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Modeling the Benzodiazepine Receptor Binding Site by the General Three-Dimensional Structure-Directed Quantitative Structure-Activity Relationship Method REMOTEDISC

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SUMMARY

A novel computer-aided receptor modeling method, REMOTEDISC [*J. Med. Chem.* **32**:746–756 (1989)], has been used to analyze the inhibition of labeled diazepam binding by 29 benzodiazepine receptor ligands. The method uses the three-dimensional structure, conformational energy, and important atom-based physicochemical properties to model the hypothetical binding site cavity. The model not only consists of the geometry of the binding cavity but also gives the weight of the various physicochemical properties of the ligands at different parts of the binding cavity responsible for their binding to the receptor. The model fitted the binding data with a correlation coefficient of 0.980, a SD of 0.223, and an explained variance of 0.898. It suggested that a small hydrophilic group is favored at position 1 of the benzodiazepine ring, the C=O region of this ring is favored by dispersive atoms and positive charge, the 4′-substituent of the 5-phenyl group is subject to strong steric repulsion, the 7-position is favored to be a hydrophilic group, and the 8- and 9-positions and their substituents are favored to be dispersive as well as hydrophilic groups. It also suggested that the substitution of the 5-phenyl group by the more dispersive 2-thiophene may increase the binding affinity. The model was allowed to predict the binding affinity data of five compounds with extensive variation of the structure from the training set; the prediction for four compounds was excellent. Some of the problems of the method have been discussed with their possible remedies.

We are in the process of developing and improving (1–5) a physically realistic 3D-QSAR method, REMOTEDISC, which is capable of deducing a model of the binding site cavity from the binding data of a series of structurally related or diverse ligand molecules. The method uses the three-dimensional structure and atom-based physicochemical properties of the ligand molecules to develop a hypothetical model for the binding site cavity. There are several 3D-QSAR approaches that have been evolving in recent years (6–11). The manual interpretation of the structural factors necessary for a biological activity or binding affinity is very difficult from molecular mechanics or dynamics conformational calculations alone. A molecule may not interact with the biological receptor in its global minimum energy conformation, and the number of possible low energy conformations often is too high for a thorough examination. The physicochemical properties related to molecular interaction and their distribution over the molecular structure often are too complex for an easy correlation with the binding affinity.

The method used here gives a complementary picture of the binding site cavity, if the biological activity is assumed to be the result of interaction with a single binding site. However, if the biological activity is a consequence of binding with more than one receptor site, the outcome of the method can be interpreted as the structural and physicochemical requirements necessary to satisfy simultaneously all the receptor sites. Such an interpretation will hold only if the relative orientations of the ligands with different receptors remain unaltered. Although the danger of getting a wrong or a chance correlation increases as the biological data become more complicated, such an approach should not be totally discouraged. The ultimate biological activity in which we are interested is the effect on the animal or human system. We can only hope that in the future all the biological receptors involved in a biological response will be characterized at the three-dimensional structural level with the identification of the binding site and that transportation, partitioning, and the metabolic factors of the drug will be clearly

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ABBREVIATIONS: 3D-QSAR, three-dimensional structure-directed quantitative structure-activity relationships; GABA, γ-aminobutyric acid; QSAR, quantitative structure-activity relationships.

understood. Even at that time, one should not be optimistic enough to think that docking the drug molecules at the receptor site(s) and performing sophisticated molecular mechanics, dynamics (12, 13), or quantum chemical (14, 15) calculation will lead to an immediate drug discovery. All our past experience shows that nature is more complex than we speculate. However, such information will definitely help in a better understanding of the biological activity data and may accelerate the drug discovery process. At present, the number of biological receptors whose structure and binding site are known is very limited; therefore, most of the biological activity or binding affinity data cannot be studied directly. To overcome this problem, not only should the biochemists and the X-ray crystallographers work closely to get the necessary information, but we should also develop some indirect way of studying the factors responsible for the biological activity. Most of the QSAR and 3D-QSAR approaches serve this purpose. Being empirical approaches, these methods have some advantages over direct molecular mechanics and dynamics studies. Even when the biochemical steps involved in a biological response are not totally understood, these methods can allow an educated guess. However, direct molecular mechanics or dynamics calculations will be more useful when the biochemical steps are clearly understood.

