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Quinazolines and 1,4-Benzodiazepines

92. Conformational Recognition of the Receptor by 1,4-Benzodiazepines

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SUMMARY

We propose that the conformation of 1,4-benzodiazepines that is recognized by the binding site on the benzodiazepine receptor complex is one in which the planes formed by the fused benzene ring and the methylene group (and the two adjoining atoms) of the diazepine ring are in the *R* configuration. The derivation of this conformation was based on comparisons of computer-generated 3-dimensional structures obtained from single-crystal X-ray data for diazepam, (*R*)- and (*S*)-1,3-dimethyl-5-(2-fluorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, and the structurally rigid ethyl (*S*)-7-chloro-11,12,13,13*a*-tetrahydro-9-oxo-9*H*-imidazo[1,5-*a*]pyrrolo[2,1-*d*][1,4]benzodiazepine-1-carboxylate. The affinity of ligands for the benzodiazepine binding site was determined using the [^{3}H]-diazepam binding assay.

INTRODUCTION[3, 4]

The high-affinity and stereospecific binding site for benzodiazepines in mammalian brain was first described by Braestrup and Squires (1) and also by Möhler and Okada (2), in 1977. This binding site, which is closely associated with a GABA[5] receptor and with a chloride ionophore channel, may be involved in the process through which benzodiazepines exert their pharmacological effects on a molecular level. Evidence for this conclusion has been summarized recently by Tallman *et al.* (3).

We have been interested in the effect of conformational changes in the 1,4-benzodiazepine ring system on the ability of benzodiazepines to bind to the receptor complex. One of the necessary criteria for differentiating binding sites from receptors is that receptor binding has been shown to be stereospecific (4). Therefore, any conformational change in the 1,4-benzodiazepine ring system would be expected to have an effect on binding affinities to the receptor complex.

MATERIALS AND METHODS

Chemicals. Diazepam, **1**, was obtained from Research Chemical Services, Hoffmann-La Roche Inc. (Nutley, N J.) Ro 11-6893 and Ro 11-6896, Compounds **2** and **3**, were obtained from Dr. E. Kyburz, F. Hoffmann-La Roche & Co., Ltd. (Basel, Switzerland). Ro 14-5975, **7**, was synthesized by Dr. R. F. Lauer, of our Chemistry Department.

[^{3}H]Diazepam binding assays. All IC_{50} values for compounds evaluated in the [^{3}H]diazepam binding assay were carried out by Dr. H. Möhler, Pharmaceutical Research Department, F. Hoffman-La Roche & Co., Ltd. (Basle, Switzerland). The assays were performed as previously described (2).

Crystallography. Intensity data for Compounds **2**, **3**, and **7** were measured on a Hilger-Watts diffractometer (nickel-filtered copper K_α radiation, θ–2θ scans, pulse-height discrimination). The data were corrected for absorption. The structures of **3** and **7** were solved by a multiple-solution procedure (5) and were refined by full-matrix least squares. The atomic coordinates for **2** were obtained by inverting those for structure **3**. In the final refinements, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. By a fortuitous quirk of nature, one molecule of methylene chloride is present in the crystals for each three molecules of **2** (and **3**). Thus, because of the presence of the chlorine atoms, it was possible to verify the absolute configurations of **2** and **3** directly from the X-ray data. The relevant data for Compounds **2** and **3** are given in Table 1, and for **7**, in Table 2.

Supplementary material available. The final atomic parameters, final anisotropic thermal parameters, bond lengths, and bond angles for Compounds **2**, **3**, and **7** are available in tabulated form. Requests should be sent to Dr. John F. Blount, Department of Physical Chemistry, Hoffmann-La Roche Inc., Nutley, N. J. 07110.

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[3] For the previous paper in the 'Quinazolines and 1,4-benzodiazepines" series, see: Fryer, R. I., W. Leimgruber, and E. J. Trybulski. Structure-activity relationship between substituted 2-amino-*N*-(2-benzoyl-4-chlorophenyl)acetamides and 1,4-benzodiazepinones. *J. Med. Chem.* **25**:1050–1055 (1982).

[4] A portion of this paper was presented at the 3rd World Congress of Biological Psychiatry in Stockholm, Sweden, June 28–July 3, 1981, by R. I. Fryer.

