic nitro-

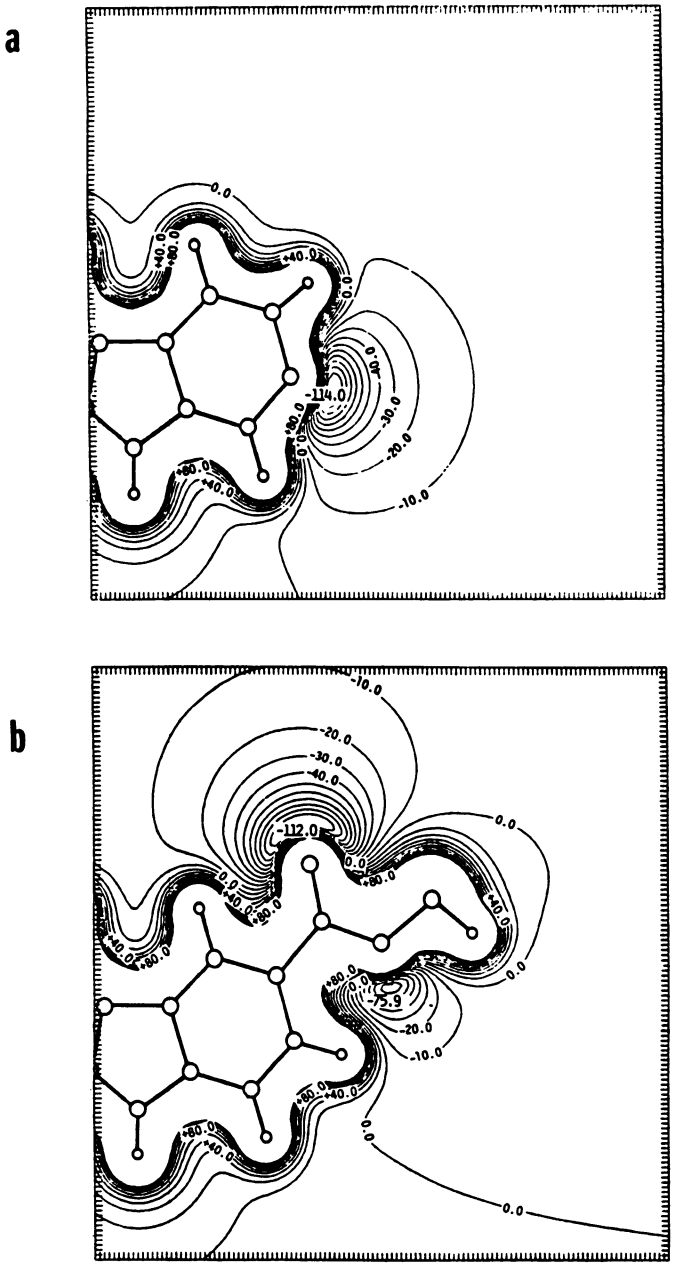


FIG. 4. *Calculated electrostatic potential contour map for (a) norharmane, 1a, and (b) ethyl ester carbazole (2a) in conformer 2.*

gen (N_2) *ortho* to C_3 did show some effect of the C_3-substituent decreasing in the order methyl ester > cyano > amide > hydrogen, this order is not the same as relative receptor affinity. The energies and compositions of the highest occupied molecular orbital also showed no correlation with the observed receptor-binding profile.

Relative Stabilities of the Anionic, Cationic, and Tautomeric Forms of the β-Carboline Analogs

The relative heats of formation of the anionic, cationic, and tautomeric forms of the analogs listed in Table 1 are given in Table 2. The relative stability of the cation formed by protonating the aromatic nitrogen is one measure of the ability of this nitrogen to function as a hydrogen acceptor in receptor interactions. By this criterion, the order of proton acceptor ability of the aromatic nitrogen is norharmane > C_3-$COOCH_3$ > C_3-$CONH_2$ > C_3-CN > C_6-NO_2. The order is consistent with the electron-withdrawing capabilities of these substituents with the parent compound being the best proton acceptor.

As shown in Table 2, electron-withdrawing groups at C_3 or C_6 also have a consistent effect on the proton-donating ability of the amine nitrogen N_9, as measured by the increased stability of the anion formed by proton abstraction. The order obtained is the reverse of that above, i.e., C_6-NO_2 > C_3-CN > C_3-$CONH_2$ ~ C_3-$COOCH_3$ > norharmane.

The simultaneous effect of substituents on both these properties, as measured by calculated tautomeric energies, shows no significant substituent impact.

Neither of the calculated effects of the C_6- or C_3-substituents, destabilization of the cation or enhanced stabilization of the anion, correlates with the relative receptor affinity of these analogs. While MNDO results sometimes encounter errors in the prediction of cationic stabilities, the effects of substituent changes in this closely related series on both proton-donating and proton-accepting ability are reasonable and self-consistent. Thus, the lack of correlation of these properties to receptor affinities does not appear to reflect an inadequacy in the methods. Rather, it appears that substituents at C_3 modulate receptor affinity primarily by their own key interactions with localized subsites of the receptor.

Electrostatic Potential Mapping

MEP created by the β-carboline can provide a good indication, by complementarity, of the position and nature of receptor subsites involved in electrostatic interaction with the drug and of requirements for high affinity.