In the past few years, the investigation of benzodiazepine has been one of the most active research fields in molecular neuropsychopharmacology (16). In the mammalian central nervous system, GABA acts as a major inhibitory neurotransmitter by affecting a chloride ion membrane channel, called the $GABA_A$ receptor. Apparently, the $GABA_A$ receptor is complexed with the benzodiazepine receptor to form an allosteric linkage, such that the binding of various benzodiazepines to their receptor can alter the binding of GABA to its receptor and vice versa (17, 18). The two receptors ($GABA_A$ and benzodiazepine receptors) occur on different proteins and both amino acid sequences are now known (19), but the detailed three-dimensional structure of the benzodiazepine binding site has yet to be determined by X-ray crystallography or two-dimensional NMR, assuming such experiments are feasible. In the meantime, our only avenue of probing the structure and energetics of the benzodiazepine receptor site is by binding studies.

Analysis of the benzodiazepine receptor binding data, however, suffers from two complicating factors, the difference in the pharmacological response (16) of the ligands (agonists, antagonists, or inverse agonists) and the heterogeneity (16, 20) of the benzodiazepine receptor. The difference in the pharmacological response may come from binding at different sites that may or may not be overlapping or from the difference in the binding mode of the ligand (conformation or orientation). Answering these questions with ligand-receptor binding affinity data alone is extremely difficult, if not impossible, and needs several assumptions. If we assume that all pharmacological responses are coming from the same overlapping binding sites and from a single binding mode, we can solve the problem using the following steps: consider the ligands having different pharmacological responses separately in the 3D-QSAR receptor mapping procedure, develop the separate receptor model, and then superimpose the receptor regions having the same complementary features. The problem of heterogeneity evolved when some evidence (16, 20) was found in favor of the existence of two receptor subclasses, called BZ_1 and BZ_2. The two receptors have slightly different recognition properties and are differently distributed in different brain regions. However, the reason for this heterogeneity is totally unknown (16). It may be due to multiple receptor species or to two conformational states of the same receptor. Because the difference between these two species is small and no extensive ligand binding data are available on the separate species, like most other workers in this field we developed a combined species model using a largely modified method of our earlier effort (21), utilizing mostly agonists.

TABLE 1

Structure and biological activity of the benzodiazepine derivatives used in the training set

