BIOORGANIC CHEMISTRY 18, 1–12 (1990)

Conformational Recognition by Central Benzodiazepine Receptors

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Received April 13, 1989

In order to distinguish conformational recognition by the receptor from steric effects brought about by substituents attached to C3 of 1,4-benzodiazepines, two series of closely related compounds were tested for binding potency. Increasing size of the 3-substituent up to isopropyl decreases both the binding and its enantioselectivity. Synthesis and X-ray determination of the molecular structure of 3,3-dimethyl derivatives possessing quasi-axial methyl substituents were followed by a mathematical separation of conformational and substituent effects for quartets with successive 3-methylation [$(H)_2$, (*S*)-Me, (*R*)-Me, $(Me)_2$ at C3]. Results indicate a very high preference for conformation M of the ligand by the receptor (the primary reason of stereoselectivity) and a large steric hindrance resulting from the axial methyl substituent. A lower but still unexpectedly substantial steric effect is exerted by the equatorial methyl group.

INTRODUCTION

One of the most widely prescribed of all classes of drugs, 1,4-benzodiazepines (1,4-BZs),[1] displays an interesting conformational enantiomerism. The seven-membered ring taking the shape of a boat (*1*) lacks any symmetry element owing to the asymmetrically positioned nitrogen atoms. Consequently, all 1,4-BZs are chiral by virtue of their nonplanarity, even if they do not contain a chiral center. When R^2 and R^3 attached to position 3 are identical (e.g., both are hydrogen), the molecule equally populates two enantiomeric conformations (P and M; cf. Fig. 1), hence the compound exists in the form of a conformational racemate. The individual enantiomeric conformations cannot be separated, since a flipping of the C3 atom through the plane of the condensed aromatic ring easily occurs (*2*) at room temperature.

If, however, only one of R^2 and R^3 is hydrogen while the other is methyl, the two conformations are not equienergetic any more. The molecule will then populate preferentially the conformation that places the bulky methyl group into the quasi-equatorial position (i.e., M for $R^2 = CH_3$, $R^3 = H$, while P for $R^2 = H$, $R^3 = CH_3$) both in the crystal (*3*) and in the solution (*4*). This preference has been

[1] Abbreviations used: BZ, benzodiazepine; GABA, γ-aminobutyric acid.

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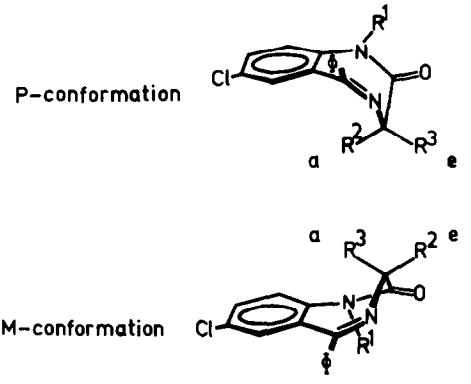


FIG. 1. 1,4-BZs exist in conformations assigned P (plus) or M (minus) on the basis of the sign of the torsion angle around C3-N4. Substituents R^2 and R^3 are located in either quasi-axial or quasi-equatorial position with respect to the seven-membered ring.

indicated to be so high that at least 97% of the dissolved molecules can be found in the preferred conformation (*4*). Thus, the chiral center at carbon induces a helical or conformational imbalance in this type of molecule.

Stereoselectivity in the binding to central benzodiazepine receptors was indicated for 3-methyl-flunitrazepam (*5*) as early as the receptor was discovered (*6*); in a synaptosomal preparation of rat cerebral cortex the (+)-(3*S*)-methyl-enantiomer displayed binding affinity more than 200 times higher than that of its optical isomer. The problem of receptor-active conformation was addressed later by applying conformationally defined analogs of 1,4-BZs. It was found again that the (3*S*)-enantiomer fixed in conformation M was more active than the (3*R*)-stereoisomer freezed in conformation P (*7*), so the question of conformational recognition appeared to be solved.