As shown in Fig. 4, low affinity analogs share the feature of having only one region of negative potential in the plane of the aromatic ring. Analog *1a*, norharmane (Fig. 4a), has a positive potential around C_3 and a single large negative potential in the plane near N_2 corresponding to the position of its lone pair of electrons (V_{max} = −114.0 kcal/mol). A similar pattern is obtained for the low affinity amide analog, *1b* (not shown), in its lowest energy form. It also has only one region of negative potential in the plane of the β-carboline ring around N_2. The methyl ester carbazole, *2a* (Fig. 4b), has a different region of low negative potential centered on the C_3-substituent with local minima near the carbonyl (V_{max} = −112 kcal/mol) and ester (V_{max} = −75.9 kcal/mol) oxygen and no negative potential in the vicinity of C_2.

Unlike the three low affinity analogs (*1a*, *1b*, and *2a*), the two high affinity analogs (*1c* and *1d*) have two regions of large negative potential. Fig. 5a shows the MEP around the high affinity β-CCM compound (*1c*) in a *trans*-planar conformer, i.e., with the carbonyl oxygen *trans* to the aromatic nitrogen. The enhanced negative potential region around N_2 and the ester oxygen in the active analog, together with the large negative region around the carbonyl oxygen, suggests a possible inter-

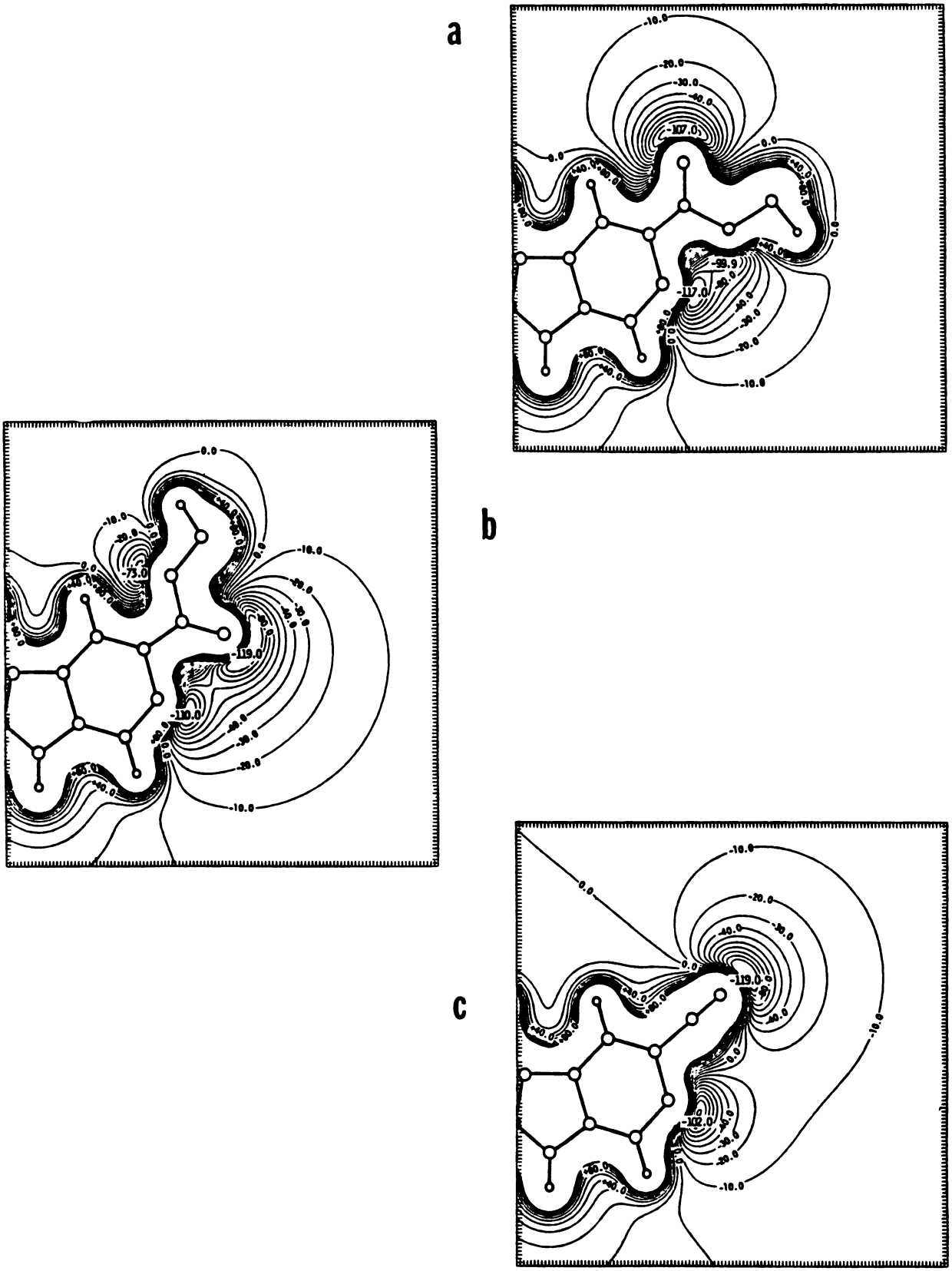


FIG. 5. *Calculated electrostatic potential contour map*
a, β-CCM *1c* in a *trans*-planar conformer (2). b, β-CCM *1c* in a *cis*-planar conformer (3). c, 3-cyano-β-carboline *1d*.