Compound[a]	Structure	Substituents	log(1/IC_{50})[b] Observed	Calculated	Δ_{c-o}
1	I	$X_7 = NO_2$; $X_{2'} = F$	8.82	8.74	−0.08
2	I	$X_7 = Cl$; $X_{X'} = X_{6'} = F$	8.80	8.67	−0.13
3	I	$X_7 = X_8 = Cl$; $X_{2'} = F$	8.44	8.56	0.12
4	I	$R_1 = CH_3$; $X_7 = F$; $X_{2'} = F$	8.29	7.82	−0.47
5	I	$X_7 = Cl$; $X_{2'} = X_{6'} = Cl$	8.15	8.36	0.21
6	I	$R_1 = CH_2CH_2NH_2$; $X_7 = Cl$; $X_{2'} = F$	8.08	8.03	−0.05
7	I	$X_7 = CH::CH_2$	7.62	7.76	0.14
8	I	$X_7 = F$	7.40	7.60	0.20
9	I	$X_7 = X_8 = Cl$; $R_1 = CH_3$	7.40	6.91	−0.49
10	I	$X_7 = NH_2$; $R_{2'} = Cl$	7.12	6.94	−0.18
11	I	$X_6 = Cl$; $R_1 = CH_3$; $X_{2'} = F$	6.82	7.46	0.64
12	I	$R_1 = CH_2CH_2NH_2$	6.59	6.58	−0.01
13	I	$R_1 = C(CH_3)_3$; $X_7 = NO_2$; $X_{2'} = Cl$	6.52	6.69	0.17
14	I	$R_1 = CH_3$; $X_{4'} = Cl$	4.00	4.03	0.03
15	II		9.05	8.98	−0.07
16	III	$X_7 = NO_2$; $R_3 = (S)CH_3$; $R_{12} = CH_3$	8.47	8.22	−0.25
17	III	$X_7 = NO_2$; $R_3 = (R)CH_3$; $R_{12} = CH_3$	5.82	5.86	0.04
18	IV	$X_7 = Cl$; $R_{14} = CONH_2$; $R_{2'} = F$	8.46	8.61	0.15
19	IV	$X_6 = Cl$; $R_{14} = COOC(CH_3)_3$	7.85	7.83	−0.02
20	V		8.41	8.46	0.05
21	VI		8.03	8.00	−0.03
22	VII		7.74	7.96	0.22
23	VIII		7.06	6.62	−0.44
24	IX		6.89	6.92	0.03
25	X		6.77	6.90	0.13
26	XI		6.51	6.45	−0.06
27	XII		6.14	6.20	0.06
28	XIII		6.06	6.06	0.00
29	XIV		8.68	8.74	0.06

[a] See Fig. 1 for the structure of the compounds.

[b] Inhibition of [^{3}H]diazepam binding. See Ref. 16 for the details about the binding data.

Methods

Because the details of the method have been presented earlier (4), only the essential features of the method are summarized here. 1) Generate a three-dimensional structure of the molecule, satisfying the normal (crystallographic) bond lengths and bond angles. 2) Assign the physicochemical properties of the atoms in the molecule. Three physicochemical properties (22) are of major importance for the binding of the ligands with the biological molecules, namely, octanol-water partition coefficient for hydrophobic interaction, molar refractivity for dispersive or steric interaction, and charge density for electrostatic interaction. 3) Locate the low energy regions of the conformational space for each ligand using a grid search, for sampling purposes. 4) Select a hypothetical binding conformation for a strongly binding ligand, the so-called reference structure, from its low energy conformations. 5) Determine the geometrically possible superpositions of the other ligands on the reference structure. 6) Decide the best relative superposition of the ligands, using physicochemical property matching.

