MOLECULAR PHARMACOLOGY, 26:19–34

Theoretical Structure-Activity Studies of Benzodiazepine Analogues

Requirements for Receptor Affinity and Activity

GILDA H. LOEW, JOHN R. NIENOW, AND MICHAEL POULSEN
Life Sciences Division, SRI International, Menlo Park, California 94025

Received April 7, 1983; Accepted March 30, 1984

SUMMARY

Conformational and electronic properties of a series of 1,4-benzodiazepine anlogues and their specific interaction with a model cationic receptor site have been calculated using both empirical energy and semiempirical molecular orbital methods. The aim of these studies was to identify molecular properties and modes of receptor interaction which are determinants of relative receptor affinities and pharmacological activities for these anxiolytics. Analogues with variations in key positions of the 7-membered (B) ring, at positions C_7, C_8, and C_9 of the fused phenyl (A) ring, and at positions 2′ and 4′ of the phenyl (C) ring were examined. The results indicate that both active and inactive analogues have similar low-energy conformations, arguing against this property as a modulator of recognition at the receptor. However, calculated molecular electrostatic potentials together with explicit model receptor interactions allowed the deduction that interactions with three cationic receptor sites are required for high-affinity analogues. The specific cationic site interactions are postulated with electron-withdrawing groups at C_7, the $C_2{=}O_1$ group, and the imine nitrogen, N_4. Moreover, interactions of N_4 with a model cationic receptor site are enhanced by halogen substituents at C_2', but only when the phenyl ring is rotated by 30° toward a more planar conformer, corresponding to an induced conformational change. If this enhancement is important, a 2′-Cl substituent on more rigid analogues of the 1,4-benzodiazepines with increased co-planarity of the phenyl C-ring and the $C_1'{-}C_5{=}N_4$ plane should have an even greater differential effect on receptor affinity.

INTRODUCTION

The 1,4-BDZs[1] are widely used in the treatment of a variety of symptoms (1–3). Recently, a specific, saturable, high-affinity receptor site has been identified for the 1,4-BDZs in rat brain homogenate (4, 5).

Before the discovery of a specific BDZ receptor, many 1,4-BDZs were synthesized and tested for anticonvulsant, muscle relaxant, sedative-hypnotic, and anxiolytic activity, and a number of theoretical calculations were made (6–8) to determine the molecular properties that correlate to their relative activities in these four different pharmacological assays. Subsequently, studies of representative BDZs indicate a good correlation of specific binding affinity in brain homogenates with the typical activities of the congeners (5, 9). Very recently (10), a linear free-energy study was reported which indicated that the presence of the $C_2{=}O$ and N_4 groups make the most statistically significant group contributions to relative receptor affinity of 39 BDZs. Since they are thought to bind and act at specific receptor sites by forming noncovalent, reversible complexes, and since binding studies are carried out *in vitro*, relative affinities and activities of closely related analogues most likely reflect differences in receptor-congener interaction.

The studies reported here are a continued effort to understand the molecular discriminants of receptor affinity and activity of a series of closely related 1,4-BDZs (Tables 1 and 2) using the techniques of theoretical chemistry. In particular, they extended previous work in four ways: (a) use of calculated molecular electrostatic potential and explicit modeling of N_4-cationic receptor site interaction to explore further the role of electronic properties in modulating receptor affinity and to characterize further the receptor site; (b) extensive investigation of conformational properties, including B-ring variations and use of total geometry optimization; (c) further investigation of the role of C_7- and C_2'-substit-

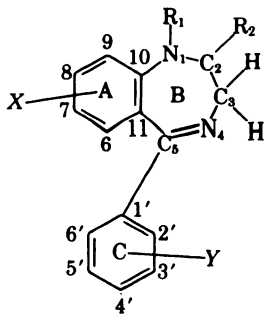
This work was supported in part by Grant DA02880-01 from the National Institute of Drug Abuse.

[1] The abbreviations used are: BDZ, benzodiazepine; 1,4-BDZ, 1,4-benzodiazepine; HOMO, highest occupied molecular orbital; LUMO, lowest occupied molecular orbital; MNDO, modified neglect of differential diatomic overlap; INDO, intermediate neglect of differential overlap; CNDO, complete neglect of differential overlap; MEP, molecular electrostatic potential; PTZ, pentylenetetrazole.

0026-895X/84/040019-16$02.00/0

TABLE 1

1,4-BDZs with known crystal structure



Compound	X	R_1	R_2	R_3	Y	ED_{50}, mouse anti-PTZ test[a]	K_i, rat whole forebrain[b]	Ref.
						mg/kg	*nM*	
1a Clonazepam	7-NO_2	H	=O	H_2	2′-Cl	0.3	1.9	15
1b Lorazepam	7-Cl	H	=O	OH	2′-Cl	0.2	2.3	17
1c Diazepam	7-Cl	CH_3	=O	H_2	H	2	8.9	11
1d Nitrazepam	7-NO_2	H	=O	H_2	H	0.7	19	16
1e	7-H	CH_3	=O	H_2	H	Inactive	90	14
1f Chlorodiazepoxide[c]	7-Cl	H	$NHCH_3$	H_2	H	8	574	13
1g Medazepam[d]	7-Cl	CH_3	H_2	H_2	H	7	3.850	12
1h	7-Cl	CH_3	=O	H_2	4′-F			14
1i[e]	7-Cl	CH_3	=O	H_2	2′,4′-Cl			18

[a] Data obtained from Randall *et al.* (2).

[b] Data from Baestrup and Squires (9).

[c] The anti-convulsant activity of medazepam is now known to be due to metabolism to diazepam [see Randall *et al.* (2)].

[d] Librium has $N_4 \rightarrow O$ and $N_2 = C_2$.

[e] This analogue has $HC_5—N_4CH_3$ in place of $C_5 = N_4$.

uents in modulating receptor affinity and activity; (d) further investigation of the role of other electronic properties and rotations about the C_5-C_1' torsion angle in determining relative receptor affinities and activities.

To examine possible conformational effects on relative affinity and activity, known X-ray structures (11–18) of the analogues listed in Table 1 with variations that could affect the conformation of the 7-membered ring or the rotation of the free phenyl ring were compared. In addition to comparisons of known X-ray structures, the presence of other low-energy conformers was explored. The analogues included in this study involve changes in rings B and C relative to diazepam (1c), particularly (a) substituents at positions 2′ and 4′ of the phenyl ring C, (b) the saturation of C_2 as in medazepam (1h), (c) the saturation of the N_4=C_5 bond as in analogue 1k, and (d) the multiple ring changes in chlorodiazepoxide (1g). In particular, B-ring variations that change the hybridization of key atoms in the 7-membered ring might be expected to have significant effects on the molecular conformation of the fused AB-ring system, and substituents on the C-ring could affect its rotational energy profile. Both possibilities were explored.

Three types of electronic properties of BDZ analogues were also calculated that could be molecular indicators of relative receptor affinities and activities. To this end, the series of 21 analogues (2a–2u) given in Table 2 were investigated. Although not all of the anticonvulsant activity and affinity data given in Table 2 are from the same laboratory, all follow similar protocols (19), so that the large qualitative differences in them are reliable. It is only these differences, rather than precise quantitative variations between high-affinity and high-activity analogues which we shall address. The analogues included in this study are active and inactive congeners with variation of substituent at position 7, 8, or 9; at position N_1; and at positions 2′ and 4′ of the C-ring. Semiempirical molecular orbital methods were used to calculate a number of properties of the isolated analogues, such as dipole moments, net atomic charges, and energies and electron distributions in the HOMO and LUMO. In addition, a second type of property, the molecular electrostatic potential, was calculated at specific points in regions and planes around selected analogues. This calculated potential is the energy of interaction of the entire molecule with a point-positive charge in the approximation that the charge does not affect the molecular potential, i.e., in a frozen-charge distribution.

Finally, explicit BDZ-model receptor interactions involving N_4 were characterized in two approximations, and the effect of a halogen in position 2′ on them determined. In one approximation, it was assumed that a proton transfer to N_4 occurs during BDZ interaction with the receptor. Proton affinities were calculated for analogues with a 2′-H, a 2′-Cl, and a 4′-Cl substituent as a function of rotation about that of the phenyl C-ring using semiempirical molecular orbital methods. In a second model, explicit interactions between N_4 and a cat-

TABLE 2

Analogues of 1,4-BDZs investigated

Compound	X	R_1	R_2	Y	ED_{50}, mouse anti-PTZ test[a]	K_i[b]
					mg/kg	*nM*
2a[c]	H	H	=O	H	>800	
2b	H	CH_3	=O	H	"Inactive"	90
2c	H	CH_3	=O	2′-Cl		
2d	H	CH_3	=O	4′-Cl		
2e	H	CH_3	=O	2′,4′-Cl		
2f	7-Cl	CH_3	=O	H	2	8.9, 6.2[d]
2g	7-Cl	CH_3	=O	2′-Cl	0.4	0.36[d]
2h	7-Cl	CH_3	=O	4′-Cl	>200	101,000
2i	7-Cl	CH_3	=O	2′,4′-Cl	"Inactive"	
2j	7-Cl	H	=O	H	6	8.8, 8.0[d]
2k	8-Cl	H	=O	H	334	
2l	8-Cl	CH_3	=O	H		
2m	7-NH_2	CH_3	=O	H	>400[e]	
2n	7-NO_2	H	=O	H	0.7	19
2o	7-NO_2	CH_3	=O	H	0.6	
2p	9-NO_2	H	=O	H	>800	
2q	9-NO_2	CH_3	=O	H		
2r	7-CF_3	H	=O	H	0.9	14
2s	7-CF_3	CH_3	=O	H		
2t	7-Cl	CH_3	H_2	H	7	3,850
2u	7-Cl	H	$-NHCH_3$	H	8	574

[a] Data compiled from refs. 2–5 and also supplied to us by Dr. W. E. Scott, of Hoffmann-La Roche.

[b] Receptor binding data compiled from Braestrup and Squires (9) K_i against [^{3}H]diazepam, where $K_i = IC_{50}/1 + \left(\frac{[^3H]\text{diazepam}}{K_d}\right)$

[c] Substitution of a CH_3 group for the phenyl C-ring in analogue 2a produces a known convulsant (RO 5-3663) with a 10-fold decrease in affinity; i.e., IC_{50} = 1000 nM in competition with [^{3}H]diazepam as communicated to us by Dr. W. E. Scott, of Hoffmann-La Roche.

[d] K_i against [^{3}H]flunitrazepam. Data from the present study.

[e] Activity reported for medazapam here has been shown to be due to metabolism to diazepam (ref. 3). Activity reported for 7-NH_2 diazepam is assumed to be due to metabolism from 7-NH_2 medazepam.

ionic receptor site, $(CH_3NH_3)^+$, were characterized assuming a hydrogen-bonded interaction between the two nitrogen atoms. The effect of a 2′-halo substituent and rotation of the phenyl group on the energy of BDZ receptor interaction was then determined using both empirical energy and semiempirical molecular orbital methods.

METHOD AND PROCEDURE

Total geometry optimizations were obtained using an empirical energy program called MOLMEC (20, 21) and the semiempirical molecular orbital method called MNDO (22) both of which are described in detail elsewhere (20–22). Both programs have been carefully parameterized using a large number of molecules with known structures to yield reliable geometries and relative energies for a variety of organic compounds. MOLMEC, developed primarily by Drs. DuChamp and Oie, uses a 7-term empirical energy expression which includes torsion angle, bond angle, and bond length variations as well as electrostatic, dispersion, and repulsion terms.