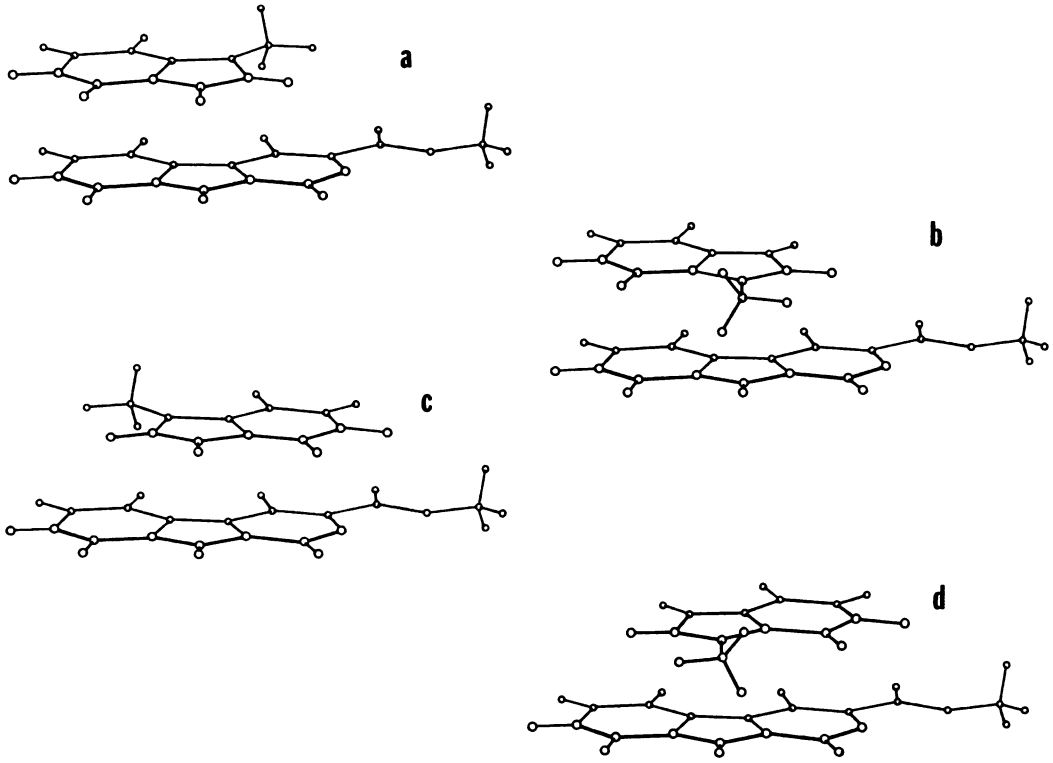


FIG. 6. *Four initial geometries selected for stacking complexes of a model tryptophan residue with β-CCM*
a, 5-membered rings and amine nitrogen groups stacked over each other. b, 5-membered rings are overlapped with carbon of indole over amine of β-carboline. c, "inverted" carbon *a*, 6-membered indole ring over pyridine ring of β-carboline. d, "inverted" *b*, 6-membered indole ring over piperidine ring of β-carboline.

action of each of these groups with separate cationic receptor subsites.

Fig. 5b shows the electrostatic potential map of the equi-energy *cis*-planar conformer of analog *1c*, i.e., with the N_2 and carbonyl oxygens "cis." In this conformation, the large potential from the carbonyl oxygen combines with the potential from N_2 to form a very large negative potential envelope (V_{max} = −119.0, −110.0 kcal/mol), larger than that seen in the *trans* conformer (Fig. 5a). β-CCM, in this conformation, appears capable of interacting with a more extended cationic receptor subsite, such as a guanidinium side chain of an arginine residue.

The potential map of the 3-cyano-β-carboline analog *1d* (Fig. 5c) shares some similarity with both the low energy conformers of analog *1c*. It contains two spatially independent potential minima, one centered on the nitrogen of the cyano group (V_{max} = −119 kcal/mol) and one centered on the ring nitrogen (V_{max} = −102.0 kcal/mol); and these distinct minima are surrounded by a common, large negative envelope. Thus, analogs *1c* and *1d* could, in principle, interact with either a single, large, complex cationic site, such as an arginine residue, or with two independent, smaller cationic residues.

Modeling the Receptor Site: Interactions of β-Carboline Analogs with Model Receptor Subsites

Stacked planar complexes with indole rings of model tryptophan residue. Four qualitatively different initial geometries, described in Methods and shown in Fig. 6, were used to investigate stacking interactions between the planar tryptophan and β-carboline rings. Two β-carboline analogs were chosen: inactive norharmane *1a* and the potent β-CCM *1c*. These four types of structures each were optimized to complexes with very similar energies of interaction (8–9 kcal/mol) and similar geometries for both analogs.

Each drug-model receptor complex was investigated for selectivity to analogs *1a* and *1c*. To this end, a series of single point energies was calculated by substituting analog *1a* into optimized complexes of analog *1c* and vice versa. All of these combinations yielded energies of interaction of ~8 kcal/mol. Thus, neither conformational nor energy specificity was found in these stacked complexes between the active (*1c*) and inactive (*1a*) analog. A possible origin of this lack of sensitivity is that a major component of the interaction energy (~90%) is a van der Waals or dispersion energy which is insensitive to the geometry of the stacked complex.

These results strongly suggest that, although a tryptophan residue may be part of the receptor site for these aromatic β-carbolines, the energy of interaction with this residue is not a discriminating factor in determining relative receptor affinities.