Since conformational restriction can only be achieved by introducing additional substituents into the molecule, the elimination of conformational mobility is always associated with additional steric requirements of the extra group of atoms. Hence, the direct comparison of enantiomeric binding affinities reflects a complexity of conformational and substituent effects and leaves conformational recognition of flexible molecules essentially uncertain. In order to resolve this complexity, an estimation of the steric effects of substituents attached to C3 of the 1,4-BZ molecule is necessary. Further, it should also be known how conformational preference in solution by 1,4-BZ enantiomers themselves influences the enantioselectivity of receptor binding.

These problems are addressed in this paper by testing the receptor binding abilities of compounds containing increasingly bulky substituents at position C3 of the diazepine ring in two independent sets of compounds. In order to have methyl groups that doubtlessly occupy the quasi-axial position, we prepared 3,3-Me_2-substituted derivatives. A quantitative treatment of the binding data of 3-unsubstituted, (3*S*)-Me, (3*R*)-Me, and 3,3-Me_2 analogs (the quartets) allowed finally the separation of the overlapping effects of conformations and methyl substituents.

EXPERIMENTAL

Materials. All 1,4-BZs (cf. Table 1) were prepared according to Refs. (*8, 9*). The optical purity of the enantiomers was verified by albumin chromatography (*10*) and CD spectroscopy. [^{3}H]Diazepam and [^{3}H]Ro 15-1788 were purchased from Amersham (UK) and from DuPont–NEN (Dreieich, FRG) with specific activities of 86 and 76 Ci/mmol, respectively.

Binding assays. Synaptosomal membranes were prepared from rat brain. The tissue was homogenized in 20 vol of 0.32 M sucrose and centrifuged at 30,000*g* for 10 min. The pellet was resuspended in 40 vol of 50 mM Tris citrate buffer (pH 7.1). Incubation mixtures contained 1.2 mg protein/ml and 1.0 nM [^{3}H]diazepam, together with varying concentrations of 1,4-BZ derivatives applied in ethanol solution. The content of ethanol in the incubation mixture amounted to 4.5% (v/v). Incubation was at 4°C for 30 min, after which the samples were filtered in triplicates through Whatman GF/B filter. Nonspecific binding determined in the presence of 1 μM diazepam was around 10% of total binding. IC_{50} values were determined from four to seven concentrations of the displacer.

For the determination of "GABA shifts" synaptosomal membranes free of endogenous GABA were prepared from rat cerebral cortex. The membranes were washed four times by centrifugations in 50 mM Tris–HCl (pH 7.1), frozen, thawed, and washed again (*11*). Incubations were in 50 mM Tris–HCl with 0.2 nM [^{3}H]Ro 15-1788 at 4°C for 90 min in the absence and presence of 10^{-4} M GABA. IC_{50} values were determined as means of three independent experiments using five different concentrations of the displacers.

The molecular structures of **IV** and **XII** have been established by X-ray crystallography from diffractometer data.

IV. ($C_{17}H_{15}ON_2Cl$) crystallizes in the monoclinic system, space group $P2_1/n$ with $a = 9.271(1)$, $b = 13.818(3)$, $c = 12.894(2)$ Å, $\beta = 92.44(2)°$, $V = 1554.76(8)$ Å^3, $Z = 4$, $D_c = 1.276$ g · cm^{-3}. The structure was solved by direct methods and refined to $R = 0.045$ ($R_w = 0.051$) for 2028 reflections taken with $I > 2\sigma(I)$ and collected by monochromated Mo$K\alpha$ radiation ($\lambda = 0.7107$ Å).

XII. ($C_{18}H_{17}ON_2Cl$) crystallizes also in the monoclinic system, space group $P2_1/c$ with $a = 18.641(3)$, $b = 9.012(1)$, $c = 25.445(3)$ Å, $\beta = 131.12(1)°$, $V = 3220.2(1.8)$ Å^3, $Z = 8$, $D_c = 1.290$ g · cm^{-3}. The structure was solved by direct methods and refined to 0.042 ($R_w = 0.063$) for 3937 reflections taken with $I > 3\sigma(I)$ and collected by monochromated Cu$K\alpha$ radiation ($\lambda = 1.5418$ Å).

