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Molecular Structure of Ro15-1788 and a Model for the Binding of Benzodiazepine Receptor Ligands

Structural Identification of Common Features in Antagonists

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SUMMARY

Ligands that bind to the benzodiazepine receptor have three possible effects. The ligand can be an agonist and reduce anxiety, an antagonist and have no biological effect, or an inverse agonist and promote convulsions. This receptor complex is unique in its spectrum of response to ligands, and conformational changes in the receptor are implicated. The x-ray crystal structure of an imidazobenzodiazepine antagonist ligand, Ro15-1788, was determined and compared to the structures of the 1,4-benzodiazepine agonists and to two other types of antagonists, β-carbolines and a pyrazoloquinolinone, CGS-8216. The antagonists were found to have similar arrangements of binding features including an aromatic ring, a carbonyl oxygen atom, and a hydrophobic side chain. The structures of these antagonists could be superimposed in a model binding site with three common features for all of the antagonists and a fourth hydrogen-bonding site for the pure antagonists (or inverse agonists), the β-carbolines, and CGS-8216. A comparison of the shapes of the antagonist benzodiazepine, Ro15-1788, and several agonists showed that RO15-1788 has a unique azepine ring conformation that distorts the usual arrangement of the aromatic A ring, carbonyl oxygen atom, and imine N atom of the agonists. A conformational adjustment in the receptor would be required to accommodate both of these types of ligands. A summary of the superpositions of typical agonists and the antagonists leads to a model with 7 conformationally mobile binding points. Inverse agonists are distinguished from antagonists by the length of the hydrophobic side chain. Antagonists are distinguished from agonists in part by the lack of a binding feature similar to the imine N atom of the diazepine ring. This model accounts for the key features found in ligands for the benzodiazepine receptor and provides an explanation for the spectrum of responses elicited by receptor binding.

INTRODUCTION

Benzodiazepine anxiolytics have a saturable stereospecific receptor in the brain (1) that is part of a receptor complex with separate but interacting sites for barbiturates, benzodiazepines, and GABA,[1] all linked to the chloride ionophore (2). Since the natural ligand for the benzodiazepine receptor is unknown, agonists have been defined as substances that mimic the action of diazepam. Agonists and antagonists can be differentiated on the basis of their effects on the interaction of GABA with its receptor as well as on the basis of their pharmacological effect. Agonists enhance the binding of GABA to the receptor complex and act as anxiolytics. There are two other responses elicited by benzodiazepine receptor ligands; either the ligand binds and produces no response and has no effect on the affinity of GABA for the receptor complex, or it potentiates convulsions and decreases the affinity of GABA. This difference for the last two ligands has prompted the classification of the former as antagonists and the latter as inverse agonists (3, 4). The multiplicity of effects elicited by ligands bound to the benzodiazepine receptor may arise from an allosteric regulation of conformational changes in the GABA receptor site (5). A study of the structures of all of the types of compounds that bind to the benzodiazepine receptor can identify the spatial and chemical criteria for differentiation among the three pharmacological effects and explain the structural basis of the mechanism for allosteric control of GABA binding.

Three structural models for the binding of benzodiazepines have been published. The model of Fryer (6) is

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[1] The abbreviations used are: GABA, γ-aminobutyric acid; βCCM, methyl-β-carboline-3-carboxylate.

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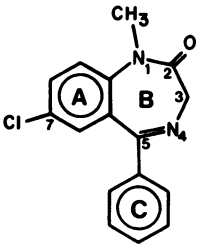
based on the three π systems that are always present in classical 1,4-benzodiazepine anxiolytics (**1**). This model



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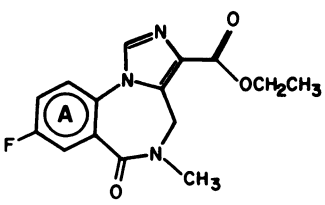

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has specific separation and orientation requirements for the aromatic fused 6-membered ring, a π system near C(2), and the 5-phenyl substituent. Another model, developed by Gilli and co-workers (7), is based on a number of x-ray structures. The authors conclude that small conformational differences among the benzodiazepines have little effect on activity and that the major determinant of activity is the electronegativity of the substituent in the 7-position since this affects the strength of potential hydrogen bonds formed at N(1). A third model (8), based on theoretical studies of the 1,4-benzodiazepines, proposes that the receptor has three cationic sites that interact with an electron-withdrawing group at C(7), the C(2)=O group and the imine nitrogen atom, N(4).