At first glance, this result appears to contradict known structure-activity profiles for the β-carbolines. Saturation or even partial saturation of the fused ring system leads to greatly reduced receptor affinities (13). However, in all such analogs, the imine nitrogen N_2 is also converted to an amine, thus destroying the ability of N_2 to participate as an H-atom acceptor in planar interactions

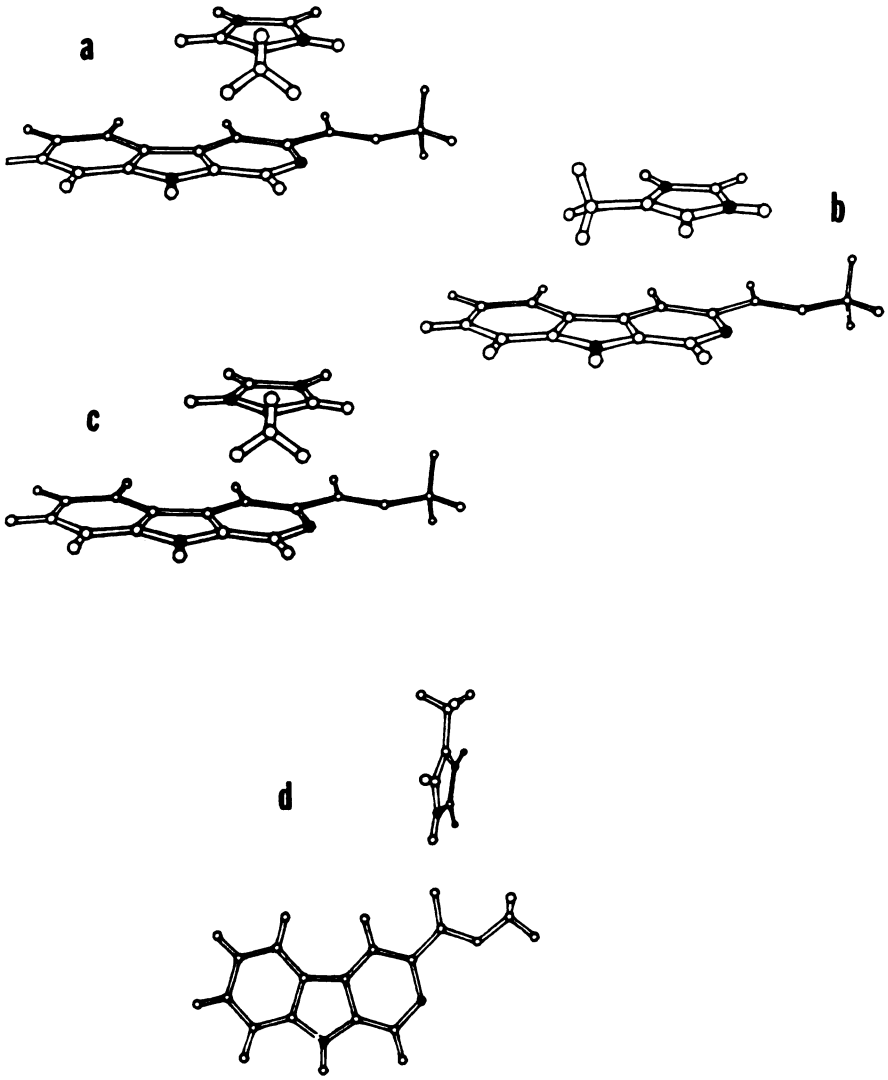


FIG. 7. *Three initial geometries (a–c) and typical optimized geometry (d) selected for stacking complexes of a model protonated histidine residue with β-CCM and the 3-carbomethoxy carbazole*

a, N_1 of imidazole over N_2 of β-CCM or C_2 of carbazole. b, N_2 of imidazole over N_2 of β-CCM or C_2 of carbazole. c, C_2 of imidazole over N_2 of β-CCM or C_2 of carbazole. d, optimized H-bonded complex obtained from all three initial geometries.

with cationic subsites. It is these interactions discussed below, rather than stacking, which we believe determine relative receptor affinity.

If this interpretation is correct, analogs in which the benzene, but not the pyridine, ring of the β-carboline system is saturated should have a much higher receptor affinity than the totally saturated ring structure or one in which the pyridine ring is saturated.

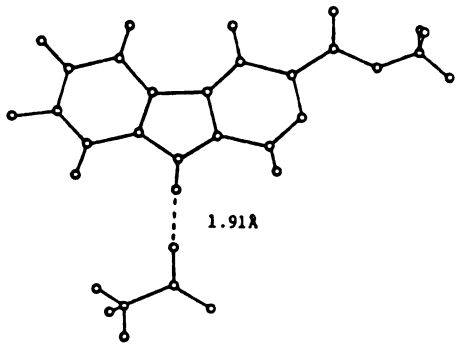
Stacked planar complexes with a model protonated histidine residue: 1-CH_3-imidazolium. Using the three initial geometries depicted in Fig. 7, a, b, and c, optimum stacking distances (d_{opt}) and interaction energies (ΔE_{int}) calculated for the active β-CCM and inactive carbazole compounds were very similar for *7a* and *7c* and disparate for *7b*. Specifically, for these analogs respectively for geometry *7a*, $d_{opt} = 3.4$ Å and the energies of interaction were −5.3 and −4.6 kcal/mol; for *7b*, $d_{opt} = 3.4$, 4.4 Å and $\Delta E_{int} = -5.8, -2.7$ kcal/mol; and for geometry *7c*, $d_{opt} = 3.4$ Å and $\Delta E_{int} = -4.9, -4.5$ kcal/mol. These results correspond to optimum energies if the interaction is constrained in a stacking mode. They indicate that such a constrained interaction in the conformation with imine nitrogens over each other as in Fig. 7b could help modulate receptor affinity.