The X-ray structure of diazepam was used as initial input to the empirical energy program MOLMEC, and total geometry optimization was performed. Additional conformations of the B-ring were also used as initial input to MOLMEC, total optimizations were performed, and resulting energies and conformations were compared. In particular, the following variations were examined: (a) B-ring inversions through C_3; (b) Various values of the torsion angle $\tau(C_9\text{-}C_{10}\text{-}N_1\text{-}C_2)$, which determines the extent of co-planarity of atoms N_1 and C_2 with the fused aromatic ring A; and (c) changes in the local geometry of N_1, depending on whether an N_1-H or N_1-CH_3 substituent is present.

The other conformational property of BDZs addressed is the rotational energy profile of the phenyl ring (C). In these studies, MNDO-optimized geometries were obtained for analogues 2f, 2g, and 2h, and MNDO was used to calculate their heat of formation as a function of the torsion angle τ_5 ($N_4\text{-}C_5\text{-}C_1'\text{-}C_2'$). Total geometry optimization was then performed for the lowest-energy conformers obtained for each analogue to characterize them more completely.

The MNDO geometry-optimized lowest-energy conformer of diazepam (2f) was used as a basis for construction of initial geometries for all other analogues in Table 2. Total geometry optimization was then performed for each analogue, using MNDO.

MNDO-optimized geometries were used to calculate all electronic properties reported. Empirical energy programs such as MOLMEC do not yield electronic structures. Thus, use of the same method, i.e., MNDO for geometry optimizations and calculation of electronic structure, was thought to yield the most internally consistent set of electronic properties for comparison among analogues. Optimized geometries for uniform comparison of electronic structures are preferable to use of X-ray structures, which are unavailable for many analogues and usually contain different amounts of ambiguity for each known compound.

Using the MNDO method together with a Mulliken population analysis, net atomic charges, bond overlap densities, π orbital densities, energies and electron distributions in the HOMO and LUMO were calculated.

Molecular electrostatic potential mapping was also performed using MNDO-optimized geometries. However, orthogonalized INDO (23) orbital function was used for MEP calculations, since the current version of the mapping program (24, 25), described elsewhere (24, 25), is orders of magnitude faster with this orthogonalized basis set as input than from any molecular orbital procedure using deorthogonalized orbitals, such as MNDO. Orthogonalized, INDO-based MEP calculations have well-known weaknesses. However, their reliability for use in qualitative comparisons in planes containing polar atoms has been confirmed in a number of studies (24).

The possible effect of 2′-halogen substituents on N_4 receptor interactions was considered in three different ways: (a) MEP mapping was done around N_4 in the presence and absence of a 2′-F group at different phenyl C-ring orientations. (b) Protonation at N_4 was considered as one model for its interaction with a cationic receptor site. Total geometry optimization of protonated analogues of 2f, 2g, and 2h were obtained using MNDO. Proton affinities were calculated as a function of rotation of the phenyl C-ring for these three analogues by single-point MNDO calculations. These proton affinities are defined as the negative of the enthalpy of the reaction:

$$BDZ + H^+ \rightarrow [H\cdot N_4BDZ]^+$$

i.e.,

$$\text{Proton affinity} = [\Delta H_f(BDZ) + \Delta H_f(H^+)] - \Delta H_f[HN_4BDZ)^+$$

(c) Explicit interaction of N_4 with a model cationic receptor site $(CH_3NH_3)^+$ was characterized assuming an H-bonded interaction: $H_4\text{—}H\text{—}NH_3CH_3$. The energy of interaction for 2′-H and 2′-*X* BDZ analogues, *X* = F,Cl, as a function of rotation of the phenyl C-ring was calculated by three different methods. One was an empirical energy method contained in a program called MOLECULE (26, 27), which is a modified Scheraga-type empirical energy function expression (26) containing five terms: electrostatic, H-bonded, dispersion (Van DerWaals), repulsion, and torsion angle energy. It has been modified in our laboratory by Dr. Stanley Burt in collaboration with colleagues at NASA-Ames to include more atom types and to calculate geometry-optimized or single-point intermolecular interactions (27). This method yields intermolecular energies directly. To verify these results, two other methods were used, CNDO/2 and INDO, both of which are semiempirical quantum mechanical methods that yield energies of interaction by differences in the calculated energies of reactants and products. The specific reaction used for complex formation was: BDZ + $(NH_3CH_3^+) \rightarrow (BDZ\cdot CH_3NH_3)^+$. In this calculation, interaction geometries were fixed in those obtained by the empirical energy program with intermolecular geometry optimization. Prior analysis of the

strengths and weaknesses of these methods in characterizing H-bonding interactions (28) indicate that they cannot be used to obtain reliable geometry, always underestimating the heavy atom distance and hence overestimating interaction energies. However, if reliable H-bonding geometries are used, these methods yield reasonable interaction energies.

RESULTS AND DISCUSSION

Conformation

The known X-ray crystal structures of analogues 1a–1i shown in Table 1 were examined for overlapping regions by use of the graphics capabilities linked to the MOLECULE structure-generating library. All nine structures were found to be essentially indistinguishable. Fig. 1 illustrates this similarity by showing the superposition of the diazepam (1c) crystal structure with that of analogues 1f, 1g, and 1i.

In addition to the conformations shown, the unit cells of analogues 1f, 1g, and 1i contain a second molecule related to the first by an inversion through atom C_3. Thus X-ray structures resembling both diazepam and "inverted" diazepam are found.

Total geometry optimizations using the empirical energy program MOLMEC and a variety of initial geometries performed for most des-7-Cl analogs of analogues listed in Table 1 led to only two types of low-energy conformers very similar to the two X-ray structures and with nearly identical energies (within 0.5 kcal/mole). To confirm these results, the MNDO method was used to optimize the geometry of many conformers for diazepam. Again, the same two conformers with enthalpies of formation differing by ~1 kcal/mole were the only minima found. These results are consistent not only with X-ray structures, but with NMR studies of diazepam (29) and des-methyl diazepam (29), indicating the presence of two interconverting conformers corresponding to B-ring inversion.

The two equal low-energy conformers are shown in Fig. 2 together with values of the B-ring torsion angles. There were no coplanar minima; i.e., no stable conformtions with $\tau(C_9\text{-}C_{10}\text{-}N_1\text{-}C_2) = 180°$ were found. Thus, differences in receptor affinity between diazepam, chlorodiazepoxide, and medazepam (values of K_i = 8.9, 574, and 3850 nM, respectively) appear to be due to stereoelectronic effects of changes of substituent in the B-ring rather than conformational differences in the B-ring itself.

From NMR studies of diazepam and N_1-H diazepam (29), it was proposed that the substituent on N_1 plays an important role in modulating the B-ring inversion and, in particular, it was suggested that the N_1 atom in the N_1-H analogue had more sp^2 character than in the N_1-CH_3 analogue, facilitating the inversion. To investigate this hypothesis further, MOLMEC-optimized geometries of analogues 2a and 2b (Table 2) were compared in detail. The results indicate that substituting a CH_3 group for a hydrogen group on N_1 does somewhat affect the B-ring geometry around the N_1 atom. However, it is in the N_1-CH_3 analogue (2b) that the hybridization of N_1 appears to be closer to sp^2. Bond angles around N_1 are close to 120°, and torsion angles of the N_1 substituents differ by about 170° as compared with the ideal sp^2 hybridization value of 180°. For the N_1-H analogue (2a), the geometry of N_1 appears to be a "mixture" of sp^2 and sp^3 hybridization. The exocyclic bond angles around N_1 decrease to values of 114° and 115°. The N_1 substituent torsion angles differ by 154°, a value between sp^2 (180°) and sp^3 (120°) hybridization. This difference in "hybridization" appears to be determined by the steric requirements of

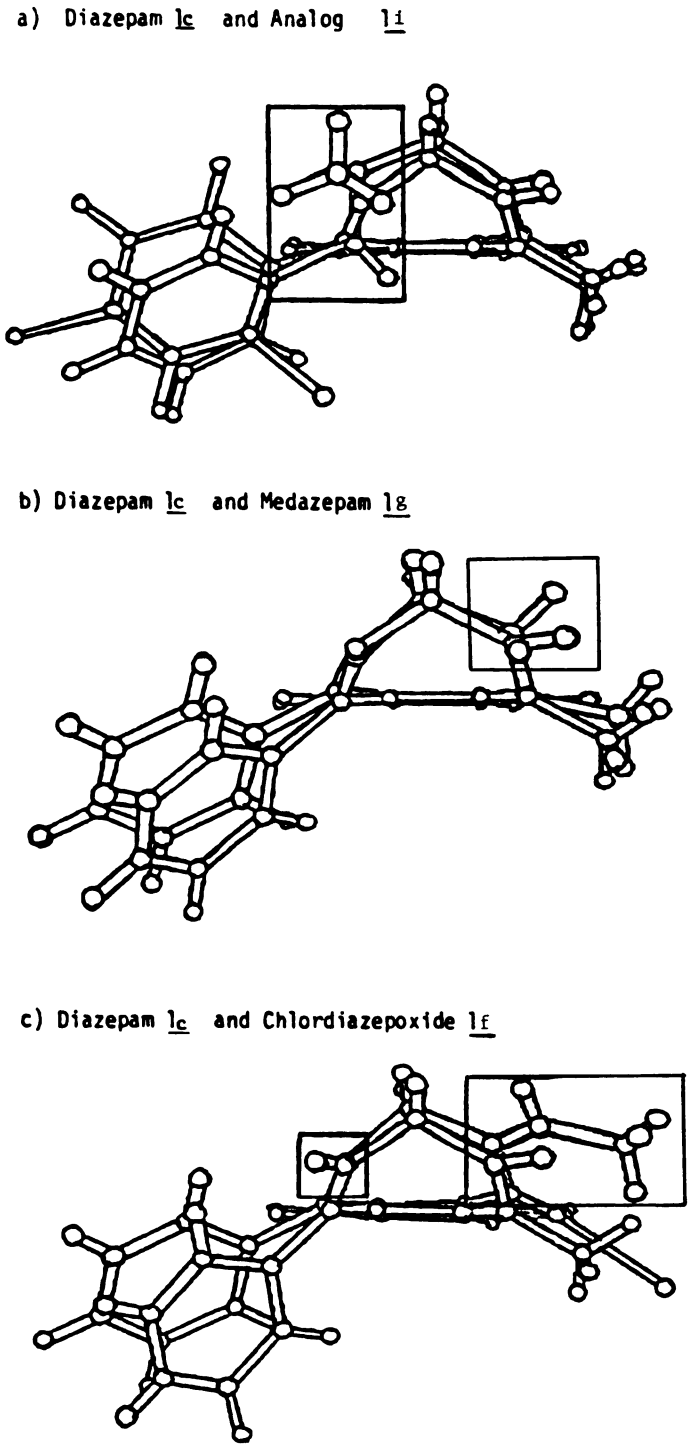


FIG. 1. *Superposition of crystal structures of three 1,4-BDZ analogues with diazepam*
Areas of conformational difference are *boxed.*