These models for the benzodiazepine receptor have not considered the binding of antagonists. Therefore, as part of a study of antagonists of the benzodiazepine receptor (9–11), the crystal structure of the title compound, ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a]-[1,4] benzodiazepine-3-carboxylate (Ro15-1788) (**2**), was determined to characterize the structural differences between antagonists and agonists. Ro15-1788 was first identified as an antagonist (12) as it has no pharmacological effect and does not change the affinity of GABA for its receptor; more recent findings have identified partial agonist properties for the drug (13).

MATERIALS AND METHODS

Single crystals were obtained by slow evaporation of a mixture of ethanol and water. The data were collected on an Enraf-Nonius CAD4F automated diffractometer; the crystal was cooled to 173 K to increase the number of observed reflections. The crystal data are in Table 1. The lattice was identified as tetragonal using the automatic centering and indexing programs of the diffractometer. The lattice and space group assignment was confirmed by examining the symmetry and the systematic absences of the intensities. The reflection intensities were measured using an $\omega/2\theta$ scan of variable speed from 0.516–6.706°/min to obtain a measurement of $I > 2.5\sigma(I)$ in less than 150 sec. Lorentz and polarization corrections were applied and E values were caculated for the observed intensities by applying a K curve.

The structure was solved using MULTAN 78 (14). The center of symmetry was chosen as the origin for this space group. The structure was refined with the restrained least squares program. RESLSQ (15–17) because the small number of observed reflections (25% of the 2389 reflections greater than zero had $I > 2.0X\sigma(I)$) were insufficient for full-matrix least squares. Use of restraints in the calculation produced a well-behaved refinement by including all nonzero reflection data. The restraints on the distances for the nonhydrogen atoms were removed during the course of the refinement so that the final geometry reflects the true molecular dimensions. The thermal parameter restraints were kept at high values to achieve convergence. The hydrogen atoms were included in the model at positions calculated from idealized geometry; the thermal parameters of the hydrogen atoms were defined as 120% of the value of the anisotropic thermal parameter of the atom to which they were attached. The parameters for the hydrogen atoms were not refined. The methyl and methylene groups were refined as semi-rigid groups. The weights were defined as $w = 1/(\sigma(F_o)^2$. The refinement converged to a final R value of 0.0712 with a maximum shift/error of 0.22. Programs from the XRAY76 (18) system were used except where otherwise mentioned. The scattering factors were those of Cromer and Mann (19) except for the hydrogen atom (20).

TABLE 1

Crystal data	
Formula	$C_{15}H_{14}N_3O_3F$
Formula weight	303.3
Space group	$P4_2/n$
$a = b$(Å)	19.395(5)
c(Å)	7.127(3)
V (Å^3)	2697(1)
Z	8
ρ_c(g cm^{-3})	1.494
Crystal dimensions (mm)	0.14 × 0.14 × 0.26
Radiation MoK$_\alpha$	λ = 0.71069 Å (graphite monochromator)
θ_{max}(°)	25.0
Scan range	$\Delta\omega = 1.5\ (0.74 + 0.347\ \tan\theta)$
Unique reflections	2389
μ (cm^{-1})	1.249

The conformation and atomic labeling scheme of Ro15-1788 are shown in Fig. 1. The fractional coordinates of the nonhydrogen atoms are given in Table 2. The anisotropic thermal parameters, the hydrogen atom parameters, and the bond distances and bond angles are available from the corresponding author.

RESULTS

Ro15-1788 differs from agonist benzodiazepines in that it lacks a C(5) phenyl group, has a saturated C(5)–N(4) bond, and has an ester group on the imidazole ring fused across the N(1)–C(2) bond. These structural modifications of the basic 1,4-benzodiazepine skeleton change both the molecular conformation of the antagonist and the potential hydrogen-bonding pattern.

The conformation of the seven-membered diazepine ring in Ro15-1788 is different from that observed in three examples of agonist compounds with a fused ring across the 1,2 bond. The two triazolo compounds alprazolam (21) and estazolam (22) have torsion angles T_o (C(5)–C–C–N(4)) of 1.89 and −3.57°, respectively, and an imidazobenzodiazepine (23) has $T_o = 0.5°$. The antagonist, Ro15-1788, has a significantly larger value of $T_o = -10.6°$; this large torsion angle twists C(5) out of the plane of the A ring of the benzodiazepine moiety by −0.20(2) Å. The twist places O(5) below the plane by −0.95(2) Å and pulls N(4) down toward the A ring plane to a height of 0.47(2) Å.