However, for all starting geometries, total geometry optimization yielded a H-bonded complex (shown in Fig. 7d) in preference to a stacked complex. As shown in this figure, typical of all optimized complexes, the N-H group of the histidine forms a H-bond with the carbonyl oxygen. Comparable energies (~12 kcal/mol) were obtained for such complexes formed by both analogs. Moreover, since protonated histidine is sterically hindered from acting as a double donor to N_2 and the ester oxygen, it could not discriminate between the carbazole ester and β-CCM in this fashion. Thus, if such a H-bonded mode is sterically feasible, protonated histidine would not contribute to discriminating interactions at the receptor site.

Complexes with model anionic (glutamate or aspartate)

TABLE 3

Modeling the receptor site: calculated energies of interaction of β-carboline analogs with a model anionic receptor subsite (CH_3COO^-)



Compound	ΔE_i[a]	ΔE_f[b]
1a	−7.6	−8.6
1c	−10.0	−11.4
1d	−9.9	−11.0
1e	−13.5	−15.6
2a	−9.4	−10.3

[a] ΔE_i, in kcal/mol, is the energy of interaction assuming a fixed receptor subsite and a single linear H-bond between COO^- of the anion and N_9—H of β-carboline. r(O--H) = 1.91 Å and r(N_9--O) = 2.9 Å.

[b] ΔE_f, in kcal/mol, is the energy of interaction after total geometry optimization assuming flexibility in the drug-receptor complex geometry. Typical final geometry (*1a*): r(O-H) = 1.85 Å. ∠ N_9HO = 161 Å. r(N_9--O) = 2.8 Å.

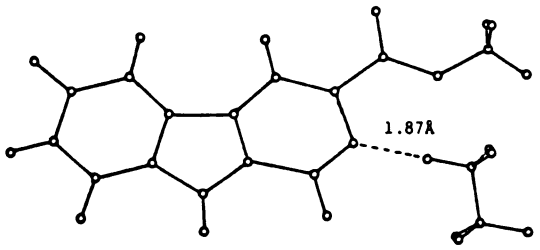
residues. β-Carboline-model receptor complexes were formed between the N_9-H group and a (CH_3COO-) ion as a model for a glutamate or an aspartate receptor residue. As shown in Table 3, the initial geometry chosen was a linear N_9---H---O bond with r(O--H) = 1.91 Å and r(N_9--O) = 2.9 Å. Table 3 gives the calculated energies of such complexes for five analogs which exhibit a spectrum of receptor affinities. Also given are the calculated energies of the optimized complexes. In these structures, the H-bonds did not remain totally linear, with a typical value for <N_9HO = 161°. Bond lengths remained similar to starting values: r(O--H) = 1.85 Å and r(N_9--O) = 2.80 Å.

The calculated interaction energies reflect the expected effect on the amine nitrogen of electron-withdrawing substituents at C_3 and C_6. The most stable complex is formed by 6-NO_2 norharmane, *1e*, reflecting the potent electron-withdrawing effect of this nitro group *para* to N_9. The high affinity analogs *1c* and *1d* have intermediate energies of interaction due to the intermediate electron-withdrawing effects of the C_3-methyl ester and cyano groups. The analogs with the lowest energy of attraction were the inactive analogs norharmane *1a* and the methyl ester carbazole *2a*, each of which is missing one of the functional groups present in the other analogs.

Thus, while an interaction of the N_9-H group with an anionic receptor site might be a component of increased receptor affinity, the differential effect of the β-carboline substituent groups on N_9-H interactions with receptors does not seem to be a discriminating factor in relative receptor affinities.

TABLE 4

Modeling the receptor site: calculated energies of interaction of β-carboline analogs with a single model lysine cationic subsite ($CH_3NH_3^+$)



Compound	$\Delta(\Delta H_f)$	ΔE_i[a]	ΔE_f[b]	ΔE_f^{net}[c]
1a	0	−10.5	−10.5[d]	−10.5
1b[e]				
Conformer 1	0	−7.1	[f]	
Conformer 2	4.1	361[g]	[f]	
Conformer 3	11.8	−1.4	−9.9[h]	+1.8
Conformer 4	8.5	−18.3	−20.2[i]	−11.7
1c[j]				
Conformer 1	0	−10.4	−13.4[i]	−13.4
Conformer 2	3.3	−12.6	−13.6[h]	−10.3
Conformer 3	3.6	−16.7	−18.6[i]	−15.0
1d	0	−10.6	−12.0[d]	−12.0

[a] ΔE_i, in kcal/mol, is the energy of interaction assuming that a fixed $(CH_3NH_3)^+$ cation subsite forms a linear H-bond with the amine N_2 of the β-carboline.

[b] ΔE_f, in kcal/mol, is the energy of interaction between a model $(CH_3NH_3)^+$ cationic subsite and the imine nitrogen, allowing total optimization of the drug-receptor complex geometry.

[c] $\Delta E_f^{net} = \Delta E_f - \Delta(\Delta H_f)$.

[d] Final complex geometry is a linear, or near-linear, H-bond (Fig. 8a).

[e] Conformers as shown in Fig. 2, a, b, and c.

[f] In optimized geometry, cation moved from imine N_2 to interaction with the carbonyl oxygen.

[g] Large repulsive interaction due to steric interference of substituent amine group hydrogens with the initial linear hydrogen bond.

[h] Final complex geometry is a "bifurcated" H-bond, i.e., a single hydrogen atom donor is shared by the imine nitrogen and the amide nitrogen (Fig. 8b).