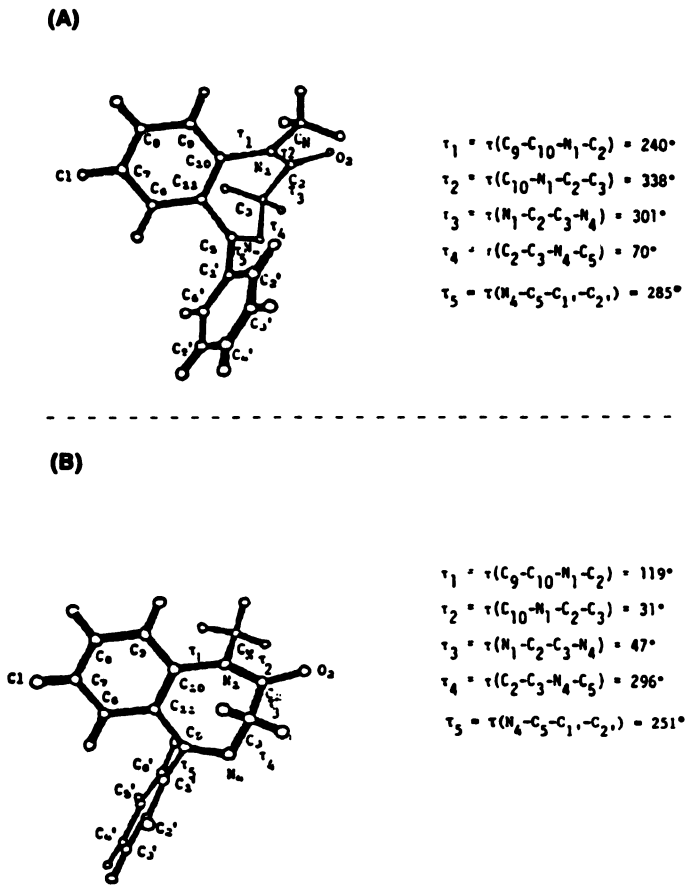


FIG. 2. *Structures of the two low-energy conformations of diazepam (analogue 1c)*

The torsion angle is measured by viewing down the axis formed from atoms 2 and 3 and rotating counterclockwise from axis formed by atoms 1 and 2 to the axis formed by atoms 3 and 4. A. Optimized X-ray crystal structure. B. Optimized "inverted" structure.

the methyl substituent. The carbon atom of the bulky methyl group does not remain in the plane of the A-ring, but moves 60° out of the plane because of repulsive interaction with the hydrogen on C_9 and with the $C_2{=}O$ carbonyl group. Since $\tau(C_9\text{-}C_{10}\text{-}N_1\text{-}C_2)$ remains at ~240°, sp^2 hybridization of N_1 results. With the N_1-H analogue, the hydrogen remains in the plane of the A-ring, thus moving the N_1-hybridization toward sp^3. This slight difference in hybridization and local N_1 geometry does not appreciably affect the π electron distribution ($\Delta\rho \leq 0.02e$) except at N_1 itself. The N_1-CH_3 analogue has a π electron density of 1.285*e*, whereas the N_1-H analogue has a π density of 1.443*e*. It is not likely that these small differences are important factors in the rate of ring inversion. It could account for the slight difference in anti-PTZ activity evidenced by the two analogues, the sp^2 hybridization being favored. However, in general, there is very little difference in affinity and activity between pairs of N_1-H and N_1-CH_3 analogues (2a, b; 2f, j; 2n, o, Table 2).

Results of systematic rotations of the phenyl ring for analogues 2f, 2g, and 2h, using MNDO with optimized geometries, are shown in Fig. 3a. These three analogues all have two essentially equal energy minima at $\tau(N_5\text{-}C_5\text{-}C_1'\text{-}C_2') \cong 120°$ and 300°. Large energy barriers between these two minima with maxima when the phenyl ring is 60° from the fused A- and B-rings are found. The enthalpic barriers increase from 18 kcal/mole for 4′-Cl-diazepam to 37 Kcal/mole for diazepam to 196 kcal/mole for 2′-Cl-diazepam. These results are consistent with previously reported values (9) using the CNDO method of a 20 kcal/mole barrier for 2′-H- and 148 kcal/mole for 2′-F-1,4-BDZ between minima at $\tau \sim 90, 270$. Total geometry optimization of the low-energy conformer with $\tau_5 = 300°$ led to a torsion angle $\tau(N_4\text{-}C_5\text{-}C_1'\text{-}C_2') \cong 284$, closer to a perpendicular conformer ($\tau = 270°$) for all three analogues. Thus, neither a 4′-Cl nor a 2′-Cl substituent affects the equilibrium conformations of the C-ring. These results suggest that the enhanced receptor affinity and activity of 2′-Cl and the greatly diminished activity of the 4′-Cl analogues are due to specific stereoelectronic requirements of local receptor subsites near these positions or to the way in which their presence affects receptor interactions in other regions.

Electronic Properties

The set of calculated electronic properties of the isolated molecules examined as discriminators of receptor affinity and pharmacological activity were (a) dipole moments; (b) net atomic charges; (c) π electron densities; (d) the energy and nature of the HOMO that could be involved in electron donation to electrophilic receptor sites, and the energy and nature of the LUMO that could be involved in electron acceptance from nucleophilic receptor sites; and (e) MEP energy contours.

Electron distributions and dipole moments were calculated for geometry-optimized structures of analogues 2a–2u using MNDO.

Calculated dipole moments. As a test of the reliability of MNDO in describing the substituted analogues, the dipole moments and heats of formation of chlorobenzene, nitrobenzene, and aminobenzene were calculated and the results compared with experimentally available values as shown in Table 3. While not perfect, agreements between experimental and calculated dipole moment values and heats of formation are good enough for reliable prediction of relative behavior of these properties.

Examining calculated dipole moments (Table 4) for geometry-optimized structures of analogues 2a–2u, we see that values for low-affinity or inactive analogues are, in general, significantly larger than those of active analogues. Substituent variation on the A-ring in particular shows this correlation; i.e., the larger the dipole moment, the lower the activity. A major exception to this correlation were analogues with a 4′-Cl substituent such as 2h, which had a K_i of 101,000 nM and a dipole moment of 2.73 Debye. This exception supports the notion that the inactivity of 4′-Cl analogues is due to a very specific localized receptor requirement at the subsite. Another exception is medazepam, with a low dipole moment (2.26 Debye) and a very low receptor affinity (K_i = 3850 nM), indicating a $C_2{=}O$ group as a specific requirement for high affinity.

This general correlation obtained confirms similar results of a previously reported study (6) in which multiple regression analysis was used to correlate calculated elec-

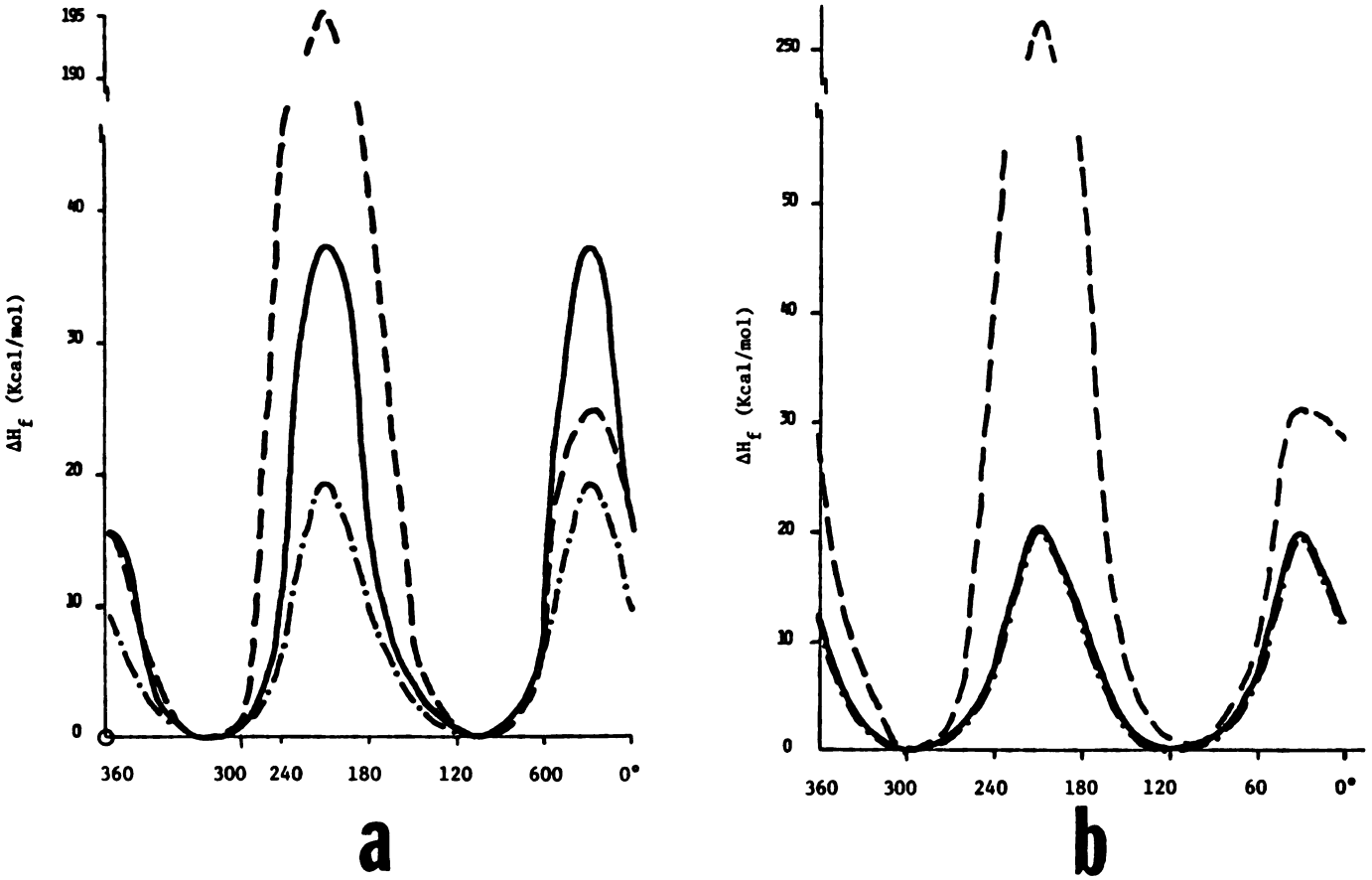


FIG. 3. *MNDO calculated C-ring rotational energy profiles for unprotonated BDZ analogues (a) and N_4-protonated BDZ analogues (b)*
——, Diazepam; - - -, C_2'-Cl diazepam; -·-·-, C_4'-Cl diazepam.

TABLE 3

Comparison of calculated and experimental dipole moments (DM) and heats of formation of chloro-, nitro-, and aminobenzene

Compound	Exp. DM[a]	Calc. DM	Exp. ΔH_f	Calc. ΔH_f
	Debye	*Debye*	*kcal/mole*	*kcal/mole*
ϕ-Cl	1.69	1.87	12.5[b]	13.3
ϕ-NO_2	4.22	5.39		
ϕ-NH_2	1.53	1.48	20.8[c]	21.7

[a] Compiled from ref. 30.
[b] Compiled from ref. 31.
[c] Compiled from ref. 32.

tronic indices with the anticonvulsant, sedative-hypnotic, muscle relaxant and anxiolytic activities of 59 BDZs. In that study, the dipole moment calculated by the CNDO method gave the only significant correlation.

This correlation has two implications: (a) It could imply a competitive, nonspecific binding process involving dipole interactions which lowers the apparent activity of BDZs. (b) It could mean that values of the molecular dipole moment reflect the presence and favorable orientation of electronegative substituents located at certain key sites required for receptor interaction, in this case, specifically at positions 7 and 2′. This latter conclusion is consistent with the results of electrostatic potential mapping and explicit drug-receptor interactions of the BDZs presented below.

Net atomic charges. Net atomic charges, typical for the 1,4-BDZs, are given for diazepam (2f) in Fig. 4. From Fig. 4, we see that the most negatively charged atoms are $N_1 > O_2 > N_4$, and the most positive atoms are $C_2 > C_5 > C_{10}$. In addition, for analogues with an H on N_1, this H is also quite positive; i.e., for analogue 2j, $q_H = +0.246$. Although this H has been proposed to be important in receptor interactions, similar receptor affinities and activities for $N_1 - CH_3$ and $N_1 - H$ analogues argue against a specific requirement for an "acidic" hydrogen.