The overall asymmetric twist of the diazepine ring conformation in Ro15-1788 is different from that found in all other agonist benzodiazepines. In an unsymmetrically substituted 1,4-diazepine ring the direction of twist determines the relative orientation of substituents and

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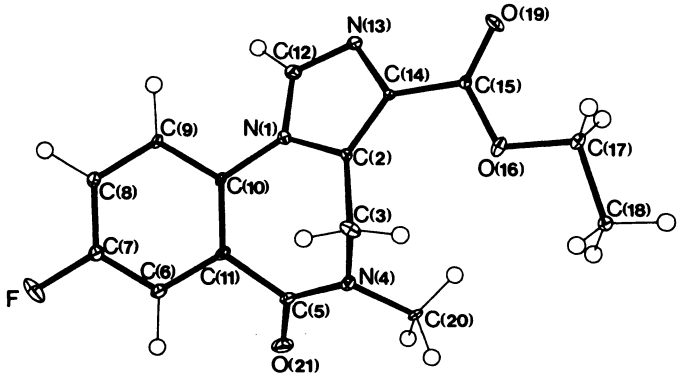

FIG. 1. *The molecular conformation and atomic labeling scheme for Ro15-1788*

This drawing was made with the computer program ORTEP (30).

TABLE 2

The fractional atomic coordinates (×10⁴) and B_{eq}(×10) for the nonhydrogen atoms of R015-1788. B_{eq} is defined as 1/3(B_{11} + B_{22} + B_{33})

Atom	x/a	y/b	z/c	B_{eq}
N1	3629(4)	−1656(4)	7813(13)	120(38)
C2	3404(4)	−1031(4)	8553(15)	85(45)
C3	3914(5)	−602(5)	9618(14)	100(47)
N4	4466(4)	−383(4)	8387(12)	123(37)
C5	4973(5)	−821(4)	7822(16)	158(45)
C6	5506(4)	−1917(5)	8741(17)	153(49)
C7	5499(4)	−2622(5)	9018(18)	182(51)
C8	4913(5)	−3019(5)	8808(16)	175(50)
C9	4293(4)	−2692(4)	8394(15)	158(47)
C10	4280(4)	−1973(4)	8185(16)	120(46)
C11	4884(5)	−1580(5)	8315(16)	151(48)
C12	3088(5)	−1948(4)	6924(14)	107(44)
N13	2525(4)	−1567(4)	7013(13)	137(38)
C14	2717(4)	−989(4)	8009(16)	89(43)
C15	2220(4)	−422(4)	8318(15)	98(46)
O16	2559(3)	172(3)	8789(10)	132(32)
C17	2116(5)	772(5)	8938(15)	108(47)
C18	2598(5)	1370(5)	9462(17)	166(49)
O19	1611(3)	−470(3)	8134(11)	167(35)
C20	4435(5)	318(4)	7635(16)	138(48)
O21	5483(3)	−641(3)	6985(11)	152(33)
F22	6104(3)	−2941(3)	9449(10)	258(30)

is, therefore, important. The value of an asymmetry parameter that indicates the direction of the twist of the azepine ring, δ, is 11.49 for Ro15-1788; the range of values for active agonist compounds is −23.24 to 8.48. The effect of this larger positive asymmetry is to twist the apical atom, C(3), toward N(4) and to push the N(4) atom closer to the mean plane of the A ring.[2]

[2] The asymmetry of a seven-membered ring can be summarized in a parameter that reflects the variation from mirror symmetry of pairs of torsion angles. A new equation, based on previous definitions (7, 24, 25) for this parameter, defines δ to reflect the direction of twist of the ring, viz.,

$$\delta = \{1/4[-S_{(To)}(To)^2 + S_{(T1+T1')}(T1 + T1')^2 + S_{(T2+T2')}(T2 + T2')^2 - S_{(T3+T3')}(T3 + T3')^2]\}^{1/2}$$

$$\text{and } S_{(A)} = +1 \quad \text{if} \quad A > 0$$
$$= 1 \quad \text{if} \quad A < 0$$

TABLE 3

Distances between six-membered aromatic ring and hydrogen bond acceptor atoms in benzodiazepine receptor ligands

Compound	Type	Distance	Reference
		Å	
βCCM	Inverse agonist	6.45(1)	11
βCCE[a]	Inverse agonist	6.47(2)	26
Ro15-1788	Antagonist	6.06(1)	This work
CGS-8216	Antagonist	6.102(5)	26
Agonists		4.95(3)	Footnote 2

[a] βCCE, ethyl-β-carboline-3-carboxylate.