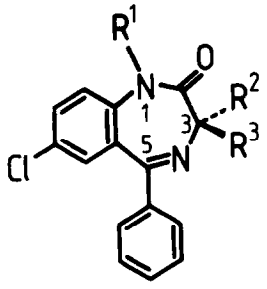
The relative atomic coordinates together with the thermal parameters for each structure are deposited at the Cambrdige Crystallographic Data Centre, Lensfield Road, Cambridge, CB2 1EW, England. The selected torsion angles are listed in Table 2 to serve conformational analyses.

RESULTS AND DISCUSSION

At C3 successively methylated, as well as ethyl and isopropyl substituted, 1,4-BZs were prepared and subjected to receptor binding assay. In order to increase

TABLE 1

Characterization of 1,4-Benzodiazepines Investigated



Code	R^1	R^2	R^3	Class of chirality	Preferred conformation	IC_{50}[a] (μM)	$\frac{IC_{50}(R)}{IC_{50}(S)}$
I	H	H	H	CR[b]	P and M	0.0100 ± 0.0018	
II	H	Me	H	S	M	0.0608 ± 0.0036	
III	H	H	Me	R	P	68 ± 11	1117
IV	H	Me	Me	CR[b]	P and M	14.3 ± 2.4	
V	H	Et	H	S	M	0.61 ± 0.05	
VI	H	H	Et	R	P	73 ± 16	119
VII	H	i-Pr	H	S	M	57 ± 12	
VIII	H	H	i-Pr	R	P	267 ± 176	4.7[c]
IX	Me	H	H	CR[b]	P and M	0.0046 ± 0.0005	
X	Me	Me	H	S	M	0.0467 ± 0.0113	
XI	Me	H	Me	R	P	12.8 ± 3.3	274
XII	Me	Me	Me	CR[b]	P and M	4.7 ± 0.5	
XIII	Me	Et	H	S	M	0.48 ± 0.03	
XIV	Me	H	Et	R	P	46 ± 18	96
XV	Me	i-Pr	H	S	M	73 ± 36	
XVI	Me	H	i-Pr	R	P	101 ± 45	1.4[c]

[a] Concentration displacing 50% of specifically bound [^{3}H]diazepam. Ratios of IC_{50} values represent ratios for the corresponding enantiomers.

[b] CR, conformational racemate.

[c] In view of the high standard deviations, these values are not significantly different from each other.

the reliability of the findings and to test a broader validity of the substituent effect at position 3, both 1-desmethyl diazepam (R^1 = H) and diazepam (R^1 = CH_3) derivatives were investigated. The structure of compounds and their IC_{50} values are collected in Table 1.

X-Ray Structure of 3,3-Dimethyl-1,4-BZs

These derivatives (**IV** and **XII**) prove that stable 1,4-BZs exist with a 3-methyl group in quasi-axial position. One of the conformations and the atomic labeling scheme for **IV** are shown in Fig. 2. Table 2 lists characteristic torsion angles for **IV** and **XII** compared with those of **II** taken from a published source (*3*).

Table 2 supports that the conformations of mono- (**II**) and dimethyl-substituted compounds (**IV** and **XII**) are very similar. Only small differences appear for the