[5] The abbreviations used are: GABA, γ-aminobutyric acid; Ro 11-6893 and Ro 11-6896, (*R*)- and (*S*)-1,3-dimethyl-5-(2-fluorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, respectively; Ro 14-5975, ethyl (*S*)-7-chloro-11,12,13,13*a*-tetrahydro-9-exo-9*H*-imidazo[1,5-*a*]pyrrolo[2,1-*c*]-[1,4]benzodiazepine-1-carboxylate.

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TABLE 1

Crystal data and experimental details for analysis of 2 and 3

Analysis	Compound	
	2	3
Formula	$C_{17}H_{14}FN_3O_3 \cdot 0.3\ CH_2Cl_2$	
Formula wt	355.63	
Space group	P $2_12_12_1$	
a	10.324(2)	10.314(3) *A*
b	16.474(2)	16.466(3) *A*
c	30.283(4)	30.273(7) *A*
Z	12	12
d_{calcd}	1.375	1.378 (g cm^{-3})
μ (Cu K_α)	18.0	18.0 (cm^{-1})
Crystal size (mm)	0.35 × 0.50 × 0.60	0.25 × 0.35 × 0.45
Maximum θ (°)	76	57
No. of reflections	5803	3902
No. of observed reflections	4789	3359
Final *R*	0.051	0.047
Final *wR*	0.0543	0.0493
Final difference map, largest peak (*e* A^{-3})	<±0.6	<±0.4

RESULTS AND DISCUSSION

Diazepam (1), a typical 1,4-benzodiazepine with a binding constant (IC_{50})[6] of approximately 5 nM in the [^{3}H] diazepam binding assay, can exist in either of the two conformations, a or b (Fig. 1). The change in conformation results from a "flipping" of the C-3 methylene group through the plane of the "A"-ring. It should be noted that these two conformations (a and b) are, in fact, mirror images, and illustrate the chiral nature of the 1,4-benzodiazepine ring system.

At ambient temperature, the proton nuclear magnetic resonance spectrum of 1 shows an AB pattern for the C-3 methylene protons with a calculated inversion barrier of 17.6 Kcal/mole (6). Thus, although this interconversion is slow on the NMR time scale (the appearance of the AB pattern), the thermodynamic barrier to inversion is too low to allow for the resolution of stable conformers. For comparison, the inversion barrier for *N*(1)-desmethyldiazepam has been found to be 12.3 Kcal/mole, and the C-3 methylene protons appear as a singlet in the NMR spectrum, indicating a rapid interconversion of conformers even on the NMR time scale (6, 7).

[6] The IC_{50} is the concentration of unlabeled ligand that reduces the binding of labeled ligand by 50%. See ref. 4, Chap. 2.

TABLE 2

Crystal data and experimental details for analysis of 7

Analysis	Compound 7
Formula	$C_{17}H_{16}ClN_3O_3$
Formula wt	345.79
Space group	P $2_12_12_1$
a	7.325(1) *A*
b	14.095(2) *A*
c	15.301(2) *A*
Z	4
d_{calcd}	1.453 (g cm^{-3})
μ (Cu K_α)	23.4 (cm^{-1})
Crystal Size (mn)	0.22 × 0.25 × 0.30
Maximum θ (°)	70
No. of reflections	1698
No. of observed reflections	1627
Final *R*	0.029
Final *wR*	0.037
Final difference map, largest peak (*e* A^{-3})	<±0.4

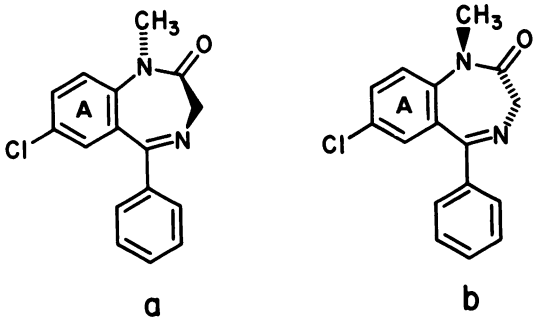

FIG. 1. *Conformations of diazepam, 1*

The X-ray structure for diazepam has been determined (8), but it obviously does not provide any information as to which conformer (a or b) is the chiral structure recognized by the benzodiazepine receptor complex.