The overall molecular shape of Ro15-1788 is curved; the imidazo ring and the C(5) carbonyl group are both tipped in the same direction away from the A ring plane. The two aromatic rings, phenyl and imidazole, are each relatively planar. The angle between these two planes is 35.0°; the angle between the phenyl ring and the carbonyl line is 36.7°. The ester side chain of the imidazole ring is twisted out of the plane of that ring by 17.7°.

The ester side chain is in an extended conformation which places the carbonyl oxygen atom in Ro15-1788 at a greater distance from the A ring than is found for the C(2) carbonyl group to A ring separation in agonists. Two of the models for benzodiazepine binding find that the interaction of either the carboxyamide, N(1)–C(2)=O, or the C(2)=O group with the receptor is important. The relative separation of the hydrophobic A ring and this group in Ro15-1788 is 6.06 Å, in contrast to the range for agonists of 4.72–4.97 Å.

Other antagonists and inverse agonists also display a larger separation between the carbonyl oxygen atom (or other electronegative site) and the ring comparable to the A ring of benzodiazepines. Table 3 summarizes these distances. The inverse agonists and antagonists all show distances greater than 6 Å, while the agonists have separations less than 5 Å. The ligands with ester side chains are usually found to be nearly planar, extended conjugated π systems and can be expected to maintain this geometry when bound to the receptor.

Nonagonist ligands Ro15-1788, CGS-8216, and the ester β-carbolines have similar three-dimensional shapes. Their common features are the six-membered fused aromatic ring, a carbonyl oxygen atom, and a hydrophobic side chain. The structures of both the methyl ((11) βCCM) and the ethyl (26) β-carboline-3-carboxylate, as found in the crystalline state, have the same conformation for the ester side chain as observed in Ro15-1788. A stereo drawing of the superimposed structures of Ro15-1788 and βCCM is shown in Fig. 2. The aromatic rings are superimposed as are atoms N(13) and O(19) (of Ro15-1788) with a corresponding imine N atom and carbonyl O atom of the β-carboline. In the

A positive δ parameter describes a twist of the ring like that described for Ro15-1788 and a negative value indicates a twist toward N(1). This parameter not only differentiates the unique antagonist conformation but also separates the agonist benzodiazepines according to their chemical formulation. Tables of torsion angles, δ values, and references are available from the corresponding author.

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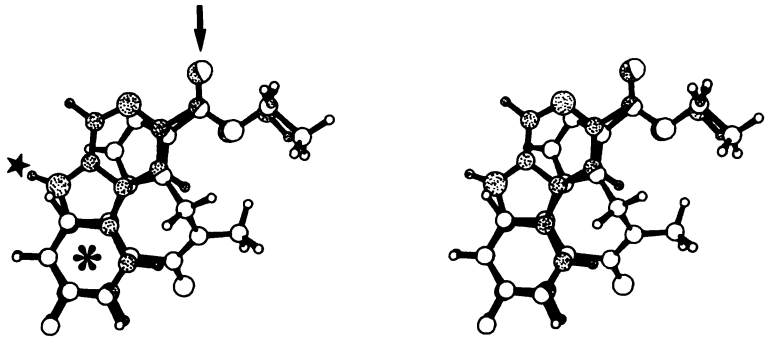

FIG. 2. *A stereoscopic drawing of the superposition of the solid-state conformations of Ro15-1788 and methyl β-carboline-3-carboxylate (stippled circles)*

The A ring of Ro15-1788 was superimposed on the ring of the β-carboline (*) and the N–C–C=O features were overlapped (marked with *arrows*). The N–H group of the β-carboline (marked with a *star*) has no similar feature in Ro15-1788. The drawing was made with the computer program PLUTO (31).

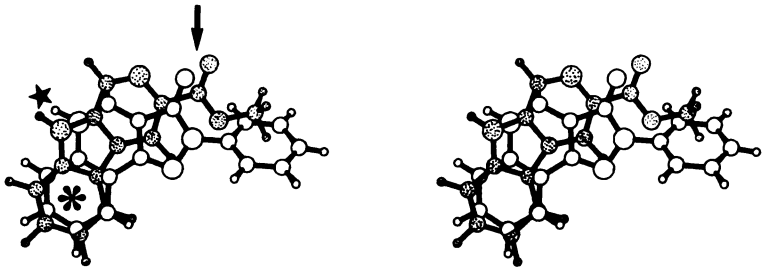

FIG. 3. *A stereoscopic drawing of the superposition of the solid-state conformations of methyl β-carboline-3-carboxylate (stippled circles) and CGS-8216*

The benzene rings in the two structures were superimposed (*) and the N–C–C=O features were overlapped (*arrow*). Both molecules have a strong hydrogen bond donating group, N–H (*star*). The drawing was made with the computer program PLUTO (31).