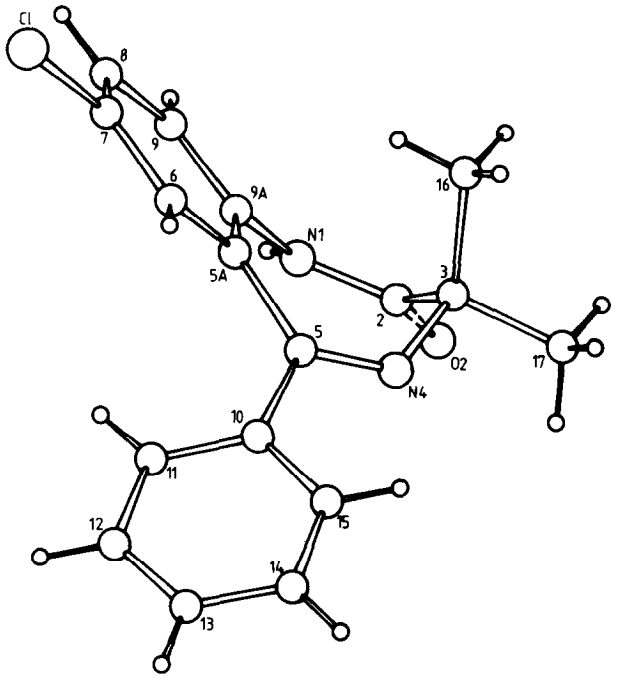


FIG. 2. The atomic labeling scheme and the shape of molecule **IV** is shown in conformation M. Fifty percent of all molecules accommodates conformation P in the crystal.

torsion angles of C2-C3 and C3-N4 bonds suggesting that the boats for **IV** and **XII** are slightly more flat than that for **II.** The additional 1-methyl group in **XII** seems to reproduce the torsions around C9A-N1 and N1-C2 in **II,** but their deviations from those of **IV** are hardly significant. Apparently, the 5-phenyl group is subject to a counterclockwise rotation of about 10° around C10-C5 in **XII.** This difference is probably not important in solution. All in all, the three molecules are of remarkably similar shape as illustrated for their seven-membered rings in Fig. 3.

Enantioselective Receptor Binding

Ratios of IC_{50} values for the corresponding enantiomers are also included in Table 1. The high enantioselectivities found for the 3-methyl-substituted derivatives are in accord with the value found for 3-methyl-flunitrazepam (*5*). Interestingly, the increasing size of 3-substituents reduces the value of enantioselectivity mainly by decreasing the binding affinity of the more active (+)-(*S*)-enantiomers. The isopropyl group abolishes both binding potency and enantioselectivity indicating the importance of steric hindrance in receptor binding of 1,4-BZs. Diazepam derivatives display binding potencies somewhat higher than those of their corresponding 1-desmethyl analogs. Hence, the 1-methyl group appears to increase the binding of 1,4-BZs.

TABLE 2

Selected Torsion Angles of **II** (*3*), **IV**, and **XII**

	II[a]	**IV**[b]	**XII**[c]
N1-C2-C3-N4	70.3(2)	65.9(3)	61.2(4)
C2-C3-N4-C5[d]	−75.7(2)	−67.2(4)	−66.2(5)
C3-N4-C5-C5A	n.a.	2.2(4)	0.6(4)
N4-C5-C5A-C9A	41.7(4)	38.3(4)	40.3(5)
C5-C5A-C9A-N1	0.7(4)	0.3(4)	2.4(4)
C5A-C9A-N1-C2	−45.7(3)	−38.0(4)	−46.3(4)
C9-C9A-N1-C2	135.7(2)	144.8(5)	134.2(5)
C9A-N1-C2-C3	4.3(3)	−1.4(4)	8.6(4)
C9A-N1-C2-O2	−175.3(2)	179.1(5)	−172.2(5)
O2-C2-C3-N4	−110.0(2)	−114.7(4)	−118.0(4)
N1-C2-C3-C17	−170.3(2)	179.1(4)	175.8(4)
C17-C3-N4-C5	162.3(2)	176.7(4)	177.3(5)
O2-C2-C3-C17	9.7(2)	−1.5(4)	−3.4(4)
C11-C10-C5-C5A	23.7(6)	26.5(4)	36.4(5)
C11-C10-C5-N4	−155.3(7)	−152.9(5)	−146.1(5)
C15-C10-C5-C5A	n.a.	−157.4(4)	−143.4(5)
C15-C10-C5-N4	n.a.	23.2(4)	34.2(5)

Note. Standard deviations are given in parentheses.

[a] Values are means of three molecules, each in conformation M; n.a., not available in Ref. (*3*). Concentration P is not populated.

[b] Data of a single molecule accommodating conformation M; conformation P is equally populated.