In an attempt to resolve this uncertainty, 3-methyl-1,4-benzodiazepines were examined. In all cases of C-3 methyl *R,S* pairs which have been studied, the *S*-enantiomer (which is formally derived from *S*-alanine) is always more active in the [^{3}H]diazepam binding assay than is the corresponding *R*-enantiomer. The same order of activity also holds for *in vivo* pharmacological tests. From single crystal X-ray analyses carried out on the

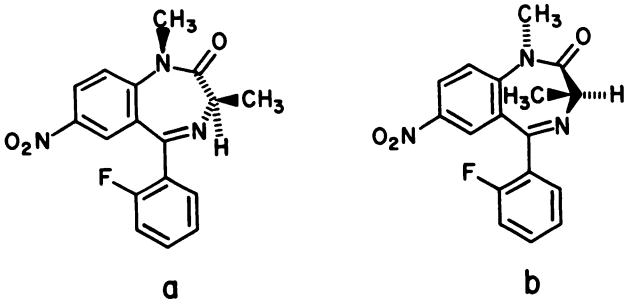

FIG. 2. *Possible conformations of 2*

Conformation a is preferred in the crystal as determined by X-ray analysis.

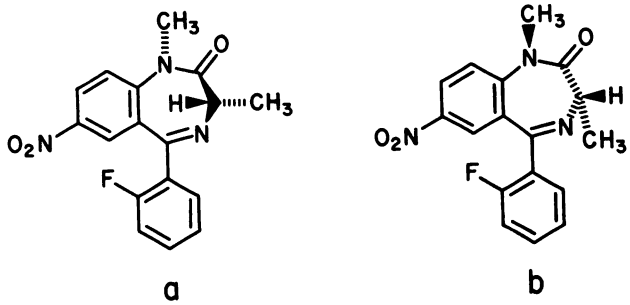

FIG. 3. *Possible conformations of 3*

Conformation a is preferred in the crystal as determined by X-ray analysis.

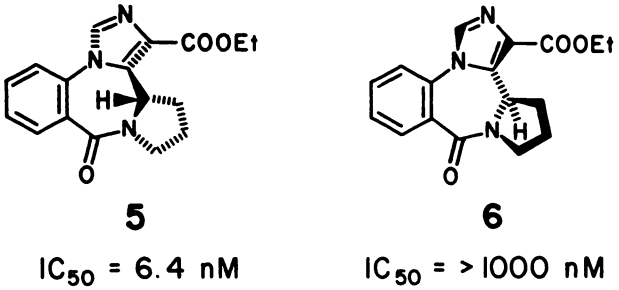

FIG. 4. *Binding constants for anthramycin-type benzodiazepines 5 and 6 derived from S-proline and R-proline, respectively*

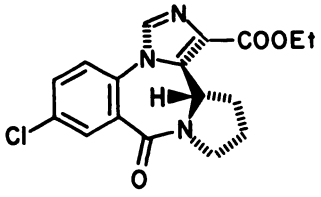

FIG. 5. *Structure of 7 as determined by single crystal X-ray analysis*

pure *R*- and *S*-3-methyl-1,4-benzodiazepines (Compounds **2** and **3**), the preferred conformation in the crystal of the inactive *R*-enantiomer (**2**) was determined to be as shown in Fig. 2a. The preferred conformation in the crystal for the active *S*-enantiomer (**3**) was as shown in Fig. 3a.

In the [^{3}H]diazepam binding assay, Compound **2** had an IC_{50} of >1000 nM and Compound **3** had an IC_{50} of 7 nM. These *in vitro* results were in good agreement with the results from the *in vivo* oral pentelenetetrazole test[7] (**2**, ED_{50} = >100 mg/kg; **3**, ED_{50} = 0.4 mg/kg).

Although, at least in the crystal, the preferred conformation of the active compound, **3**, is as shown in Fig. 3a, with the 3-methyl group pseudoequatorial, the possibility exists that flipping may occur *in vivo*. Thus, the crystal structure data alone cannot lead to assignment of the conformation which actually binds to the benzodiazepine receptor. After the completion of our work, Šunjić *et al.* (9) reported that the preferred conformation of 7-chloro-5-phenyl-d_5-3(*S*)-methyl-dihydro-1,4-benzodiazepin-2-one (**4**) in solution had the 3-methyl group in an equatorial position, based on NMR data.

The activity observed for some *R*-3-methyl-1,4-benzo-

[7] Testing carried out at Hoffmann-La Roche Inc. (Nutley, N. J.) by Dr. W. Dairman, Department of Toxicology.