βCCM structure, this *cis*-N–C–C=O group accepts a three-center hydrogen bond. There is a good fit of the entire ethyl ester side chain and of the overall molecular shape. The regions occupied by N(4) and by substituents on C(5) in Ro15-1788 are not present in the β-carboline structure. A similar feature to the β-carboline hydrogen bond donor atom, N(9), is not found in the Ro15-1788 structure (see Fig. 2). Fig. 3 shows an overlap of βCCM with the pyrazoloquinolinone antagonist, CGS-8216 (26). Again, the six-membered aromatic rings overlap as do the hydrogen bond acceptor regions: =N–C–C=O in βCCM with C=O in CGS-8216. In this overlap the ethyl ester side chain occupies the same region as the phenyl side chain in CGS-8216. Unlike the overlap with Ro15-1788, the hydrogen bond donor atom, N(9), of βCCM has a parallel in the CGS-8216 structure, a strongly positive N atom in the central ring (26).

DISCUSSION

Larger conformational changes in the receptor when antagonists or inverse agonists are bound, over those due to the binding of agonists, may be the key to distinguishing among ligands with positive, negative, or no efficacy. Chiu *et al.* (27) have shown that the conformational changes in the receptor induced by Ro15-1788 are different from those induced by agonists. This finding may be universal for both the negative and the no-efficacy ligands. If this hypothesis is true, these ligands should have common structural features that resemble the agonists, since they all bind to the same receptor but are different in the relative orientation of their functional groups.

The similarities in the observed structures of the three types of nonagonist ligands can be summarized in a model binding site as shown in Fig. 4. This model has sites that 1) interact with the aromatic portions of the antagonists, 2) bind to the carbonyl oxygen atom of either the ester side chain in β-carbolines and Ro15-1788 or of the pyrazole ring of CGS-8216, 3) bind the hydrophobic side chain, and 4) accept a hydrogen atom from a N–H donor (this last site is not present in the imidazobenzodiazepine). The six-membered aromatic ring (A ring) is the common feature used as an anchor in these comparisons. The relative orientation and separation of the other features can then be examined with respect to the A ring. Site 2 is present in all of the ligands. The extensive structure-activity data available on β-carboline derivatives indicates both that an electronegative atom on the C(3) side chain is required for high affinity and that the β-carboline must be aromatic which enhances the extended coplanarity of the ring and the side chain. The separation of sites 1 and 2 is the same in all of the antagonists as shown in Table 3. The hydrophobic side chains (site 3) superimpose easily in the overlap drawings. The hydrogen bond formation invoked in site 4 is consistently observed in all crystal structures of the β-carbolines and CGS-8216 (26). Indeed, in the CGS-8216 crystal structure there is evidence that this N atom has a partial positive charge, so it is a strong H atom donor.

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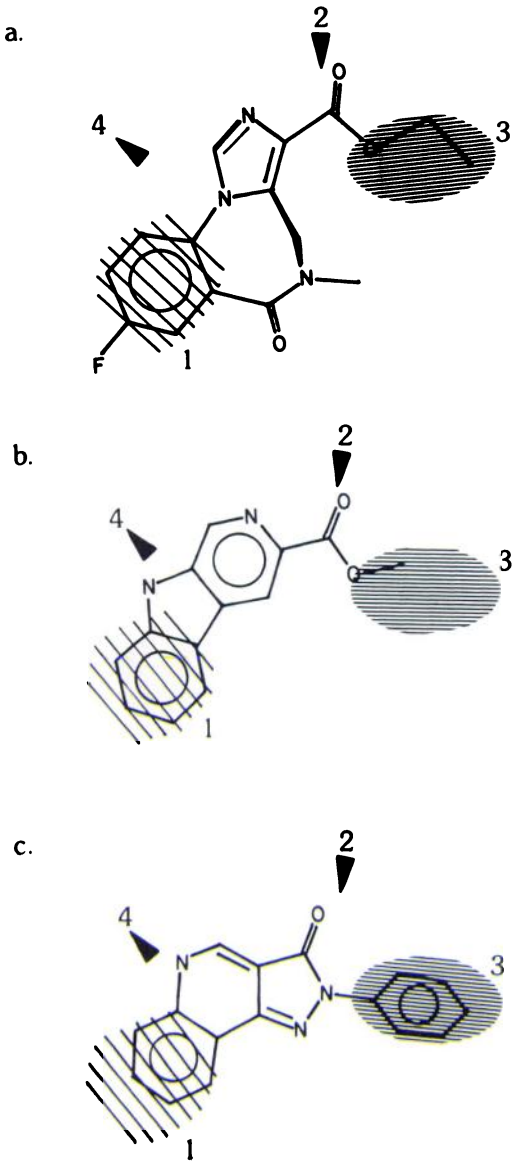