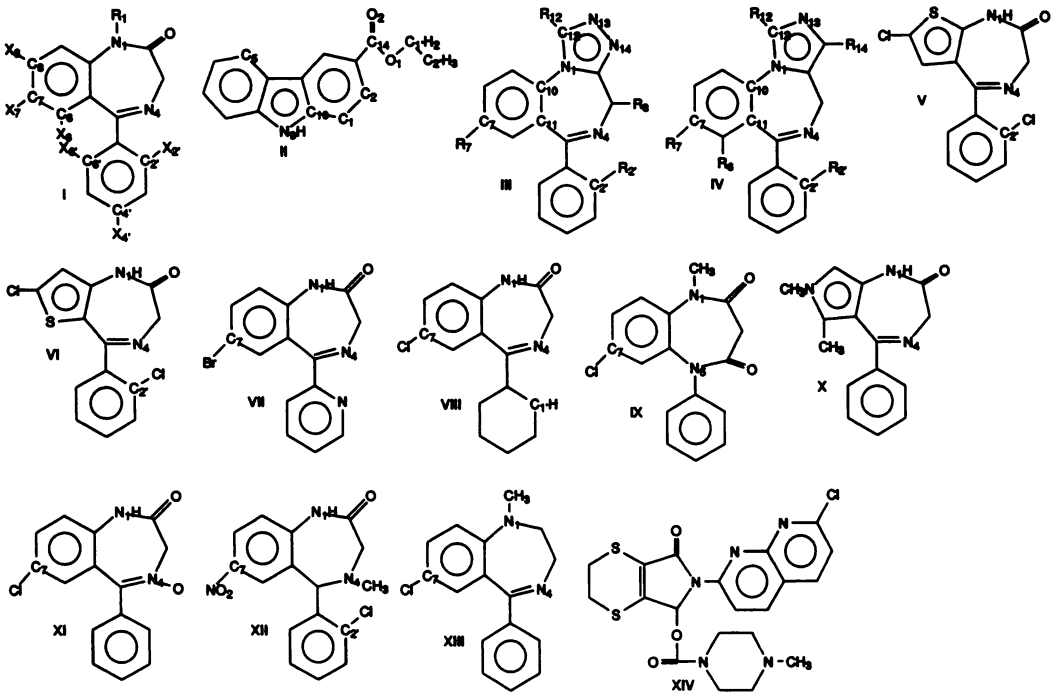

Fig. 1. General structure and atom numbering of the various benzodiazepine receptor ligands used in the training set.

TABLE 2

Description of the minimum energy conformations of the molecules

Compound	Structure[a]	Torsion angles[b]
1	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—O) = 0°$
2	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$
3	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$
4	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—R_1—H) = 40°$
5	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 60°$
6	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—C—C) = 140°$, $\omega(N_1—C—C—N) = 300°$, $\omega(C—C—N—H) = 180°$
7	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 0°$, $\omega(C_6—C_7—C{=}C) = 200°$
8	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 200°$
9	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C_2—N_1—C—H) = 40°$
10	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—H) = 340°$
11	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—C—H) = 40°$
12	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C_2—N_1—C—C) = 140°$, $\omega(N_1—C—C—N) = 300°$, $\omega(C—C—N—H) = 300°$
13	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—O) = 0°$, $\omega(C_2—N_1—C—C) = 40°$
14	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 180°$, $\omega(C_2—N_1—C—H) = 40°$
15	II	$\omega(N_2—C_3—C_{14}{=}O) = 180°$, $\omega(C_3—C_{14}—O_1—C_{1'}) = 180°$, $\omega(C_{14}—O_1—C_{1'}—C_{2'}) = 180°$
16	III	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(N_1—C_{12}—C—H) = 100°$, $\omega(C_2—C_3—C—H) = 60°$, $\omega(C_6—C_7—N—O) = 0°$
17	III	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$, $\omega(N_1—C_{12}—C—H) = 20°$, $\omega(C_2—C_3—C—H) = 60°$, $\omega(C_6—C_7—N—O) = 0°$
18	IV	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—C_{14}—C{=}O) = 0°$, $\omega(C_{14}—C—N—H) = 0°$
19	IV	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 120°$, $\omega(C_2—C_{14}—C{=}O) = 0°$, $\omega(C_{14}—C—O—C) = 40°$, $\omega(C—O—C—C) = 80°$
20	V	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 240°$
21	VI	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 240°$
22	VII	$\omega(N_4—C_5—C_{1'}—N) = 180°$
23	VIII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 300°$
24	IX	$\omega(C_4—N_5—C_{1'}—C_{2'}) = 20°$, $\omega(C_2—N_1—C—H) = 20°$
25	X	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C—CH_3) = 0$; $\omega(N—CH_3) = 60°$
26	XI	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$
27	XII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 120°$, $\omega(C_6—C_7—N—O) = 0°$, $C_3—N—C—H) = 60°$
28	XIII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$, $\omega(C_2—N_1—C—H) = 60°$
29	XIV	$\omega(C—N—C—N)$°k = 0°, $\omega(N—C—O—C) = 140°$, $\omega(C—O—C—N) = 200°$, $\omega(O—C—N—C) = 0°$, $\omega(C—N—C—H) = 60°$

[a] See Fig. 1 and Table 1 for the structural details and atom numbering.

[b] For the sign of the torsion angle, we followed the IUPAC convention (45); the only difference is that our angles vary from 0° to 360° but, in IUPAC convention, angles greater than 180° are considered negative.