[i] Final complex geometry is a "double" H-bond, i.e., one hydrogen atom of the cation interacts with the imine nitrogen (N_2) and another with the ester oxygen (Fig. 8c).

[j] Conformers are shown in Fig. 1, a, b, and c.

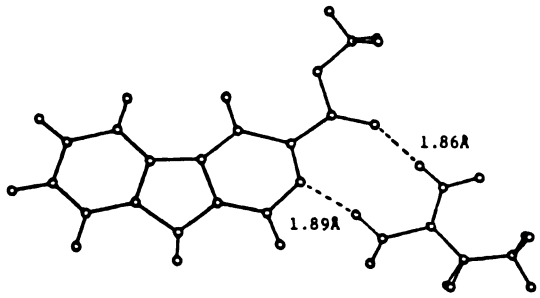
[k] Final complex geometry is a "bifurcated" H-bond, i.e., a single hydrogen atom of the cation donor is shared by the imine nitrogen (N_2) and the ester oxygen (Fig. 8b).

Complexes with model cationic receptor subsites. Lysine-type $(CH_3NH_3)^+$. Table 4 summarizes the calculated energies of interaction of the aromatic ring nitrogen (N_2) with a model lysine cationic subsite, $(CH_3NH_3)^+$. Two low affinity analogs, *1a* and *1b*, and two high affinity analogs, *1c* and *1d*, were chosen for these studies. The initial geometry for each interaction was the linear H-bond complex shown in Table 4 (with an r(N--H) distance of 1.87 Å and an r(N_2--N) distance of 2.90 Å). Energies of interaction were calculated for these fixed geometry complexes (ΔE_i) and for totally optimized complex geometries (ΔE_f).

We see from Table 4 that the low affinity norharmane

TABLE 5

Modeling the receptor site: calculated energies of interaction of β-carboline analogs with a model arginine cationic subsite ($CH_3NH_2CN_2H_4^+$)



Compound	ΔE_i[a]	ΔE_f[b]	$\Delta(\Delta H_f)$	ΔE_i^{net}[c]	ΔE_f^{net}[d]
1a	−7.5	−11.8	0	−7.5	−11.8
1b (conformer 4)	−17.1	−18.7	8.5	−8.6	−10.2
1c (conformer 3)	−15.6	−17.3	3.6	−12.0	−13.7
1d	−12.4	−13.5	0	−12.4	−13.5
2a (conformer 3)	−9.2	−14.1	2.5	−6.7	−12.6

[a] ΔE_i, in kcal/mol, is the energy of interaction assuming a fixed receptor subsite and two linear H-bonds, one between the cation and N_2, and the other between the cation and the C_3-substituent of the β-carboline.

[b] ΔE_f, in kcal/mol, is the energy of interaction assuming flexibility in the drug-receptor complex geometry. Analogs 1*b*, 1c, and *1d* optimized to a complex very similar to that of the starting geometry. In optimized complexes with *1a* and *2a*, the guanidinium cation became perpendicular to the molecular plane of the β-carboline and C_3 group.

[c] $\Delta E_i^{net} = \Delta E_i - \Delta(\Delta H_f)$.

[d] $\Delta E_f^{net} = \Delta E_f - \Delta(\Delta H_f)$.

1a and the high affinity nitrile compound *1d* maintain this linear H-bond in an optimized complex, and have energies of interaction differing by only 1.5 kcal/mol.

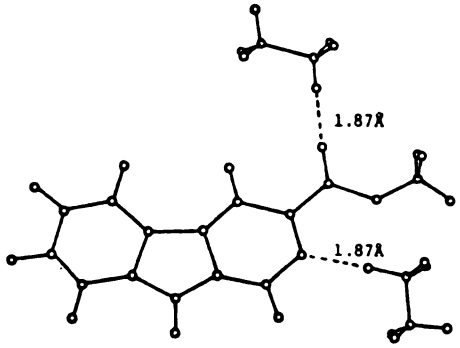
For the other two analogs, the energies of interaction and type of H-bonded complex formed are dependent on the conformer in which each analog interacts at the receptor site. For the high affinity β-CCM analog *1c*, the most energetically favorable optimized complex is formed by the *cis*-planar conformer (conformer 3, Fig. 1c). This conformer allows a complex to form that involves a "double" H-bond, i.e., between two donors and two acceptors, one hydrogen atom of the protonated amine interacts with the N_2 while another interacts with the carbonyl oxygen (see Fig. 8c). The energy of interaction of this complex is −18.6 kcal/mol, while the conformational energy (enthalpy of formation) required to form this conformer is +3.6 kcal/mol. Thus, an estimate of a net energy of interaction ΔE_f^{net} of −15.0 kcal/mol is obtained.

For the 3-amide-β-carboline, neither its single stable conformer (1) nor any of its three possible induced conformers, with different amounts of conformational energy required for them, gives as good net energies of interaction as β-CCM. In fact, all but one of these conformers led to a repulsive interaction. Thus, while a single interaction with a single lysine residue could account for differences in affinity between *1b* and *1c*, it does not seem to account for the high affinity of the 3-cyano-β-carboline *1d*, which has a net energy of interaction similar to that of the low affinity *1a* and *1b* analogs.

TABLE 6

Modeling the receptor site: calculated energies of interaction of β-carboline analogs with two model lysine cationic subsites ($CH_3NH_3^+$)

Numbers in parentheses indicate that the hydrogen bond did not remain after geometry optimization due to repulsive interaction between the two cations, if no protein constraints remain at the binding site.