FIG. 4. *Model binding site for antagonists that recognizes 4 features 1*, an aromatic six-membered ring; *2*, a carbonyl oxygen atom *or* a combination of imine N atom and carbonyl O atom; *3*, a hydrophobic side chain; and *4*, a hydrogen atom donor group N–H. (This last feature is not present in Ro15-1788.) a. Ro15-1788 in the model binding site; b. methyl β-carboline-3-carboxylate in the site; c. CGS-8216 in the binding site.

The efficacy of antagonists and inverse agonists is sensitive to the size and the hydrophobicity of the side chain that binds at site 3. The β-carboline ligands change from convulsant to proconvulsant to weak agonist as this side chain increases in size from methyl to ethyl to propyl esters. The pyrazoloquinolinone compounds show a trend from antagonist to partial agonist to agonist as the *para* substituent of the phenyl ring is changed from H to methoxy to chlorine (28). This side chain is not the only feature that determines the response to the ligand since the two ligands with ester side chains, Ro15-1788 and ethyl β-carboline-3-carboxylate, produce different responses, antagonist *versus* proconvulsant. The common diazepine ring in Ro15-1788 and the agonists, not present in the β-carbolines, may explain this difference.

Some of the features of this model for the binding of antagonists and inverse agonists also apply to the agonist benzodiazepines, but not without changes in the relative orientation and separation of the sites. Fig. 5 shows an overlap of Ro15-1788 with an agonist benzodiazepine, flunitrazepam (29). The overall fit of the benzodiazepine portion of the molecule is good, but the positions of the common carbonyl groups in the two structures are quite different. Agonist benzodiazepines have a consistent arrangement of this carbonyl oxygen atom, the aromatic A ring, and the imine N atom, N(4). The two electronegative atoms (O and N) are always on the same side of the A ring and are within 0.2 Å of the same distance from the ring. In Ro15-1788 these two atoms, O(19) and N(4), are on the same side of the ring, but they are at different distances from the plane of the A ring, 1.27 and 0.47 Å, respectively. In addition, the relative separation of these two potential binding sites is always the same in the agonist benzodiazepines, 3.30 ± 0.04 Å for 30 examples; in Ro15-1788 this distance is much longer at 5.54 Å. Thus, a conformational change of the receptor from the shape that binds agonists would be required to bind to both of these sites on the antagonist.

A model for the benzodiazepine receptor that has seven possible interactions with a ligand can account for the structural similarities and binding data for the antagonists *and* the agonists. Fig. 6 shows a superposition of Ro15-1788 and flunitrazepam in this binding site. The addition of three features to the antagonist model accounts for the binding data on agonists: 5, a cationic site that binds to the imine N atom; 6, a site that recognizes

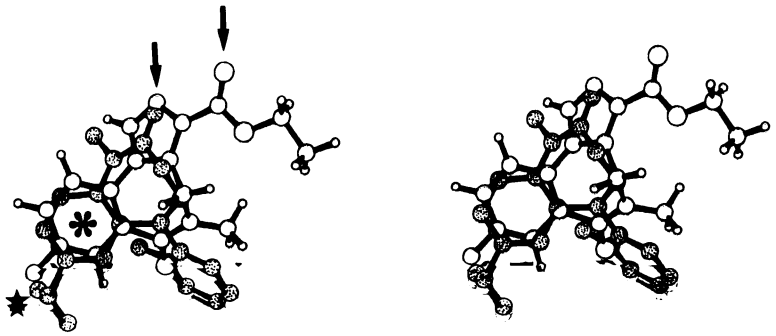

FIG. 5. *A stereoscopic drawing of the superposition of the solid-state conformations of Ro15-1788 and flunitrazepam (stippled circles)*
The A rings of the two structures were superimposed (*) and the best fit of the diazepine ring and the electronegative atoms bound to C(2) was sought. The carbonyl oxygen atoms (*arrow*) are in different positions in the two structures while the C(7) substituents (*star*) are in similar positions. The drawing was made with the computer program PLUTO (31).