[c] The rotation of the pyridopyridine group with respect to the amide bond.

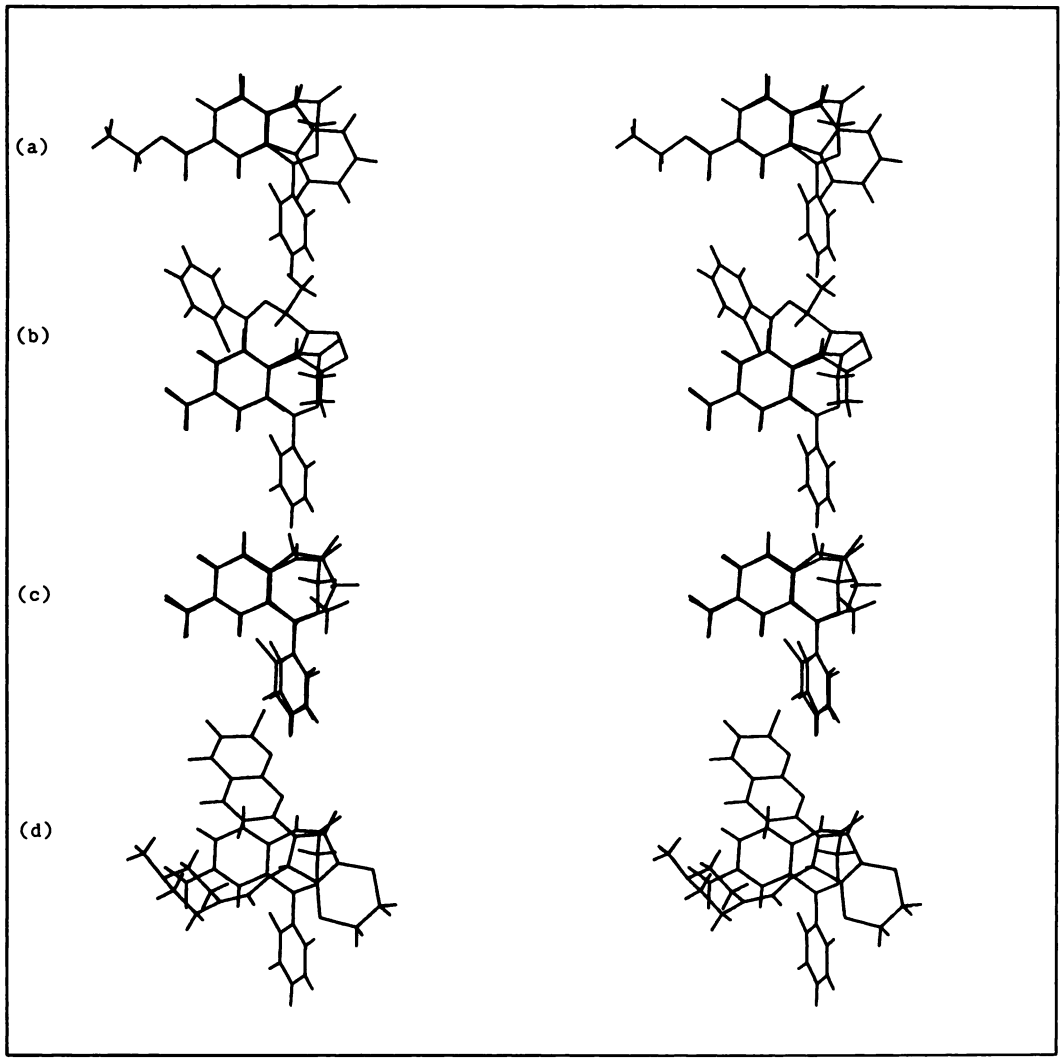

Fig. 2. Stereo view of the superpositions of some of the structurally different compounds on the reference compound **1**. a, compound **15**; b, compound **17**; c, compound **27**; d, compound **29**.