A Cl substitutent in any position has a slightly negative charge and orbital population that resembles atomic chlorine: i.e., in atomic Cl, $(3s)^2\ (3p^5)$; in BDZ Cl, $(3s)^{1.98}\ (3px)^{1.79}\ (3py)^{1.35}\ (3pz)^{1.98}$.

Net atomic charges and π electron distributions of the A-ring, N_1, and C_5 are summarized in Table 5 for five analogues closely related to diazepam. As seen in Table 5, the presence or absence of a Cl substituent on position 7 or 8 of the A ring has only a local effect on total charge distribution and π electron distribution. Similarly, the presence of a Cl substitutent at position 2′ or 4′ of the C-ring has only a local effect on total charge distribution.

The other 7-substituted analogues (2m, 2n, and 2r) show results expected for electron-donating and electron-withdrawing groups, respectively.

π Electron densities. The π electron distribution for the A-ring, C_5, and N_1 in diazepam (Fig. 4) indicates two

TABLE 4

Correlation of calculated dipole moment (DM) with anticonvulsant activity of 1,4-BDZ analogues

A. A-Ring substituent variation

Analogue[a]	X	DM	ED_{50}, mouse anti-PTZ test	K_i
		Debye	*mg/kg*	*nM*
1. 2n (2o)	7-NO_2	2.2	0.7 (0.6)	19
2. 2j (2f)	7-Cl	2.6 (2.6)	6 (2)	8.8 (8.9)
3. 2r (2s)	7-CF_3	(2.8)	0.9	
4. 2k (2l)	8-Cl	3.4 (3.5)	334	
5. 2a (2b)	7-H	4.9 (4.4)	>800 ("inactive")	(90)
6. — (2m)	7-NH_2	(6.7)	>400	
7. 2p (2q)	9-NO_2	7.3	>800	

B. B-Ring substituent variation

Analogue	DM	ED_{50}, mouse anti-PTZ test	K_i
	Debye	*mg/kg*	*nM*
1. 2t Medazepam	2.26	7	3,850
2. 2f Diazepam	2.6	2	8.9
3. 2u Chlordiazepoxide	13.1	8	574

C. C-Ring substituent variation

Analogue	X	Y	DM	ED_{50}, mouse anti-PTZ test	K_i
			Debye	*mg/kg*	*nM*
1. 2f	7-Cl	2′,4′-H	2.6	2	8.9
2. 2h	7-Cl	4′-Cl	2.7	>200	101,000
3. 2i	7-Cl	2′,4′-Cl	2.7	"Inactive"	
4. 2g	7-Cl	2′-Cl	3.4	0.4	0.36
5. 2d	7-H	4′-Cl	4.3		
6. 2b	7-H	2′,4′-H	4.4	"Inactive"	90
7. 2e	7-H	2′,4′-Cl	4.6		
8. 2c	7-H	2′-Cl	5.0		

[a] Results are given for pairs of N_1-H and N_1-CH_3 analogues, labeled as in Table 2, with the value for the N_1-CH_3 analogue shown in parentheses.

electrons in the Cl(π) orbital, an electron-rich $N_1(\pi)$ orbital, and an electron-deficient C5(π) orbital. These characteristics of $N_1(\pi)$ and $C_5(\pi)$ orbitals persist for all analogues investigated (Table 5).

Energy and nature of HOMO and LUMO. All of the HOMO and LUMO are essentially π orbitals, centered on either the A-ring or the C-ring, with some heteroatom participation. The energies and nature of the four highest filled and four lowest empty orbitals are given in Table 6 for diazepam. The nature of the orbitals is expressed as percentage of electron density on the indicated atomic orbitals of the molecular orbital calculated from a Mulliken population analysis.

The nature and energy of the lowest-energy unoccupied orbitals and highest-energy occupied orbitals (HOMO −3 to LUMO +3) do not vary in any consistent or significant manner with the presence or absence of substituents on the A- or C-rings. A typical example of the effects of these substituents is given in Table 7, using variations in the nature and energy of HOMO. These results are consistent with those of previous investigations in which no significant correlation was found between measure of the four biological activities and lipophilicity, and total electronic charge on any atom, HOMO or LUMO energies (6).

The results from the electronic investigations, taken together with the conformational results, strongly indicate that the striking modulation of affinity and activity of A-ring and C-ring substituents is not primarily due to the effect of these substituents (a) on the charge distribution of the remaining portions of the benzodiazepines, or (b) or their conformation. Rather, the effect of these substituents appears to be due to their own localized interaction with specific receptor subsites, or to modulation of receptor interactions of other polar regions of the molecule.

MEP energy contours. MEP mapping was performed for active and inactive analogues with $C_7 - X$, where X = H, NH_2, F, CF_3, NO_2, and $C_2' - Y$, where Y = H and F, to investigate further the postulated role of substituents in interactions with specific receptor subsites and in modulation of receptor interactions of other polar groups of the 7-membered ring.

The focus of the investigation centered on two themes: the nature of the MEP contour near the 7-substituent of the A-ring, and the effect of A-ring and C-ring substituents on the MEP in the vicinity of the heteroatoms N_1, O_2, and N_4 of the 7-membered B-ring. To this end, electrostatic potential contour maps were obtained in three planes: (a) in the plane of the A-ring, (b) in the C_3—N_4═C_5 plane, and (c) in the N_1—C_2═O plane.

Figure 5A and B shows the results of electrostatic potential mapping in the plane of the A-ring, with the C_7 substituent at the center of the region mapped, for two inactive analogues (C_7-H and C_7-NH_2). As seen in Fig. 5, these inactive analogues were found to have a largely positive potential around the area of the 7-substituent. In both cases the molecule exhibits a uniformly decreasing positive potential and around the A-ring hydrogens. The shapes of the potentials are very similar, the only difference being the extension of the positive potential away from C_7 owing to the more bulky NH_2 group.

By contrast, all of the low-energy conformers of the three active analogues shown in Fig. 6A, B, and C were found to have a large negative potential in the plane of the A-ring. For both the 7-F and 7-NO_2 analogues, two symmetrical potential minima are found at distances of about 4.7 AU and 6.1 AU, respectively, from the C_7 atom (1 AU = 0.529Å). Although the magnitudes of the minima for the C_7-CF_3 analogues are smaller, at a distance of 5.6 AU from the C_7 atom, they increase substantially in the plane just below the A-ring that contains the other two fluorine atoms. In all three analogues, the orientation of these two minima, the shape of the zero contour, and the area covered by the negative potential envelope are very similar. It is this similar directionality of the two minima, as well as their large values, that could be efficacious for interaction with a specific cationic receptor subsite.

Electrostatic potential mapping in the N_1—C_2═O_2 plane, as shown in Fig. 7A and B, reveals a large negative potential in this plane, and a long-range effect of C_7-substituents. As shown in Fig. 7A, with a C_7-H substituent, there is a large negative potential region around O_2

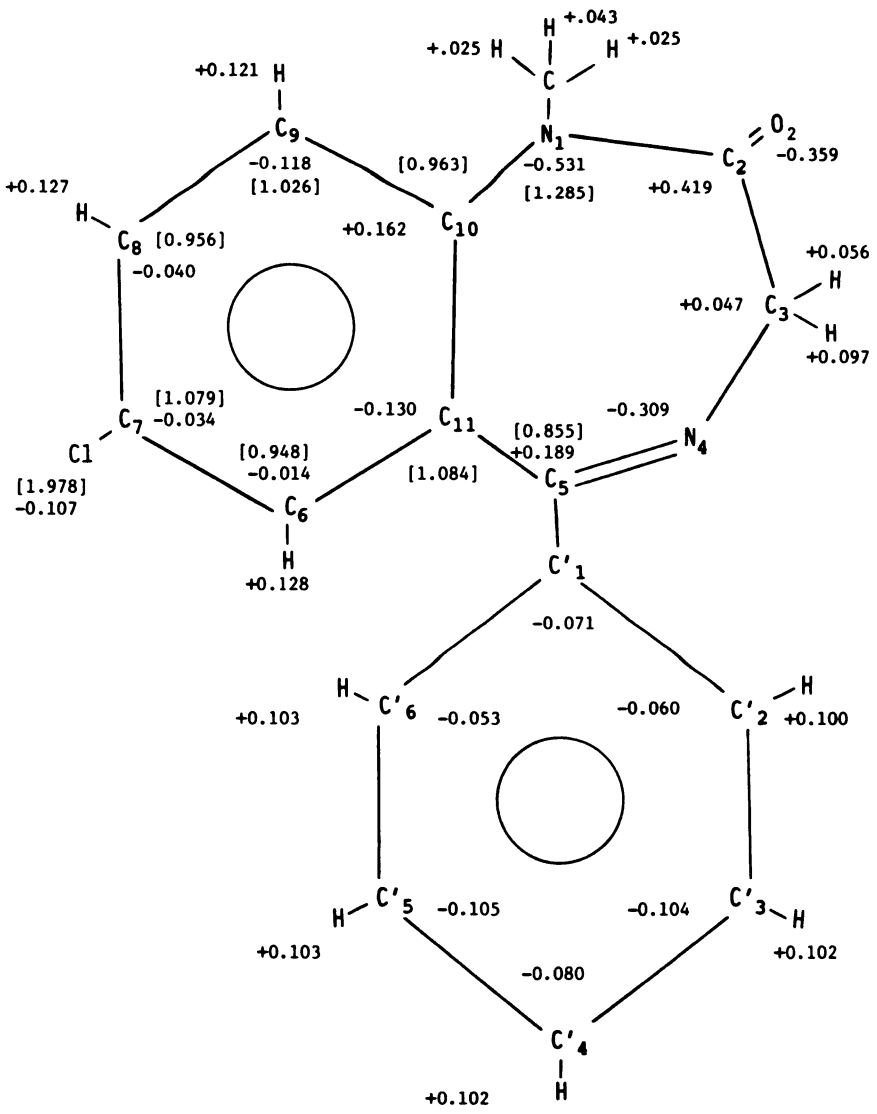


FIG. 4. *Calculated net atomic charges and π orbital densities for diazepam*
Net atomic charges are preceded by plus or minus signs, and π orbital electron densities are shown in *brackets*.

with a maximal value of −111 kcal/mole. With a C_7-F substituent (Fig. 7B), this maximum is reduced to −103 kcal/mole.

For medazepam and chlorodiazepoxide, there is no negative potential around position C_2 in the N_1-C_2-*X* plane, since the carbonyl group is replaced by C_2-H_2 in medazepam, and by C_2-$NHCH_3$ in chlordiazepoxide. Their greatly diminished receptor affinity relative to diazepam-type BDZs strongly indicates that the large negative potential generated by the C_2═O group is very important to receptor affinity, and implicates another cationic receptor site in this region.

Electrostatic potential contours around N_4═C_5 in the plane defined by C_3—N_4═C_5 are given in Fig. 8A and B for C_7—H and C_7—F analogues. For both analogues, a large negative potential was found around N_4, due mainly to the lone pair of electrons of the imine N in this plane. Shown in Fig. 8A and B is the diminished magnitude of this potential due to the electron-withdrawing group at C_7. As shown in Fig. 8B, the 7-F analogue was found to have a minimum reduced by 6–7 kcal (107 kcal/mol versus 101 kcal/mole, respectively).

There was no significant effect of 4′-F on the electrostatic potential of any polar groups on the A- or B-ring. Thus, the greatly diminished affinity and activity of the 4-halo analogues appears to be due to a specific unfavorable interaction at that site, presumably with an anionic receptor subsite.