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FIG. 6. *A model binding site for the benzodiazepine receptor with the structures of Ro15-1788 and flunitrazepam superimposed in the site*

The site has all the features of the antagonist binding site shown in Fig. 4 and three additional features. These are a site (*) to bind the imine N atom of the diazepine ring, a site (*large asterisk*) for the C(5) phenyl ring, and a site for the C(7) electronegative substituent (+). The *arrows* mark the conformational change required to accommodate the differences between antagonists (Ro15-1788) and agonists (flunitrazepam).

the C(5)-phenyl group; and 7, a cationic site for the C(7) electron-withdrawing substituent on the A ring. A binding point for site 5 is not present in either the β-carbolines or pyrazoloquinolinone compounds and is not in the same position for the Ro15-1788 structure as for the agonist. (This difference between the two types of antagonists may be coupled to the difference in effect of the hydrophobic side chain mentioned above.) A binding point for site 6 is not present in any of the antagonists considered, although the C(5) carbonyl group of Ro15-1788 does occupy part of this space. An electron-withdrawing group at site 7 is present in the Ro15-1788 compound and does enhance activity. This binding site model (Fig. 6) predicts that a similar substituent on the β-carbolines or the pyrazoloquinolinones would increase the affinity of these compounds.

The model-binding site in Fig. 6 agrees, in part, with the other models for agonists. The relative orientation of the aromatic groups in this model is approximately the same as that proposed by Fryer (6). Gilli's model for the importance of the N(1)–C(2)=O group would be consistent with this model as well. The cationic sites of the theoretical model proposed by Loew and co-workers (8) correspond to sites 2, 4, and 5. The crystal structure interactions caused us to identify site 4 as a hydrogen-bonding site, but this identification is not unambiguous.

This model specifically identifies the positions and the types of binding groups that characterize the differences between antagonist and agonist ligands for the benzodiazepine receptor. It is notable that the two major differences between agonists and antagonists, sites for the imine N atom and the C(5) phenyl ring, are on the same side of the ligand; it may be that interaction with this portion of the receptor determines the biological response to the ligand.