[c] Values are means of two molecules both accommodating conformation M; conformation P is equally populated.

[d] Torsion angle defining conformation M.

Separation of Conformational Recognition from Steric Effects via the Methyl Quartets

Both the 3-3-dimethyl-substituted derivatives (**IV** and **XII**) are about 1000-fold weaker in binding potency than their respective unsubstituted counterparts (**I** and **IX**). This is all the more remarkable since these compounds in solution do not show preference to any of the two possible conformations. The reason can only be the steric effect hindering the suitable fit of **IV** and **XII** into the receptor cavity. Closer observation of the two quartets (**I–IV** and **IX–XII**) reveals that in terms of potency we are dealing with two pairs of compounds; while the unsubstituted and (+)-(*S*)-methyl derivatives are highly potent, both (−)-(*R*)-methyl and 3-3-dimethyl derivatives possess low affinity. In other words, the appearance of a methyl group in the R^3 position (Table 1, Fig. 1) is decisive for a sharp decrease of binding. Such quartets were earlier applied (*12, 13*) in order to demonstrate and interpret stereoselectivity in terms of steric substituent constants. The treatment, however, cannot account for the characteristic feature of 1,4-BZs that they can exist in one of the two conformations (Fig. 1). An adequate treatment should

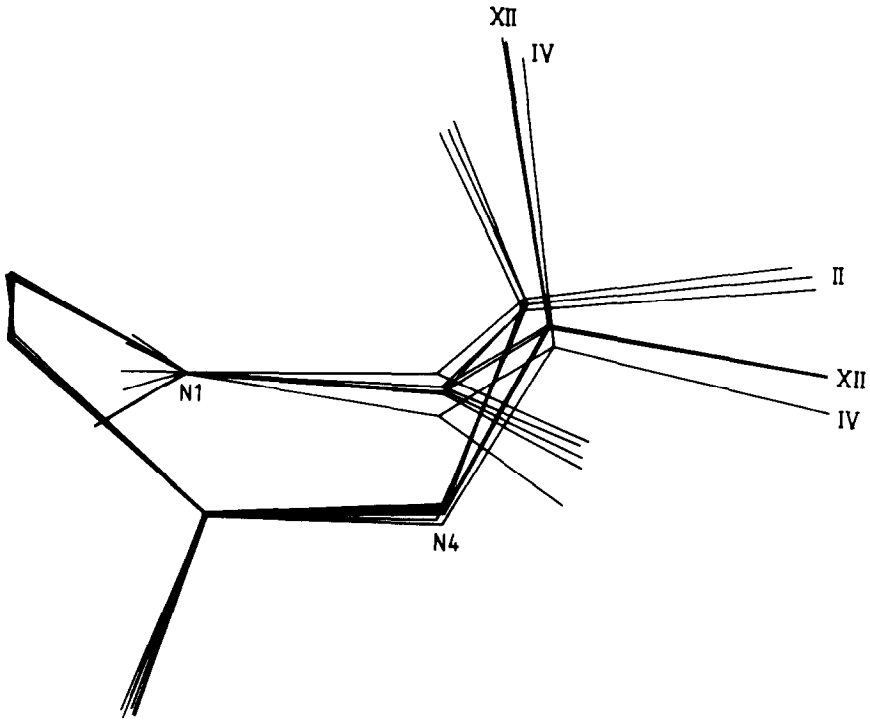
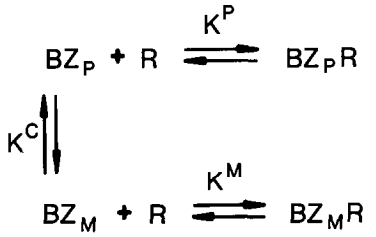


FIG. 3. Superposition of the seven-membered rings of **II** (three molecules, Ref. (*3*)), **IV** (single molecule, this work), and **XII** (two molecules, this work), as found in the crystal.