To investigate fully the effect of a 2′ substituent on the MEP around N_4, three phenyl ring conformers of a C_7-F, C_2′-F analogue were mapped, as shown in Fig. 9a, b, and c: (a) the minimum conformer, corresponding to τ_5 (N_4-C_5, C_1′-C_2′) = 284° with the C-ring nearly perpendicular to the B-ring; (b) a conformer with τ_5 (N_4-C_5-C_1′-C_2′) = 316°, which is only 2.3 kcal/mole higher in energy than the minimum energy conformer and represents the optimum energy of interaction with a cationic receptor site; and (c) an energy conformer with forced planarity [τ_5 (N_4-C_5-C_1′-C_2′) = 0] of the C-ring and N_4 of the B-ring, which is 15 kcal/mole higher in energy

TABLE 5

Net atomic charges and π electron density distributions in five BDZ analogues

qi (πi)	Analogue				
	2f	2l	2g	2h	2m
N_1	−0.53 (1.29)	−0.53 (1.28)	−0.53 (1.29)	−0.53 (1.29)	−0.50 (1.30)
C_1	+0.17	+0.17	+0.17	+0.17	+0.16
C_{10}	+0.16 (0.96)	+0.16 (0.97)	+0.17 (0.96)	+0.16 (0.96)	+0.09 (1.06)
C_9	−0.12 (1.03)	−0.10 (1.03)	−0.12 (1.03)	−0.12 (1.03)	−0.06 (0.98)
C_8	−0.04 (0.96)	+0.02 (1.03)	−0.04 (0.95)	−0.04 (0.95)	−0.15 (1.08)
C_7	−0.03 (1.08)	−0.09 (1.01)	−0.03 (1.08)	−0.03 (1.08)	+0.20 (0.93)
X^a	−0.12 (1.98)	−0.10 (1.98)	−0.11 (1.98)	−0.10 (1.98)	−0.54 (1.86)
C_6	−0.01 (0.95)	−0.04 (0.96)	−0.02 (0.95)	−0.01 (0.95)	−0.13 (1.07)
C_{11}	−0.13 (1.08)	−0.13 (1.07)	−0.13 (1.09)	−0.13 (1.09)	−0.08 (1.04)
C_5	+0.19 (0.86)	−0.19 (0.86)	+0.19 (0.86)	+0.19 (0.86)	+0.15 (0.86)
N_4	−0.31	−0.31	−0.31	−0.31	−0.28
C_3	+0.05	+0.05	+0.05	+0.05	+0.02
C_2	+0.42	+0.42	+0.42	+0.42	+0.43
O_2	−0.36	−0.36	−0.36	−0.36	−0.39
C_1'	−0.07	−0.07	−0.04	−0.06	−0.07
C_2'	−0.06	−0.06	+0.03	−0.06	−0.06
C_3'	−0.10	−0.10	−0.08	−0.08	−0.10
C_4'	−0.08	−0.08	−0.08	+0.00	−0.08
C_5'	−0.11	−0.11	−0.10	−0.08	−0.11
C_6'	−0.05	−0.05	−0.05	−0.05	−0.06
Y^b	—	—	−0.12	−0.11	—

[a] X = Substituent atom on C_7 or C_8 in the different analogues.
[b] Y = Cl substituent on C_2' or C_4'.

than the minimal energy conformation. Both of these higher energy conformers would correspond to induced conformational changes at the receptor site.

Comparing the MEP in Fig. 9a with that of Fig. 8B (with a C_2'-H substituent), we see that the presence of the C_2'-F substituent diminishes the maximal energy of attraction with a point charge from −101 to −97.4 kcal/mole. This energy remains very similar (−95 kcal/mole) when the phenyl C-ring is rotated by 180° to its other local minimum.

TABLE 6

Nature and energy of HOMO and LUMO of Diazepam

Diazepam orbital	Energy of orbital	Electron distribution in orbital[a]
	eV	
HOMO −3	−10.1	84% Ring-A, 6% N_4
HOMO −2	−9.6	92% Ring-C
HOMO −1	−9.6	85% Ring-C, 9% ring-A, 2% N_1
HOMO	−9.5	53% Ring-A, 11% ring C, 4% Cl, 17% N_1
LUMO	−0.8	85% Ring-A, 6% N_4, 4% C_5
LUMO +1	−0.5	95% Ring-A
LUMO +2	−0.07	86% Ring-C, 2% N_4
LUMO +3	+0.1	96% Ring-C

[a] Orbital distributions are defined in terms of percentage of π electron density contribution from each participant atom in the three ring systems (rings A, B, and C; see Table 1.) as calculated from a Mulliken population analysis. Substantial π contributions from the heteroatoms have also been included.

However, when the phenyl group is allowed to relax toward planarity by 30° (Fig. 9b) and then by the full 76° from its minimum energy conformer (Fig. 9c), the maximal value of the negative potential increases to −102 and then to −125 kcal/mole. Thus, "induced" conformations requiring 2.3 and 15 kcal/mole result in increases of 4.6 kcal/mole and 27.6 kcal/mole, respectively, in the maximal value of MEP obtained. This enhanced negative electrostatic potential is indicative of an enhanced affinity of N_4 to a point-positive charge. However, steric factors involved in the explicit interactions of an extended cationic site with N_4 could be important in determining the net effect of the 2′-halogen group on receptor affinity.

Model Drug-Receptor Interactions

Drug-receptor interactions were modeled in two approximations. (a) In one, the assumption was made that protonation of N_4 is a good model for its interaction with a cationic receptor site. (b) In the second, this drug-receptor interaction was explicitly characterized by an H-bonded type intermolecular complex between N_4 and a model cationic receptor site $(CH_3\ NH_3)^+$ of the form N_4—H-NH_2 CH_3. In the first study the proton affinity of complex formation and in the second study the stability of complex formation were calculated as a function of rotation of the phenyl C-ring for BDZ analogues with a 2′-H and a 2′-halogen substituent.

Protonation of BDZ at N_4. As shown in Fig. 3b, both the 2′-H and 2′-Cl protonated analogues at N_4 have the same minimum energy conformers. Moreover, in comparing Fig. 3a with Fig. 3b, we see that these two mini-

TABLE 7

Comparison of the nature and energy of the HOMO in various BDZ analogues

X[a]	R_1	R_2	Y	Energy of HOMO	% Ring-A[b]	% Ring-C	Other[c]
				eV			
H	CH_3	=O	H	−9.39	65	0	20% N_1, 4% O_2
7-Cl	CH_3	=O	H	−9.57	64	16.5	8.2% N_1, 3% O_2, 4.0% Cl_7, 1.2% N_4
8-Cl	CH_3	=O	H	−9.60	37	38.4	12% N_1, 1.6% O_2, 2% N_4, 1.2% Cl_8
7-Cl	CH_3	=O	2′-Cl	−8.52	62	0	21% N_1, 3.6% O_2, 4.8% Cl_1, 8% Cl_2
7-Cl	CH_3	=O	4′-Cl	−9.67	58	0	20% N_1, 3.5% O_2, 5.0% Cl_7, 8% Cl_4, 1% N_4
7-Cl	CH_3	=O	2′,4′-Cl	−9.64	59	0	22% N_1, 5% Cl_7, 4% O_2
7-NH_2	CH_3	=O	H	−8.68	64	0	24.1% N_7, 6.7% N_2, 2% O_2
7-CF_3	CH_3	=O	H	−9.78	1.1	88	2.4% N_4
7-NO_2	CH_3	=O	H	−9.88	0	92	
9-NO_2	CH_3	=O	H	−9.94	0	88	2.7% N_4

[a] Substituents reference to the BDZ figure, Table 1.

[b] Orbital natures are defined in terms of % π electron density contribution from each participant atom in the three-ring systems (see Table 1), as calculated from a Mulliken population analysis.

[c] π-Contributions from important heteroatoms have been included.

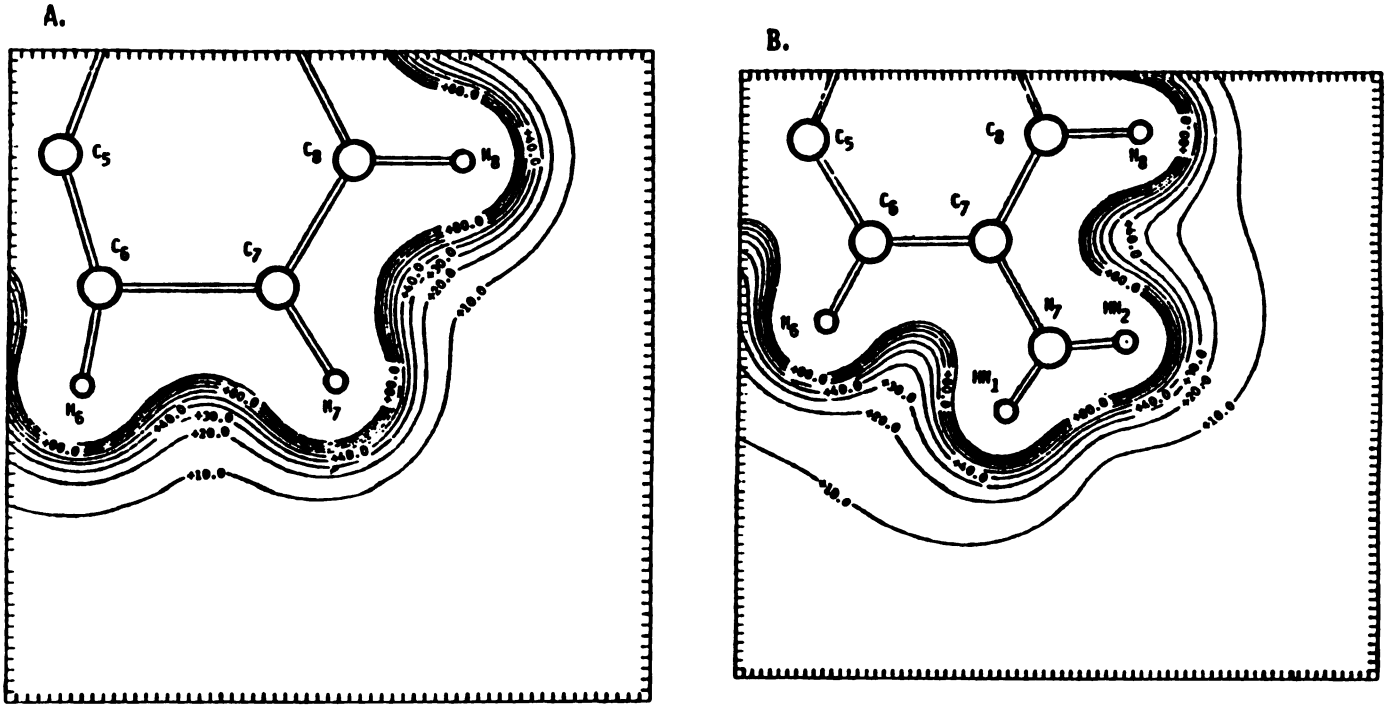


FIG. 5. *Calculated MEP maps of two inactive 1,4-BDZ analogues*

Shown are contours in energy units of kilocalories per mole within a 6 AU (1 AU = 0.529 Å) square region around the C_7-*X* substituent, in the plane of the A-ring. A. C_7-H Diazepam analogue (2b). B. C_7-NH_2 analogue (2m).

mum energy conformers are the same as for the unprotonated analogues. Only the barrier between the minima is substantially increased for the protonated species. Since this barrier is already high for the parent compounds, analogues protonated at N_4 are predicted to have the same equilibrium conformers as the parent compounds.

Proton affinities as a function of phenyl group rotation were also calculated for 2′-H, 2′-Cl, and 4′-Cl analogues using MNDO. As shown in Table 8, the presence of either a 2′-Cl or 4′-Cl group decreases the proton affinity of N_4 at every calculated value of C-ring rotation. Thus, if protonation is a good model for N_4 interaction with a receptor site, the presence of a 2′-Cl or 4′-Cl weakens this interaction, and partial rotation toward planarity does not change this result. Since Cl substituents in these two positions have opposite effects on affinity, these results argue against full protonation as a key feature of drug-receptor interaction.