REFERENCES

1. Squires, R. F., and C. Braestrup. Benzodiazepine receptors in rat brain. *Nature* **266**:732–734 (1977).
2. Snyder, S. H. Neurotransmitter receptor binding and drug discovery. *J. Med. Chem.* **26**:1667–1672 (1983).
3. Tallman, J. F., J. W. Thomas, and D. W. Galleger. GABA-ergic modulation of benzodiazepine binding site sensitivity. *Nature* **274**:383–385 (1978).
4. Braestrup, C., and M. Nielsen. GABA reduces binding of H-methyl β-carboline-3-carboxylate to brain benzodiazepine receptors. *Nature* **294**:472–474 (1981).
5. Braestrup, C., R. Schmiechen, G. Neef, and M. Nielsen. Interaction of convulsive ligands with benzodiazepine receptors. *Science* **216**:1241–1243 (1982).
6. Fryer, R. I. Benzodiazepine ligand-receptor interactions, in *The Benzodiazepines: From Molecular Biology to Clinical Practice* (E. Costa, ed.). Raven Press, New York, 7–20 (1983).
7. Gilli, G., P. A. Borea, V. Bertolasi, and M. Sacerdoti. Qualitative and quantitative aspects in the structure-activity relationships in benzodiazepines, in *Molecular Structure and Biological Activity* (J. F. Griffin and W. L. Duax, eds.). Elsevier Science Publishing Co. Inc., New York, 259–276 (1983).
8. Loew, G. H., J. R. Nienow, and M. Paulson. Theoretical structure-activity studies of benzodiazepine analogues. Requirements for receptor affinity and activity. *Mol. Pharmacol.* **26**:19–34 (1984).
9. Codding, P. W. Structure-activity studies of β–carbolines. 1. Molecular structure and conformation of cis-3-carboxylic acid-1,2,3,4-tetrahydroharmane dihydrate. *Can J. Chem.* **61**:529–532 (1983).
10. Muir, A. K. S., and P. W. Codding. Structure-activity studies of β-carbolines. 2. Crystal and molecular structures of *N*-ethyl-3-carbamoyl-β-carboline. *Can. J. Chem.* **62**:1803–1806 (1984).
11. Muir, A. K. S., and P. W. Codding. Structure-activity studies of β-carbolines. 3. Crystal and molecular structures of methyl β-carboline-3-carboxylate. *Can. J. Chem.*, in press (1985).
12. Hunkeler, W., H. Mohler, L. Pieri, P. Polc, E. P. Bonetti, R. Cunien, R. Schaffner, and W. Haefley. Selective antagonists of benzodiazepines. *Nature* **290**:514–516 (1981).
13. Kawasaki, K., M. Kodama, and A. Matsushita. An imidazodiazepine derivative, Ro15-1788, behaves as a weak partial agonist in the crossed extensor reflex. *Eur. J. Pharmacol.* **102**:147–150 (1984).
14. Germain, G., P. Main, and M. M. Woolfson. The application of phase relationships to complex structures III. The optimum use of phase relationships. *Acta Crystallogr. Sect. A.* **27**:368–376 (1971).
15. Hendrickson, W. A., and J. H. Konnert. in *Biomolecular Structure, Conformation, Function and Evolution* (R. Srinivasan, ed.). Pergamon Press, New York, 43–57 (1979).
16. Konnert, J. H., and W. A. Hendrickson. A restrained-parameter thermal-factor refinement procedure. *Acta Crystallogr. Sect. A.* **36**:344–350 (1980).
17. Flippen-Anderson, J., R. Gilardi, and J. H. Konnert. Program RESLSQ. NRL Memorandum Report 5042. Naval Research Laboratory, Washington, D.C. (1982).
18. Stewart, J. M. The XRAY system of crystallographic programs. Computer Science Center, University of Maryland, College Park, MD (1976).
19. Cromer, D. T., and J. B. Mann. X-ray scattering factors computed from numerical Hartree-Fock wave functions. *Acta Crystallogr. Sect A.* **A24**:321–323 (1984).
20. Stewart, R. F., E. R. Davidson, and W. T. Simpson. Coherent X-ray scattering for the hydrogen atom in the hydrogen molecule. *J. Chem. Phys.* **42**:3175–3187 (1965).
21. Hester, J. B., Jr., D. J. Duchamp, and C. C. Chidester. A synthetic approach to new 1,4-benzodiazepine derivatives. *Tetrahedron Lett.* **20**:1609–1612 (1971).
22. Kamiya, K., Y. Wada, and M. Nishikawa. Molecular structures of 8-chloro-6-phenyl-4H-8-triazolo [4,3-a][1,4]benzodiazepine, 2-acetoxyamino-4-acetyl-8-chloro-3,4-dihydro-6-phenyl-1,4,5-benzotriazocine and 8-chloro-4,11-diacetyl-4,11-dihydro-2-methyl-6-phenyloxazolo [4,5-b]-[1,4,5]benzotriazocaine. Formation of an eight membered ring from a quinazoline derivative on treatment with hydrazine evidenced by X-ray analysis. *Chem. Pharm. Bull. (Tokyo)* **21**:1520–1529 (1973).
23. Butcher, H., and T. A. Hamor. Structure of 8-chloro-1-(dimethylamino)methyl]-6-phenyl-4H-imidazo-[1,2a][1,4]benzodiazepine, $C_{20}H_{19}ClN_4$. *Acta Crystallogr. Sect. C.* **40**:848–850 (1984).
24. Duax, W. L., C. M. Weeks, and D. C. Rohrer. Crystal structures of steroids, in *Topics in Stereochemistry*, Vol. 9 (E. L. Elial and N. Allinger, eds.). John Wiley and Sons, New York, 271–383 (1976).
25. Hamor, T. A., and I. L. Martin. The Benzodiazepines. in *Progress in Medicinal Chemistry*, Vol. 20 (G. P. Ellis and G. B. West, eds.). Elsevier Science Publishers, New York, 157–223 (1983).
26. Muir, A. K. S. Structural studies of benzodiazepine receptor ligands. Ph.D. thesis, University of Calgary (1985).
27. Chiu, T. H., and H. C. Rosenberg. Conformational changes in benzodiazepine

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receptors induced by the antagonist Ro15-1788. *Mol. Pharmacol.* **23**:289–294 (1983).
28. Yokoyama, J. W., B. Ritter, and A. D. Neubert. 2-Arylpyrazolo[4,3-c]quinolin-3-ones: novel agonist, partial agonist, and antagonist of benzodiazepines. *J. Med. Chem.* **25**:337–339 (1981).
29. Butcher, H., T. A. Hamor, and I. A. Martin. Structures of 7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one (Bromazepam) and 5-(2-fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2H-1,4-benzodiazepin-2-one (Flunitrazepam). *Acta Crystallogr.* **C39**:1469–1472 (1983).
30. Johnson, C. K. ORTEP. Report ORNL-3794. Oak Ridge National Laboratory, Oak Ridge, TN (1965).
31. Motherwell, S. PLUTO. A program for plotting molecular and crystal structures. University Chemical Laboratory, Cambridge, England (1979).

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