involve a conformational equilibrium for the free molecules and the possibility that both conformations may participate in overall receptor binding, as shown in Scheme 1. Since only the total of bound molecules can be measured, the binding constant determined experimentally (K_{app}) is a composite quantity related to the conformation-specific equilibrium constants, as given by

$$K_{app} = \frac{[BZ_MR] + [BZ_PR]}{\{[BZ_M] + [BZ_P]\}[R]} = \frac{K^M[BZ_M] + K^P[BZ_P]}{[BZ_M] + [BZ_P]} = \frac{K^M + K^PK^c}{1 + K^c}. \quad [1]$$



SCHEME 1. A general scheme for receptor binding of 1,4-BZs. BZ_M and BZ_P refer to free molecules accommodating M and P conformations, respectively. Each binds to a homogeneous population of BZ receptors (R) with binding constants K^M and K^P. $K^c = [BZ_P]/[BZ_M]$ stands for the conformation equilibrium constant in solution.

Hence, Eq. [1] gives K_{app} as a weighted average of K^M and K^P; if $K^c = 0$, no molecule is found in conformation P and $K_{app} = K^M$. At the other extreme, when $K^c \rightarrow \infty$, all molecules populate conformation P, and K_{app} approaches K^P. Thus, Eq. [1] expresses how the conformational equilibrium for free BZ molecules influences the contribution of the conformations to overall receptor binding.

Next, explicitly define steric hindrance arising from 3-methyl substituents in receptor binding. In order to describe the binding of quartets, let K_0^M and K_0^P refer to unsubstituted compounds ($R^2 = R^3 = H$; $K^c = 1$). We introduce steric factors f^a and f^e which characterize the effect of methyl substituents attached to C3 when they accommodate quasi-axial and quasi-equatorial positions, respectively. Then for $R^2 = Me$, $R^3 = H$, i.e., for (3*S*)-methyl derivatives that prefer conformation M in solution ($K^c < 1$), equilibrium constants of the individual conformations are expressed by

$$K^M = f^e K_0^M; \qquad K^P = f^a K_0^P. \qquad [2]$$

Applying the above principle, we can describe K_{app} values for the quartets, as given in Table 3.

Table 1 gives IC_{50} values for the quartets, but it is not straightforward to convert them into K_{app} affinity constants, since IC_{50} values are not identical to K_d dissociation constants. It is, however, reasonable to assume that ratios of IC_{50} values can be taken as reciprocal ratios of the corresponding affinity constants. This is also in line with our interest focused on conformational recognition that can be expressed as K_0^M/K_0^P. Therefore, the reciprocal of the IC_{50} values can be used in place of K_{app} for computation.

Another problem is posed by the value of K^c for 3-Me enantiomers, since we have only limiting values from NMR measurements (Table 3). When these were used for computation, they unexpectedly resulted in negative values for K_0^P which is, of course, unacceptable. Sensible results could only be obtained if sharper limits of K^c were used as input parameters. The results are collected in Table 4.

TABLE 3

Mathematical Description of the Quartets

Substituent in position 3	K^c	K_{app}
None	1	$\frac{K_0^M + K_0^P}{2}$
(*S*)-Me	<0.03[a]	$\frac{f^e K_0^M + f^a K^c K_0^P}{1 + K^c}$
(*R*)-Me	<32[a]	$\frac{f^a K_0^M + f^e K^c K_0^P}{1 + K^c}$
Me_2	1	$f^a f^e \frac{K_0^M + K_0^P}{2}$

[a] According to Ref. (*4*).

TABLE 4

Conformational Recognition and Steric Factors Computed from Equations in Table 3 for the Quartets

Parameter	$R^1 = H$	$R^1 = CH_3$	Condition
K^c for (3*S*)-Me	<0.0088	<0.0092	$K_0^P > 0$
K^c for (3*R*)-Me	>114	>109	$K_0^P > 0$
K_0^M/K_0^P	$>10^4$	2910	$K^c = 120^a$
K_0^M/K_0^P	4641	1001	$K^c = 150^a$
K_0^M/K_0^P	2605	603	$K^c = 200^a$
K_0^M/K_0^P	1450	351	$K^c = 500^a$
lim (K_0^M/K_0^P)	1117	274	$K^c \rightarrow \infty^a$
f^a	0.00843	0.0197	$K^c = 120^a$
f^a	0.00848	0.0198	$K^c = 500^a$
f^e	0.0829	0.0497	$K^c = 120^a$
f^e	0.0825	0.0495	$K^c = 500^a$

[a] Conditions defined by K^c values refer to (3*R*)-Me enantiomers; corresponding reciprocal values for the (3*S*)-Me enantiomer are implied.