Compound	$\Delta(\Delta H_f)$	$\Delta E_i^{(1)}$[a]	$\Delta E_i^{(2)}$[b]	ΔE_i^{net}[c]
1a	0	−10.5	[d]	−10.5
1b				
Conformer 1	0	(−7.1)	−15.3	−22.4
Conformer 2	4.1	(361.3)	−14.5	
Conformer 3	11.8	−1.4	−13.9	−3.5
1c				
Conformer 1	0	(−10.5)	(−13.9)	−24.4
Conformer 2	3.3	(−12.6)	(−13.5)	−22.8
1d	0	(−10.6)	(−14.1)	−24.7
2a				
Conformer 2	2.6	[d]	14.2	−11.6

[a] Calculated energy of interaction, assuming a fixed receptor subsite (cation 1) and geometry of interaction, with N_2.

[b] Calculated energy of interaction, assuming a fixed receptor subsite (cation 2) and geometry of interaction, with the carbonyl oxygen analog of 1*b*, 1c, and *2a*, and with the cyano nitrogen of *1d*.

[c] $\Delta E_i^{net} = (\Delta E_1 + \Delta E_2) - \Delta(\Delta H_f)$.

[d] *Analogs 1a* and *2a* each have only one of the two possible hydrogen-bonding sites.

Arginine-type $(CH_3NH_2CN_2H_4)^+$ cationic site. Interactions of the same four analogs with a larger cationic site, a guanidinium cation $(CH_3NH_2CN_2H_4)^+$ to model arginine, were examined to possibly account for their relative receptor affinities. Such a large cationic site with multiple amine groups could, in principle, interact simultaneously with N_2 and the electron-rich groups of each C_3-substituent, including the cyanonitrogen of *1d* and *cis*-conformers of *1c* and *1b*.

The initial geometry chosen for this interaction, shown in Table 5, consists of two simultaneous, nearly linear hydrogen bonds, with two hydrogens of the cation as donors, and with N_2 and the C_3-substituent as acceptors. These complexes are similar to the "double" H-bond complexes found for the $(CH_3NH_3)^+$ cation. In both model cations, each of two amine hydrogen atoms forms a hydrogen bond, one with the aromatic N_2 and the other

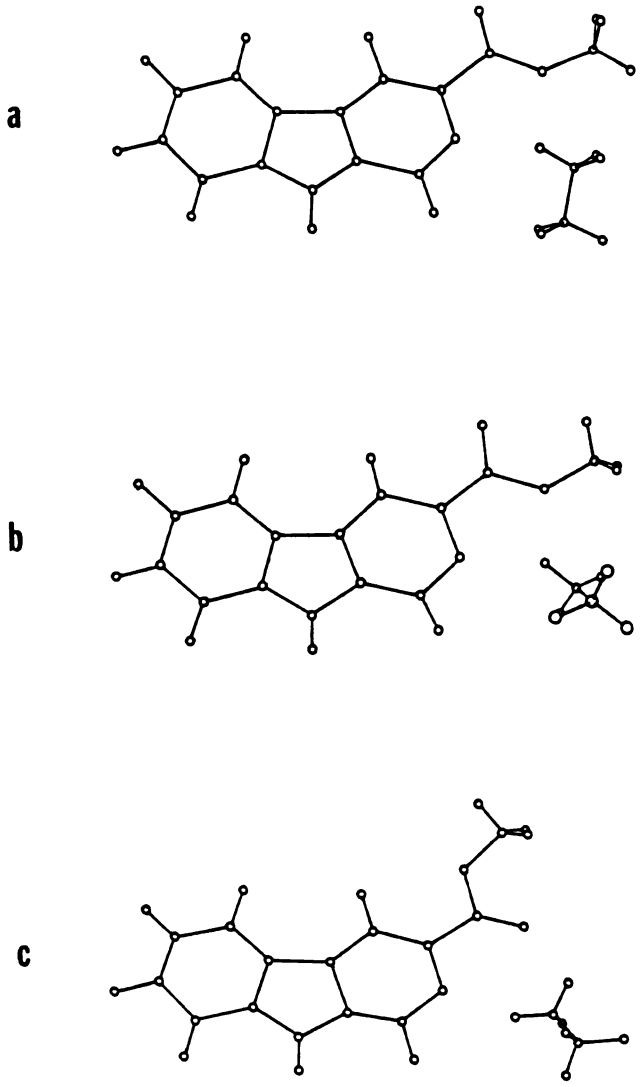


FIG. 8. *Three types of analog cation receptor complexes formed by β-CCM and model lysine $(CH_3NH_3)^+$ receptor site*

a, linear "H-bond" between aromatic N_2 and H-N of $(CH_3NH_3)^+$. b, bifurcated H-bond formed between two hydrogen acceptors (the aromatic nitrogen and the carbonyl oxygen) and one donor, an H-N of $(CH_3NH_3)^+$. c, "double" H-bond formed between two acceptors (the aromatic nitrogen and the carbonyl oxygen) and two donors, i.e., two H-atoms of the $(CH_3NH_3)^+$ cation.

with a carbonyl oxygen or cyano nitrogen. However, for the guanidinium cation, the hydrogen donors are located on different nitrogen atoms, while for the methyl ammonium cation they are on the same nitrogen.