Model cationic receptor site BDZ interactions. Using $CH_3NH_4^+$ as a model for a lysine-type cationic receptor, its energy of interaction with N_4 in the H-bonded complex shown in Table 9 was calculated as a function of phenyl ring rotation and $C_3N_4H_c$ bond angle variation, using the empirical energy program MOLECULE (26, 27). As shown in Table 9, three significant results were obtained: (a) In the minimum energy conformer of the phenyl ring (τ = 284°), the presence of a C_2′-Cl group

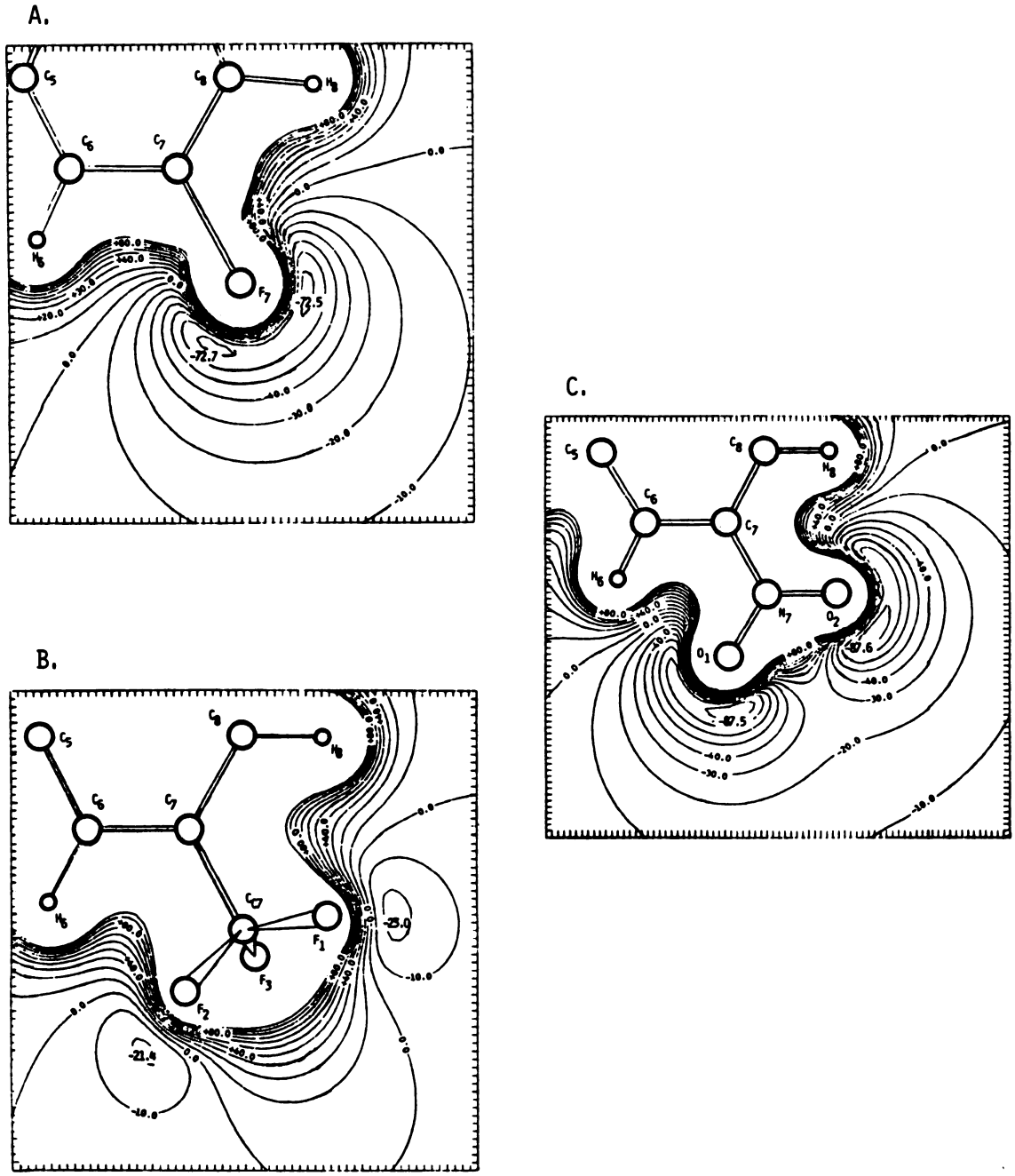


FIG. 6. *Calculated MEP maps of three active 1,4-BDZ analogues*

Shown are contours in energy units of kilocalories per mole within a 6-Bohr square region around the C_7-*X* substituent, in the plane of the A-ring. F substituents are substituted for Cl substituents. A. C_7-F analogue. B. C_7-NO_2 analogue (2o). C. C_7-CF_3 analogue (2s).

diminishes N_4 interaction with a cationic site. This result is in keeping with the electron-withdrawing ability of the Cl substituent. (b) Rotation of the phenyl C-ring toward planarity in the 2′-H analogue does not enhance the N_4-cationic receptor interaction. (c) Rotation of the phenyl C-ring toward planarity of the 2′-Cl does enhance the N_4-cationic receptor interaction, with optimal interaction occurring at $\tau = 316°$.

These results suggest that, in its minimum energy conformer, the 2′-halogen substituent of a 1,4-BDZ would diminish the interaction of N_4 with a model cationic receptor site. This result is in keeping with its effect on the proton affinity at N_4 and on the MEP. However, rotation of the phenyl C-ring toward planarity enhances the N_4-cation interaction of a 2′-halogen analogue but not that of a 2′-H analogue. Although the MEP contours in Fig. 9a–c suggest that total planarity is more effective for point cationic interactions, with an extended cationic site, interaction is a maximum when the phenyl group is rotated 30° toward planarity, apparently representing an optimal balance between steric and electronic factors.

In order to verify these results further, two related semiempirical molecular orbital methods (CNDO, INDO) were used to calculate interaction energies using two optimized intermolecular geometries obtained from

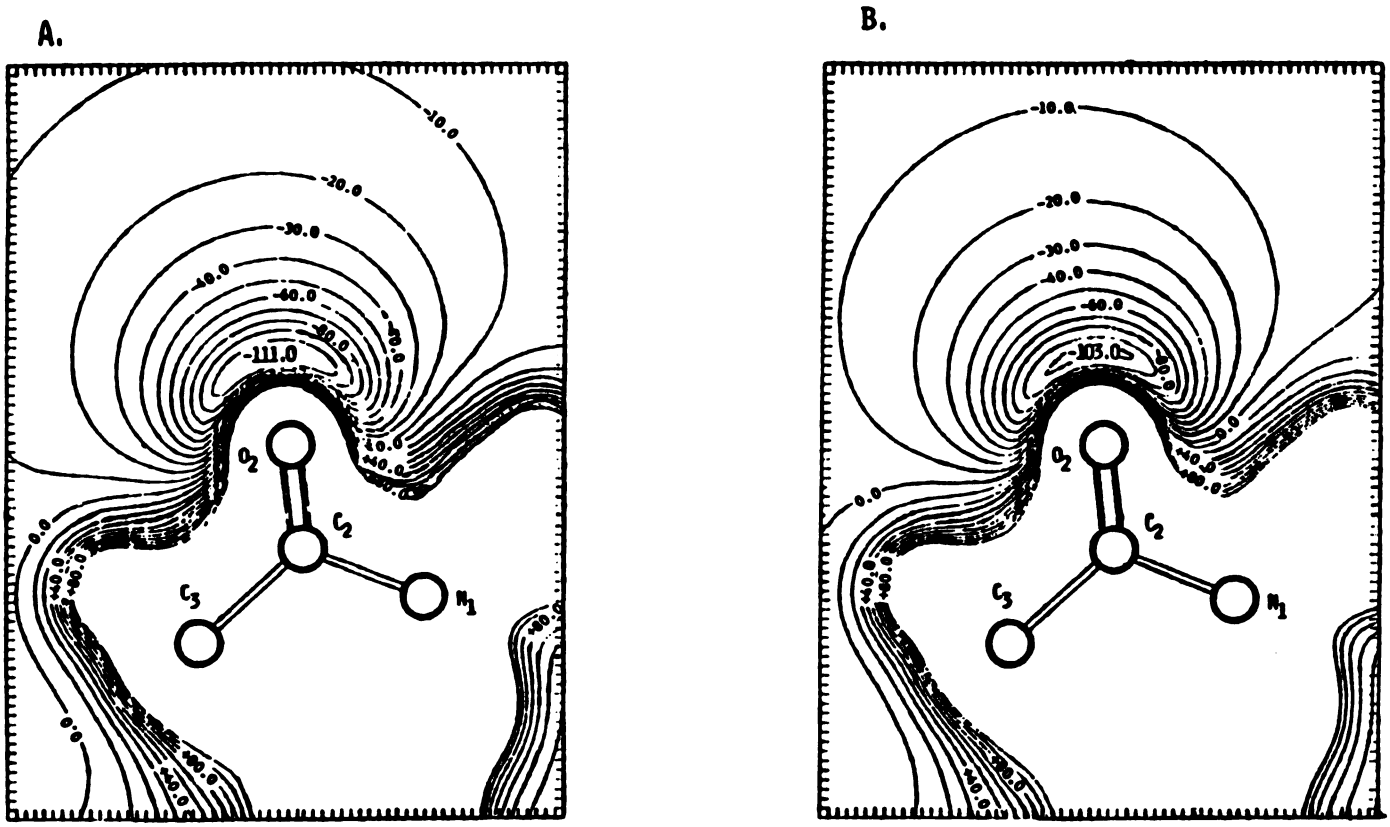


FIG. 7. *Calculated MEP maps in kilocalories per mole of two 1,4-BDZ analogues showing the effect of A-ring substitutents with a 4-Bohr square region around C_2═O, in the $N_1C_2O_2$ plane*

A. C_7-H analogue. B. C_7-F analogue.

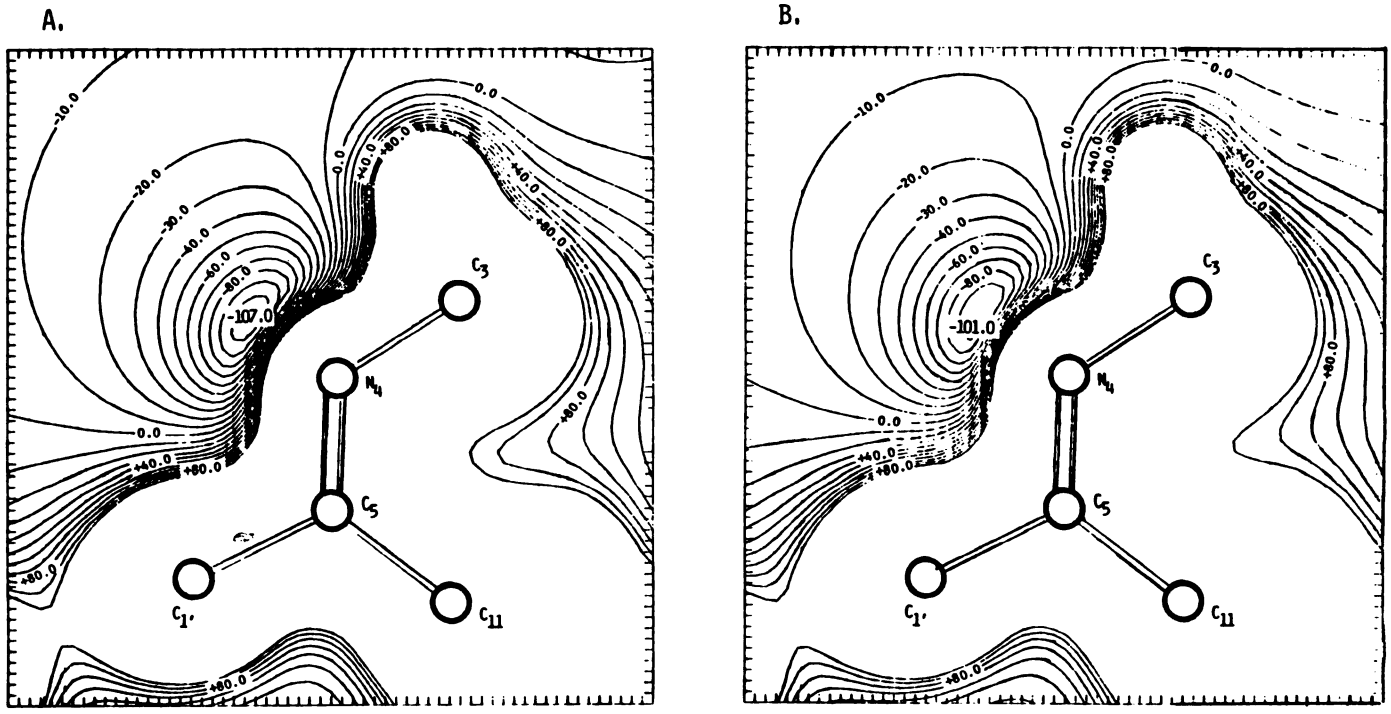


FIG. 8. *Calculated MEP maps in kilocalories per mole of two 1,4-BDZ analogues showing the effect of the C_7-substituent within a 6-Bohr square region around N_4, in the $C_3N_4C_5$ plane*