The K^c values in Table 4 indicate that in solution less than 1% of the 3-methyl-1,4-BZ enantiomers can adopt the conformation possessing a quasi-axial methyl substituent. In other words, the population of free enantiomers in their preferred conformation exceeds 99%. Note, that this result follows directly from the binding potencies (Table 1) and the treatment of quartets (Table 3). It agrees well with the limit of 97% indicated by NMR measurements (*4*). The exact value of conformational recognition by the receptor as expressed by the ratio K_0^M/K_0^P depends heavily on the actual value of K^c. Table 4 lists a few values for K_0^M/K_0^P which decrease when K^c for the (*R*)enantiomer increases. It is interesting to note that the apparent values of enantioselectivity (**III** and **XI** in Table 1) are reproduced as lower limits for K_0^M/K_0^P implying infinitely large K^c. The reason can be seen in Table 3. If $K^c \rightarrow \infty$ for (*R*)-Me and, accordingly, $K^c \rightarrow 0$ for (*S*)-Me, the ratio of the two K_{app} values yields directly K_0^M/K_0^P. Hence, even the lowest possible degree of conformational selectivity is high enough to indicate a total preference by the receptor for conformation M of the ligand.

This trend is also clearly seen by comparison of the (3*R*)-methyl and 3,3-dimethyl derivatives (**III** vs **IV** and **XI** vs **XII** in Table 1); the 3,3-Me_2 compounds are more potent since in solution they do not prefer the conformation unfavorable for the receptor. The steric effects having been separated, this result offers a strong confirmation of the earlier proposal based on the binding potencies of conformationally defined 1,4-BZs (*7*). Although the favorable conformation alone is not sufficient for biological activity (*1, 14*), our results demonstrate the conformation of 1,4-BZs to be the chiral property from which the high enantioselectivity of receptor binding mainly originates. The recognition of conformation M by the receptor implies that compounds which are conformational racemates in solution become optically active upon binding. A direct proof that **IV** and **XII** bind to human serum albumin exclusively in conformation M has recently been obtained

by demonstrating binding-induced optical activity (*10*). Similar results were also demonstrated for **I** and **IX** (*15*).

Interestingly, the steric factors f^a and f^e are practically independent of the value of K^c (cf. Table 4). As shown, f^a is small expressing that the receptor cavity does not tolerate axial extrusion from the bow of the boat (C16 in Fig. 2). It agrees with receptor models predicting a rather flat cavity providing steric confinement around the C3 atom of 1,4-BZs (*16*) and with the supposition that the axial substituent would hinder the good fit to a dominant binding domain (*17*). Our small f^a disagrees with the notion that potent (3*S*)-Me-1,4-BZ enantiomers bind with an axial methyl group (*16*). A combination of the quasi-axial methyl group with conformation M (*18*) is not possible for (3*S*)-methyl-1,4-BZs, if we apply the Cahn-Ingold-Prelog convention correctly.

Although f^e is larger than f^a (Table 4), its value is still low compared to unity. Steric hindrance to receptor binding as a consequence of an equatorial methyl group attached to C3 is a rather unexpected result of the present work. The effect is inevitably real; the decrease of binding in going from unsubstituted to (3*S*)-methyl derivatives (**I** → **II** and **IX** → **X**) is corroborated by the further decrease from (3*S*)-methyl to (3*S*)-ethyl (**II** → **V** and **X** → **XIII** in Table 1) and by the extremely weak binding for the 3-isopropyl derivatives. No earlier receptor model indicated such a property mainly as a consequence of efforts to define a common receptor cavity accommodating structurally diverse sets of compounds (*16, 17*). Previous approaches led to the identification of different kinds of interactions that are important components of receptor binding (*17, 19*). On the other hand, the superposition of compounds of different pharmacological classes in order to define a common binding site at central BZ receptors unavoidably lends a static character to the models derived (*16, 17, 19*) which is at variance with the dynamic aspects of receptor function (*20*).