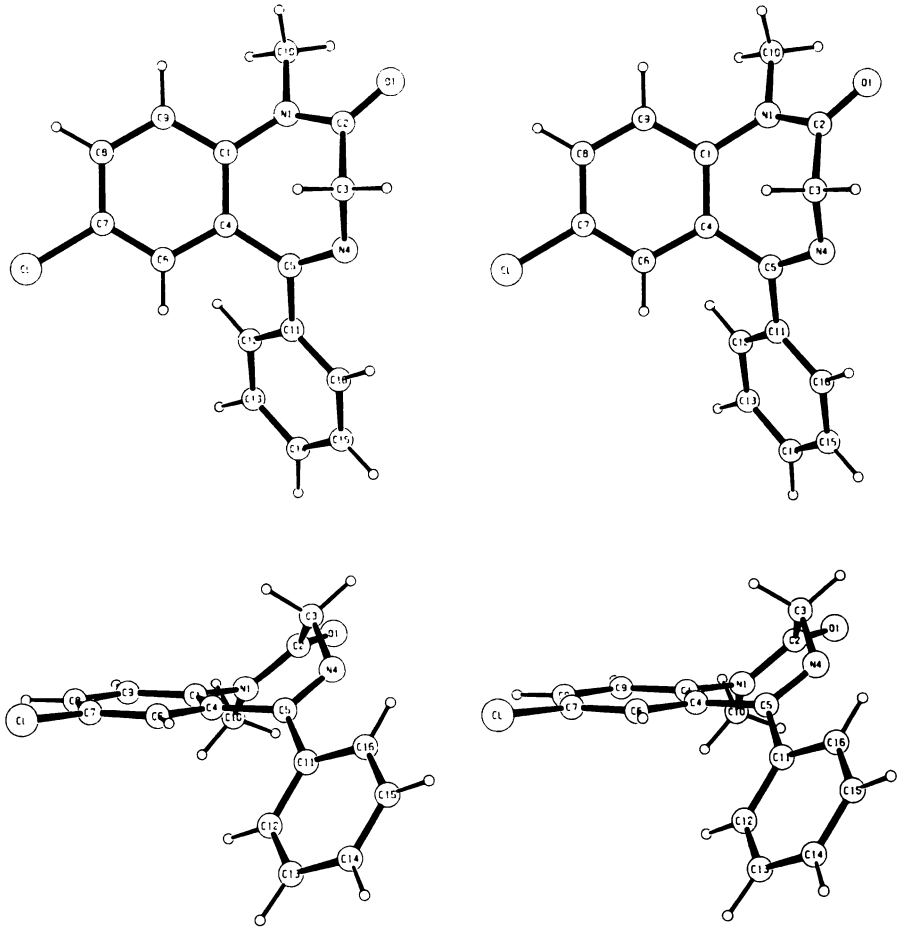

FIG. 6. *Proposed conformation of diazepam responsible for binding to the benzodiazepine receptor*
The top perspective drawings have the substituted benzene ring (the A-ring) in the plane of the paper. In the bottom perspective drawings, this ring has been rotated approximately 85°. The bottom drawings clearly show that the C-3 methylene group is above the A-ring.

diazepines, which is always of a lower magnitude than that of the corresponding *S*-enantiomers, may be due to the presence of Conformer **2b** (3-methyl group in a pseudoaxial position) in solution.

Further insight to the question of conformational preference of benzodiazepines in diazepam receptor binding was provided by a study of the *conformationally fixed* anthramycin-type benzodiazepines. As is the case with the 3-methyl compounds discussed earlier, the anthramycin analogue derived from *S*-proline (*S*-alanine for the 3-methyl compounds) is the active species (e.g., **5**), whereas the *R*-enantiomer prepared from *R*-proline (e.g., **6**) is inactive (results obtained from the [^{3}H]diazepam binding assay; see Fig. 4).

An X-ray analysis of **7**, the 8-chloro analogue of Compound **5**, gave the structure as shown in Fig. 5, in complete agreement with the expected conformation for an active compound.

On the basis of all of the above data, we would propose that the conformation of 1,4-benzodiazepines that is recognized by the binding site on the benzodiazepine receptor complex is as shown in Fig. 6 for diazepam—a typical 1,4-benzodiazepine.

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