A. C_7-H analogue (2b). B. C_7-F analogue.

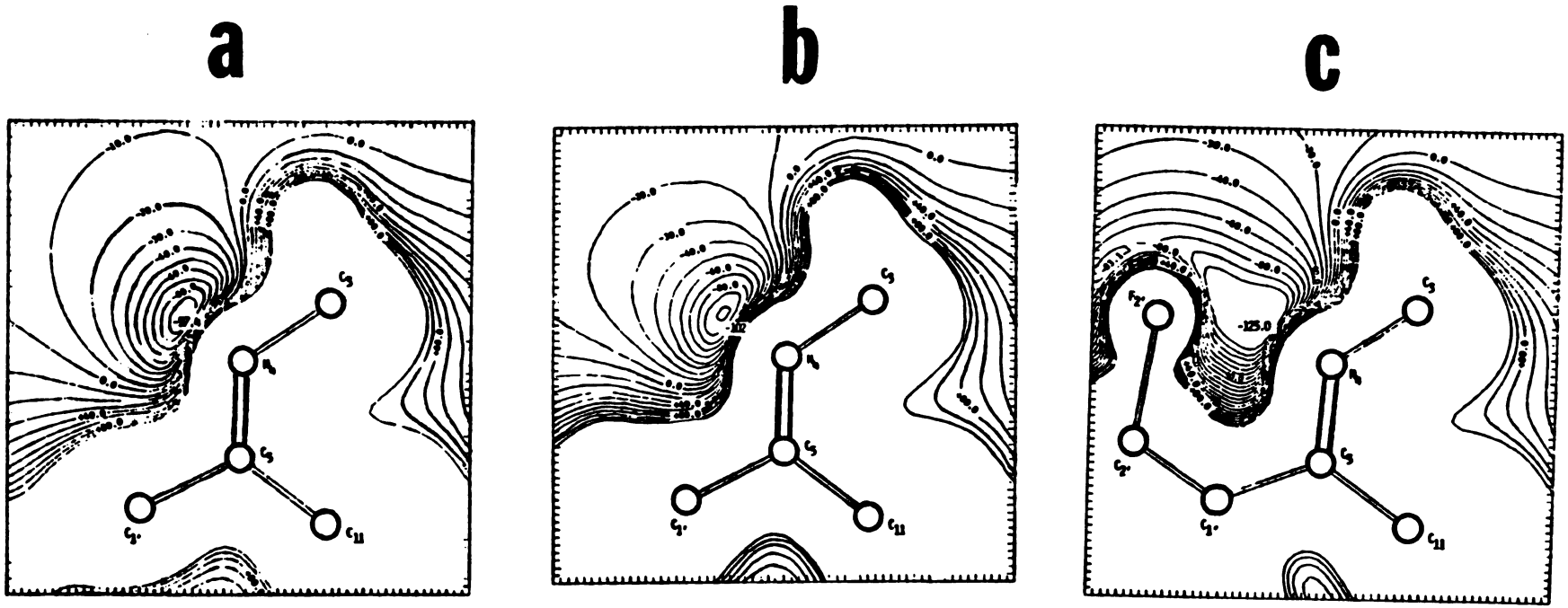


FIG. 9. *Calculated MEP maps in Kcal/mol of C_2'-F diazepam, showing the effect of C_2'-F on the electrostatic potential around N_4, as a function of rotation of the phenyl C-ring within a 6-Bohr square region around N_4, in the $C_3N_4C_5$ plane*

a. Minimum energy conformation of C-ring. b. Conformation of C-ring for greatest energy of interaction with model cationic receptor site, $\tau_5 = 316°$. c. Forced planarity of the C-ring with $N_4, \tau_5 = 0°$.

TABLE 8

MNDO calculated proton affinities[a] of three BDZ analogues as a function of rotation of the C_5-phenyl ring

τ_5[b]	Proton affinity		
	Diazepam	4′-Cl Diazepam	2′-Cl Diazepam
	kcal/mole		
0	226.3	204.1	197.2
30	223.8	206.6	206.6
60	223.1	207.0	208.3
90	223.1	206.2	208.4
120[c]	230.8	206.9	209.5
150	240.0	205.9	202.6
180	226.3	204.1	195.9
210	223.8	206.6	206.8
240	223.1	207.0	209.3
270	223.1	206.2	208.4
300[c]	230.8	206.9	211.9
330	240.0	205.9	153.1

[a] Proton affinities are defined as the negative enthalpy for the reaction BDZ + $H^+ \rightarrow$ [BDZH]$^+$; i.e., proton affinity = $\Delta H_f(BDZH)^+ + \Delta H_f(H^+) - \Delta H_f(BDZ)$. $\Delta H_f(H^+)$ = 365.6 kcal/mole. Protonation is at N_4. The larger the value, the greater the proton affinity of the parent compound (i.e., the more exothermic the above reaction).

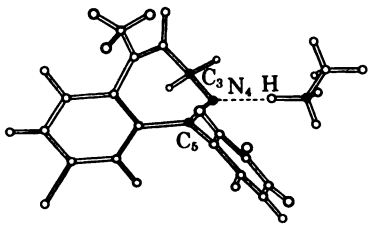
[b] $\tau_5 = \tau(N_4\text{-}C_5\text{-}C_1'\text{-}C_2')$.

[c] Minimum energy conformers.

the empirical energy calculations: (a) the minimum energy phenyl C-ring conformer and (b) the conformer of the phenyl C-ring which gave the greatest enhancement of N_4-cationic interaction. Since the INDO method does not contain Cl atom capabilities, calculations were done for a 7-F, 2′-F analogue for which the MEP mapping was also done. The calculated energies of interaction are given in Table 10. CNDO and INDO results are more reliable when used with reasonable intermolecular geometries, for example, obtained from the empirical

TABLE 9

Calculated energies of model cation receptor-BDZ interactions as a function of phenyl ring rotation by empirical energy method (26, 27)



$\tau^a(\phi)^b$	E_{int}	
$\tau^c(N_4\text{-}C_5\text{-}C_1'\text{-}C_2')$	C_2'-H	C_2'-Cl
	kcal/mole	
285[b] (120°)	−11.18	−9.49
310 (123°)	−10.25	−13.10
314 (129°)	−10.26	−13.73
316[c] (129°)	−10.70	−13.89
318 (129°)	−5.38	−13.75

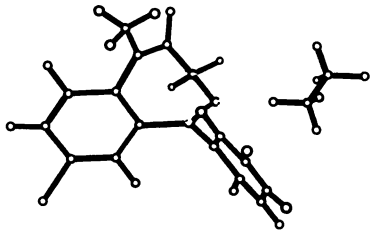
[a] $\tau = \tau(N_4\text{-}C_5\text{-}C_1'\text{-}C_2')$. $\phi = C_5\text{-}N_4\text{-}H$ bond angle. The angle $C_3\text{-}N_4\text{-}H$ was also varied. Optimum $N_4\text{-}N_c$ distance = 2.90 Å. N_c-H distance = 1.08 Å.

[b] Minimum energy conformation.

[c] Conformer which gives optimal interaction for C_2'-Cl analogue.

TABLE 10

Interaction energies, calculated by three different methods, of des-Cl diazepam and 2′-Cl(F) diazepam with a model cationic receptor site $(CH_3NH_3)^+$



7-X	2′-Y	$\tau(\phi)$[a]	ΔE_{int}			ΔE_{conf}[d]	
			Empirical	INDO[c]	CNDO[c]	MNDO	CNDO
			kcal/mole			*kcal/mole*	
H	H	284 (120)	−11.2	−21.0	−18.4		
H	H	316 (129)	−10.7	−22.4	−19.1		
Cl(F)	Cl(F)	284	−9.5	−16.9	−16.6	0	0
Cl(F)	Cl(F)	316	−13.8	−28.2	−28.1	2.3	0.50

[a] τ = the torsion angle: $\tau(N_4C_5C_1'C_2')$; $\phi = C_5\text{-}N_4\text{-}H$ bond angle.

[b] Molecule empirical energy program gives interaction energy directly. X,Y = H with CNDO calculated charge and geometries for Cl.

[c] INDO and CNDO results from "supermolecule" calculations give interactions energy from $\Delta E_{int} = E_{(AB)^+} - E_{(A)} - E_{(B^+)}$ X,Y = F for INDO,; X,Y = Cl for CNDO, using optimized intermolecular geometry from empirical energy calculation.

[d] ΔE_{conf} is the energy (CNDO) or enthalpy (MNDO) difference between the minimum energy (τ = 284°) and the induced conformation (τ = 316°) with constrained geometry optimization. Without optimization, the MNDO difference is $\Delta(\Delta H_f)$ = 3.1 kcal/mole.

method. Although they could still be overestimated (29, 33), these results confirm that qualitatively similar results are obtained from all three methods. In the minimum energy conformation, a 2′-halo substituent diminishes N_4-cationic site interactions as compared with a 2′-H analogue. However, rotation of the phenyl C-ring ~30° toward planarity enhances this interaction in the presence of a 2′-halo substituent but not a 2′-H substituent. Moreover, the enhancement of the N_4 model cationic site interaction in the induced 2′-halo conformation calculated by any method exceeds the energy required to make the conformational change. These results, taken togehter, strongly suggest that the enhanced affinity and activity of the 1,4-BDZ analogues with halogen substituents at position 2′ could be due, in part, to their enhancement of the interaction of the $C_5{=}N_4$ region with a cationic receptor site.

If this effect is important, than analogues with enhanced planarity of the C-ring, for example, by a covalent bond(s) between C_6 and C_6' forming a 5- or 6-membered ring, and with a C_2'-F or Cl substituent, should have an even higher differential receptor affinity, because no energy would be required to keep the phenyl ring in a nearly planar position for optimal receptor interaction. However, if the C-ring is interacting with its own receptor subsite, and the near-perpendicular conformation is essential to the steric requirements of the receptor, the near-planar analogues will have diminished affinity. We are in the process of synthesizing such analogues to distinguish further between these two possibilities.