Comparing either initial or final energies in Tables 4 and 5, we see that multiple H-donor-acceptor-type interactions with a single cationic site are somewhat stronger with a lysine than with an arginine model receptor. For example, an optimized β-CCM-$(CH_3NH_3)^+$ complex has an energy of −18.6 kcal/mol while with a guanidinium cation the energy is −17.3 kcal/mol. This slight decrease in interaction energy could be due to an increased delocalization of the positive charge on the larger guanidinium cation. The net charge on each amine hydrogen in the guanidinium cation is +0.27, while for $(CH_3NH_3)^+$ it is +0.32, leading to the somewhat stronger interactions found since a large component of this interaction is electrostatic.

As indicated in Table 5, the two low affinity analogs (*1a* and *2a*) have weaker interaction with guanidinium whether or not the guanidinium cation is free to rotate at the receptor site (i.e., E_i *vs.* E_f in Table 5). Optimized geometries for these analogs (*1a* and *2a*) which cannot be double H-acceptors, involved a large movement of the guanidinium cation (90° relative rotation of the cation). From interaction energies alone, the three other analogs which can act as double H-acceptors should all have a higher affinity at the receptor site. However, the amide (*1b*) is "penalized" because of the energy required to attain the conformation necessary for optimum interaction. Preliminary investigations indicated that, when rotations away from *cis*-oid planarity of the aromatic nitrogen and the carboxyl oxygen are allowed, the energy of interaction is greatly diminished. While the energy differences between the inactive amide and the active ester are not very great, variations in these energies for the 5-analogs, particularly if the arginine does not have a large degree of rotational flexibility, could account for the observed relative affinities of these analogs.

Two lysine-type $(CH_3NH_3)^+$ cationic sites. A third model of receptor interaction at the aromatic nitrogen (N_2) and C_3-substituent considered was that with two independent model lysine sites $(CH_3NH_3)^+$, a model that involves maximum separation between the two cationic sites. The corresponding conformations of each analog with which such sites can interact are those which have the greatest separation of the N_2 and carbonyl oxygen functional groups, i.e., conformers 1, 2, and 3 of *1b* (Fig. 3), and 1 and 2 of *1c* (Fig. 1). An initial interaction geometry was constructed for each analog as shown in Table 6. It consisted of two linear hydrogen-bonded complexes: one involving N_2, and the other the C═O group of analogs *1a*, *1b*, *1c*, and *2a*, and the C≡N group of analog *1d*.

The energies calculated for these two-site interactions, $\Delta E_i^{(1)}$ and $\Delta E_i^{(2)}$, are summarized in Table 6, together with the energies (heats of formation) required to form the conformer involved in the receptor interaction. When total intermolecular complex geometry optimization was attempted, none of the complexes remained intact because of the proximity of the two cations. Thus, if two such cationic residues do exist at the receptor site, they would remain in place only by the steric constraints of the protein. From the net energies of interaction, ΔE_i^{net}, given in the table, we see that strong attractive interactions are achieved both at N_2 and the C_3-substituent in both high affinity analogs *1c* and *1d*.

The observed low affinity in analogs *1a* and *2a* is also readily explained by the complete lack of one of the necessary binding sites.

However, this two-cation model for receptor interaction serves less well in explaining the low affinity of the 3-amido-β-carboline analog *1b*. In its most stable conformer, it has an interaction energy only 2 kcal/mol less than that of the high affinity analog *1c*. While in the right direction, this difference is somewhat smaller than

the net interaction energy difference between *1b* and *1c* with the model arginine residue.

Conclusion

Conformational and electronic properties of a series of β-carboline analogs have been calculated using a number of theoretical methods, in order to determine the molecular characteristics involved in high affinity binding to the brain benzodiazepine receptor, and, by complementarity, to map the various amino acid residues located at the receptor-binding site. Analogs with variations at the N_2, C_3, and C_6 positions were investigated, focusing on the difference in binding affinity displayed by analogs with similar C_3-substituents.

The results indicate that a ring nitrogen at position 2, and an electron-rich C_3-substituent, are specifically required for high affinity binding. Electron-withdrawing substituents at C_3 appear to have two effects. 1) They create a large negative potential capable of interaction with a unique local receptor subsite; and 2) they enhance the stability of interaction of an anionic receptor site with the amine nitrogen (N_9-H). Electron-withdrawing C_6-substituents, such as a nitro group, also located *para* to N_9, share this enhancing effect on N_9. However, since they do not lead to high receptor affinity binding, it appears that the local interactions of N_2 and the C_3-substituent with nearby cationic residues dominate the smaller electronic effect on interactions at N_9. Electrostatic potential mapping confirms the necessity of two larger, adjacent negative potentials for high affinity binding.

From these results, some aspects of the receptor site itself may be inferred. A model of the receptor site emerges that includes a large cation in the vicinity of N_2 and the C_3-substituent, such as an arginine residue or perhaps two lysine residues as the major discriminant of receptor affinity. Other interactions such as with an anionic aspartate or glutamate residue in the vicinity of N_9 and stacking interactions with a neutral tryptophan could also contribute to binding affinity but would not discriminate between analogs. Stacking interactions with a protonated histidine residue could be contributing if the histidine is sterically constrained.

If these results are correct, other analogs with electron-rich, H-bonding substituents at C_3, such as a 3-nitro-β-carboline, should also demonstrate high affinity binding. By the same reasoning, a 3-halo-β-carboline should be less active. Although electron-rich, it would not be able to form the specific H-bonding complexes with a guanidinium cation that the C_3-ester or C_3-cyano groups can.

Analogs with an oxygen or a sulfur atom substituted for the amine nitrogen in the β-carbolines might also have at least moderate receptor affinity similar to the N_9-CH_3-β-CCM analog.

ACKNOWLEDGMENT

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