The Central BZ Binding Site

Until the X-ray structure of receptor-bound molecules cannot be determined, methods to characterize the receptor cavity will remain indirect. A principal assumption of recent approaches has been that the geometry of BZ binding sites is uniform. This hypothesis may be questioned on pharmacological grounds, since central BZ receptors are known to be heterogeneous especially when prepared from whole brain. Nevertheless, heterogeneity of BZ receptors has not been observed for diazepam (compound **IX**), so it is reasonable to assume that BZ receptors are homogeneous for the two series of structurally closely related compounds used in this study.

Another uncertainty may arise, since the pharmacological profile of 3,3-dimethyl derivatives has never been reported. The transition of efficacies of BZs from agonists to inverse agonists correlates with a decrease in the potency of γ-aminobutyric acid to enhance the binding of BZ ligands. Therefore, the ratio of IC_{50} values in the absence and presence of GABA (known as GABA shift, e.g., Ref. (*21*)) was determined for a representative pair of compounds. As Table 5 illustrates, regardless of its low potency, compound IV is characterized by the

TABLE 5

Displacing Potencies and GABA Shifts of a Representative Pair of 1,4-BZs Investigated

Compound	IC_{50} (μM)[a]		GABA shift[b]
	Control	$+10^{4}$ M GABA	
1-Desmethyldiazepam (I)	0.029 ± 0.007	0.019 ± 0.006	1.53 ± 0.12
3,3-Dimethyl-1-desmethyldiazepam (IV)	84 ± 29	55 ± 16	1.53 ± 0.17

[a] Fifty percent displacement of [^{3}H]Ro 15-1788 specifically bound.

[b] The ratio of IC_{50} values in the absence (control) and presence of GABA.

same magnitude of GABA shift as the highly potent 1-desmethyldiazepam (I). This suggests that members of the quartets are agonists and their binding is associated with similar ligand-induced changes in receptor conformation.

In view of earlier BZ receptor models (*16, 17, 19*) indicating a lack of steric hindrance in equatorial direction at the static receptor site, it might be assumed that the steric confinement of the receptor cavity may become more stringent upon the binding of BZ agonists, mainly as a consequence of changing receptor conformation brought about by the agonist itself. An equatorial 3-methyl substituent may partly hinder the change of receptor conformation resulting in a somewhat weaker binding, hence the steric effect expressed by f^e. In accordance, larger ω-phenyl-acyloxy substituents in position 3 not only decrease the affinity to BZ receptors but also turn the compounds into antagonists (*22*). The pharmacological profile of the ligand is determined by the distance between the aromatic ring of BZs and the carbonyl in the side chain (*23*). Therefore, a steric effect expressed by f^e is clearly not observed if a common activity is statically defined for agonist and antagonist ligands having a variety of chemical structures (*16, 17, 19*).

Close analogies for both structures (Table 2, Fig. 3) and pharmacological efficacies (Table 5) of our model compounds allowed the quantitative treatment of binding potencies in terms of quartets implying steric factors for quasi-axial and quasi-equatorial methyl groups and conformation-specific equilibrium constants. Results obtained from two independent quartets indicate (1) selective recognition of conformation M of 1,4-BZs by the receptor; (2) a stronger steric effect originating from a quasi-axial 3-methyl substituent; and (3) a weaker, but still highly significant steric effect brought about by the equatorial 3-methyl group. Thus, the primary source of stereoselectivity in the binding of 3-methyl-1,4-BZ enantiomers is the differential recognition of chiral ligand conformations by the receptor.

ACKNOWLEDGMENT

Financial support from the Project OTKA 1021 by the Hungarian Academy of Sciences is acknowledged.

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