CONCLUSION

Conformational and electronic properties, MEPs, and explicit model receptor interaction have been calculated for a sereies of 1,4-BDZ analogues using both empirical energy and semiempirical molecular orbital methods, in order to identify molecular properties relevant to their relative receptor affinities and pharmacological activities. Analogues with variations in key positions of the 7-membered ring, at positions C_7, C_8, and C_9 of the A-ring, and at positions 2′ and 4′ of the C-ring were examined. The results indicate that electronic rather than conformational differences are responsible for variations in relative receptor affinities.

Electron-withdrawing substituents at C_7 appear to have two opposing effects: they create a large local negative potential with a well-defined directionality in their vicinity, and they diminish the negative potential around the O_2 and N_4 heteroatoms of the B-ring. Since the presence of these groups has the net effect of greatly enhancing receptor affinity (compare analogues 2b and 2f), the specific and local interaction of the C_7 substituents with a cationic receptor site, such as the protonated amine terminal group chain of lysine residue, appears to dominate the smaller effect on distant interactions of $C_2{=}O$ and $C_5{=}N_4$ with other cationic receptor sites.

Large negative potential regions are also generated near the $C_2{=}O$ group (in the $N_1{-}C_2{=}O$ plane) and near the N_4 atom (in the $C_3{-}N_4{=}C_5$ plane). It is the absence of these negative potential regions, rather than conformational changes, that most likely causes the greatly diminished receptor affinity relative to diazepam evidenced in medazapam (1g), chlorodiazepoxide (1f), and analogue (1i) (with a saturated $N_4{=}C_5$ bond). These results are consistent with the recently reported results of a statistical linear free-energy analysis of the relative affinities of 39 BDZ analogues which indicated that the presence of $C_2{=}O$ and N_4 were the most important correlates to affinity (10).

The negative potential around N_4 is somewhat diminished by halogen substituents at C_2' when the phenyl C-ring is in its minimum energy conformer. However, when the phenyl ring is relaxed toward planarity, a 2′-F group but not a 2′-H group greatly enhances the negative potential around the N_4 atom. Explicit N_4 interactions with a model cationic receptor site confirm this result. Such interactions are maximally enhanced when the phenyl group with a 2′-halo is rotated about 30° toward planarity from its minimum energy conformer.

From these results some aspects of the receptor site of the BDZs emerge. The binding site appears to include three specific cationic subsites near C_7, $C_2{=}O$, and the imine nitrogen N_4, and an anionic subsite near C_4'. An indication that these results are correct is that 1,4-BDZ analogues with a saturated $N_4{=}C_5$ bond but without a 4′-substituent are inactive (33), and we predict similar behavior for 1,5-BDZs with a saturated $C_4{=}N_5$ bond. The role of the phenyl C-ring in enhancing affinity and agonist activity requires further elucidation. One possibility is that the low-energy conformer of the phenyl group, a conformation nearly perpendicular to the fused-ring system, is interacting with a key receptor subsite, perhaps a protonated histidine aromatic side chain. The enhanced affinity produced by a 2′-halo substituent and the diminished affinity produced by a 4′-halo substituent could be due to the effect of the halogen on interactions of the low-energy phenyl ring conformer with such a nearby aromatic receptor subsite. Alternatively, as explicitly indicated by the results obtained here, enhancement of affinity by the addition of a 2′-halo group could be due to an enhanced potential near N_4, created by an induced conformation of the phenyl C-ring. Rigid analogues of the 1,4-BDZs with a more nearly co-planar phenyl C-ring and 1,4-BDZ with an aromatic N in place of the C_2' carbon are being synthesized to sort out further the possible roles of the phenyl C ring in receptor binding.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Dale Spangler for many helpful discussions. Thanks also to Dr. W. E. Scott of Hoffmann-La Roche for supplying samples of diazepam, des-methyl diazepam, and 2′-Cl diazepam (2f, 2g, and 2j) for receptor binding studies and to Dr. Lawrence Toll for performing these studies.

REFERENCES

1. Sternbach, L. H. The benzodiazepine story. *Prog. Drug Res.* **21**:229–266 (1978).
2. Randall, L. O., W. Schallek, L. H. Sternbach, and R. Y. Ning. Chemistry and pharmacology of the 1,4-benzodiazepines, in *Psychopharmacological Agents* (M. Gordon, ed.), Vol. III. Academic Press, New York, 175–281 (1974).
3. Sternbach, L. H. Chemistry of 1,4-benzodiazepines and some aspects of the structure-activity relationship, in *The Benzodiazepines*. Raven Press, New York, 1–51 (1975).
4. Squires, R. F., and C. Braestrup. Benzodiazepine receptors in rat brain. *Nature* (*Lond*) **266**:732–734 (1977).
5. Mohler, H., and T. Okada. Benzodiazepine receptor: demonstration in the central nervous system. *Science* (*Wash. D. C.*) **198**:849–851 (1977).
6. Blair, T., and G. A. Webb. Electronic factors in the structure-activity relationship of some 1,4-benzodiazepin-2-ones. *J. Med. Chem.* **20**:1206–1210 (1977).
7. Lucek, R. W., W. A. Garland, and W. Dairman. CNDO/2 Molecular orbital study of selected 1,3-dihydro-5-phenyl-1,4,-benzodiazepin-2-ones. *Fed. Proc.* **38**:541 (1979).
8. Kaufman, J. J., and E. Kerman. The structure of psychotropic drugs (including theoretical prediction of a new class of effective neuroleptics). *Int. J. Quantum Chem. Quantum Biol. Symp.* **1**:259–287 (1974).
9. Braestrup, C., and R. F. Squires. Pharmacological characterization of benzodiazepine receptors in the brain. *Eur. J. Pharm.* **48**:263–270 (1978).
10. Borea, P. A. De novo analysis of receptor binding affinity data of benzodiazepines. *Arzneim. Forsch.* **33**:1086–1088 (1983).
11. Camerman, A., and N. Camerman. Sterochemical basis of anticonvulsant drug action. II. Molecular structure of diazepam. *J. Am. Chem. Soc.* **94**:268–272 (1972).
12. Gilli, G., V. Bertolasi, and M. Sacerdoti. 7-Chloro-2,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepine (medazepam). *Acta. Crystallogr. Sect. B. Struct. Crystallogr. Cryst. Chem.* **34**:3793–3795 (1978).
13. Hernstadt, C., D. Mootz, H. Wunderlich, and H. Mohrle. Protonation sites of organic bases with several nitrogen functions: crystal structures of salts of chlordiazepoxide, dihydralazine, and phenformin. *J. Chem. Soc. Perkin Trans. II*, 735–740 (1979).
14. Sternbach, L. H., F. D. Sancilio, and J. F. Blount. Quinazolines and 1,4-benzodiazepines. 64. Comparison of the stereochemistry of diazepam with that of close analogs with marginal biological activity. *J. Med. Chem.* **17**:374–377 (1974).
15. Chananont, P., and T. A. Hamor. 5-(2-Chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one (clonazepam): $C_{15}H_{10}ClN_3O_3$. *Cryst. Struct. Commun.* **8**:393–400 (1979).
16. Gilli, G., V. Bertolasi, M. Sacerdoti, and P. A. Borea. 7-Nitro-1,3-dihydro-5-phenyl-2H-1,4 benzodiazepin-2-one (Nitrazepam). *Acta Crystallogr. Sect. B. Struct. Crystallogr. Cryst. Chem.* **33**:2664–2667 (1977).
17. Bandoli, G., and D. A. Clemente. Crystal, molecular, and electronic structure of an antianxiety agent: 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-1,4-benzodiazepin-2-one. *J. Chem. Soc. Perkin Trans. II*, 413–418 (1976).
18. Karle, J., and I. L. Karle. The crystal and molecular structure of 7-chloro-5-(2,4-dichlorophenyl)-4,5-dihydro-1,4-dimethyl-3H-1,4-benzodiazepin-2-one: $C_{17}H_{15}Cl_3N_2O$. *J. Am. Chem. Soc.* **89**:804–807 (1967).
19. Pier, L., R. Schaffner, R. Scherschlicht, P. Pole, J. Sepinwall, A. Davidson,

H. Möhler, R. Cumin, M. DaPrada, W. P. Burkhard, H. H. Keller, R. K. M. Müller, M. Gerold, M. Pieri, L. Cook, and W. Haefly. Pharmacology of midazolam. *Arzneim. Forsch.* **31**:2180–2210 (1981).
20. Oie, T., G. M. Maggiora, R. E. Christoffersen, and D. J. DuChamp. Development of a flexible intra- and intermolecular empirical potential function for large molecular systems. *Int. J. Quantum Chem. Quantum Biol. Symp.* **8**:1–48 (1981).
21. Oie, T., G. M. Maggiora, and R. E. Christoffersen. Structural characterization of a special-pair chlorophyl II dimer model of P_{700}. *Int. J. Quantum Chem. Quantum Biol. Symp.* **9**:157–171 (1982).
22. Dewar, M. J. S., and W. Theil. Ground states of molecules. 38. The MNDO method approximations and parameters. *J. Am. Chem. Soc.* **99**:4899–4907 (1977).
23. Pople, J. A., D. L. Beveridge, and P. A. Dobosh. Approximate self-consistent molecular-orbital theory. V. Intermediate neglect of differential overlap. *J. Chem. Phys.* **47**:2026–2033 (1967).
24. Weinstein, H., S. Maayani, S. Srebrenik, S. Cohen, and M. Sokolovsky. Psychotomimetic drugs as anticholinergic agents. II. Quantum-mechanical study of molecular interaction potentials of 1-cyclohexylpiperidine derivatives with the cholinergic receptor. *Mol. Pharmacol.* **9**:820–834 (1973).
25. Srebrenik, S., H. Weinstein, and R. Pauncz. Analytic calculation of atomic and molecular electrostatic potentials from the Poisson equation. *Chem. Phys. Lett.* **20**:419–423 (1973).
26. Momany, F. A., R. F. McGuire, A. W. Burgess, and H. A. Scheraga. Energy parameters in polypeptides. VII. Geometric parameters, partial atomic charges, nonbonded interactions, hydrogen bond interactions, and intrinsic torsional potentials for the naturally occurring amino acids. *J. Phys. Chem.* **79**:2361–2381 (1975).
27. Egan, J. T., J. Hart, S. K. Burt, and R. D. MacElroy. The display of molecular models with the Ames interactive modeling system (AIMS). *Comput. Graphics* **6**:177–199 (1982).
28. Weller, T. PCILO calculations on hydrogen bonded complexes: dimers involving second row atoms. *Int. J. Quantum Chem.* **12**:805–811 (1977).
29. Romeo, G., M. C. Aversa, P. Giannetto, M. G. Vigorita, and P. Ficarra. Nuclear magnetic resonance of 1,4-benzodiazepines. *Org. Magn. Res.* **12**:593–597 (1979).
30. Weast, R. C. (ed.). *CRC Handbook of Chemistry and Physics*, Ed. 56. CRC Press, Cleveland (1975–1976).
31. Benson, S. W., F. R. Cruickshank, D. M. Golden, G. R. Haugen, H. E. O'Neal, A. S. Rogers, R. Shaw, and R. Walsh. Additivity rules for the estimation of thermochemical properties. *Chem. Rev.* **69**:279 (1969).
32. Cox, J. D., and D. Pitcher. *Thermochemistry of Organic and Organometallic Compounds.* Academic Press, New York (1970).
33. Darvas, F., J. Rohricht, Z. Budai, and B. Bordas. Belfree: a new method for predicting biological activity using indication variables, in *Proceedings of the Third Conference of the Hungarian Pharmacological Society* (F. Darvas, ed.). Oxford, England, 25–38 (1980).

Send reprint requests to: Dr. Gilda H. Loew, Life Sciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, Calif. 94025.