7) Generate the approximate structure of the active site cavity from the superimposed ligands by creating one site point for the coincident atoms and one site point for each atom of each molecule that is not superimposed on other atoms. 8) Dissect the active site cavity to differentiate the relative importance of the various physicochemical properties of the ligands at different regions of the binding site cavity, using reverse stepwise regression (23). The calculated binding energy or the biological activity is given by:

$$E_{calc} = -W \cdot E_c + \sum_{i=1}^{n_s} \sum_{j=1}^{n_p} [C_{ij} \sum_{k=1}^{n_o} P_{jk}] \quad (1)$$

where E_c is the conformational energy with some weight factor W, C values are the site pocket- and physicochemical property-dependent coefficients determined by the regressional method or any optimization procedure, n_s is the number of site pockets, n_p is the number of physicochemical properties active in the site pocket, n_o is the number of ligand atoms occupying the site pocket, and P_{jk} is the jth physicochemical property of the kth occupying atom of the ligand. Eq. 1 is based on the following idea: when an atom or atoms of the ligand enters a site pocket, it interacts with the receptor and the interaction energy depends not only on the physicochemical properties of the atom but also on the region of the binding site cavity where it entered. The weight factor for charge density to account for the electrostatic interaction, for example, will be high where the receptor atoms are charged.

When an independent variable is used during regressional analysis, it is very important that the variable should be uniformly distributed within its range. If only a few compounds (equations) have very different values for a particular independent variable (structural or physicochemical properties) and their dependent variables (biological activities) also vary considerably from those of the other compounds, the regression method uses this variable as the reason for the difference in the dependent variables. Although such interpretation may be reasonable, it creates another problem in the model. Because the independent variable in that range has not been tested in an adequate number of systems, its validity within the entire range may be uncertain. In our experience, a t-test or F-test does not always respond to this problem. The equations (compounds) differing considerably in the values of the dependent variable are commonly known as outliers. In a massive regression, one may often find that outlying independent variables were used for the outliers. Although we do not totally discourage the use of such variables during the regression analysis, we have defined a numerical function that will make an investigator aware of the outlying variables used during the regression process. He can then rectify his data set or test the outlying variable in some additional systems (see Appendix).

Critique of the method. The concept in Eq. 1 is that, when a ligand atom is in a particular site pocket, it is in a constant hydrophobic, dispersive, and electrostatic field. This may be true when the site pocket is sufficiently small (22). However, due to the statistical limitations, the number of different types of site pockets should be very limited and, hence, the size of the site pocket ought to be large. To get a more physically realistic potential, we should use distance-dependent potential. According to this formalism, the calculated binding energy is given by:

TABLE 3
Description of the active conformations of the various molecules

Compound	Structure[a]	Torsion angles[b]	Energy
1	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—O) = 0°$	0.00
2	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$	0.00
3	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$	0.00
4	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—R_1—H) = 40°$	0.00
5	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$	3.33
6	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—C—C) = 140°$, $\omega(N_1—C—C—N) = 300°$, $\omega(C—C—N—H) = 180°$	0.00
7	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 0°$, $\omega(C_6—C_7—C{=}C) = 40°$	0.27
8	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$	0.25
9	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C_2—N_1—C—H) = 40°$	0.00
10	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—H) = 200°$	4.01
11	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—N_1—C—H) = 40°$	0.00
12	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C_2—N_1—C—C) = 140°$, $\omega(N_1—C—C—N) = 300°$, $\omega(C—C—N—H) = 300°$	0.00
13	I	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_6—C_7—N—O) = 0°$, $\omega(C_2—N_1—C—C) = 40°$	0.00
14		$\omega(N_4—C_5—C_{1'}—C_{2'}) = 20°$, $\omega(C_2—N_1—C—H) = 40°$	1.48
15	II	$\omega(N_2—C_3—C_{14}{=}O) = 180°$, $\omega(C_3—C_{14}—O_1—C_{1'}) = 180°$, $\omega(C_{14}—O_1—C_{1'}—C_{2'}) = 180°$	0.00
16	III	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(N_1—C_{12}—C—H) = 100°$, $\omega(C_2—C_3—C—H) = 60°$, $\omega(C_6—C_7—N—O) = 0°$	0.00
17	III	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$, $\omega(N_1—C_{12}—C—H) = 20°$, $\omega(C_2—C_3—C—H) = 60°$, $\omega(C_6—C_7—N—O) = 0°$	0.00
18	IV	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$, $\omega(C_2—C_{14}—C{=}O) = 0°$, $\omega(C_{14}—C—N—H) = 0°$	0.00
19	IV	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 120°$, $\omega(C_2—C_{14}—C{=}O) = 0°$, $\omega(C_{14}—C—O—C) = 40°$, $\omega(C—O—C—C) = 80°$	0.00
20	V	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 240°$	0.00
21	VI	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$	1.87
22	VII	$\omega(N_4—C_5—C_{1'}—N) = 40°$	3.12
23	VIII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 220°$	2.10
24	IX	$\omega(C_4—N_5—C_{1'}—C_{2'}) = 40°$, $\omega(C_2—N_1—C—H) = 20°$	0.97
25	X	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 40°$, $\omega(C—CH_3) = 0°$, $\omega(N—CH_3) = 60°$	0.00
26	XI	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$	0.00
27	XII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 260°$, $\omega(C_6—C_7—N—O) = 0°$, $\omega(C_3—N—C—H) = 40°$	3.01
28	XIII	$\omega(N_4—C_5—C_{1'}—C_{2'}) = 140°$, $\omega(C_2—N_1—C—H) = 60°$	0.00
29	XIV	$\omega(N—C—O—C) = 140°$, $\omega(C—O—C—N) = 200°$, $\omega(O—C—N—C) = 0°$	0.00

[a] See the footnote *a* of Table 2.
[b] See the footnote *b* of Table 2.

$$E_{calc} = -W \cdot E_c + \sum_{i=1}^{n_s} \sum_{j=1}^{n_a} [C_{1i}Q_j/(1 + r_{ij}) + C_{2i}P_j/(1 + r_{ij})^m + C_{3i}R_j/(1 + r_{ij})^n] \quad (2)$$

The first term within brackets is the electrostatic interaction between the *i*th site type and the *j*th atom and is dependent on the atomic charge Q_j and its distance r_{ij} from the 'center' of the site pocket; the second term is the hydrophobic interaction, which is dependent on the atomic octanol-water partition coefficient P_j. The distance dependence of the hydrophobic potential is not a well studied subject. Utilizing our atomic parameters, Furet *et al.* (24) recently proposed an expression for the hydrophobic potential with m equal to 1, but an extensive research is needed before we come to an appropriate value. The third term may be either for the dispersive interaction or for the steric repulsion. For the dispersive interaction the suggested value of n is 6 and for the steric repulsion it may be 12. We added 1 to the distance term, because ligand atoms can always occupy the 'center' of the site pocket and can experience a finite limiting interaction. C values are the site-dependent coefficients to be determined by some optimization technique, n_s is the number of site pockets, and n_a is the number of atoms in the ligand. Such distance-dependent hydrophobic, dispersive, and electrostatic potentials can be coupled with the regular molecular mechanics potentials for docking studies at the hypothetical active site, using the standard approach of molecular dynamics. Such an approach will give a true 'dynamic QSAR.' In the present work, however, we used Eq. 1 for the calculation of binding affinity.

Although the physicochemical properties used to model the ligand-receptor interactions can cover most of the physical interactions and the entropic factor arising from the structuring of the water molecules before and after binding, they are not sufficient to cover the entropic factor coming from the fixing of the ligand in its active conformation from the regular conformational distribution. Such a factor is important when the ligand molecule has an energetically flat global minimum conformational region or a large number of minima energetically close to global minimum. Although, in theory it is not very difficult to calculate such a factor from statistical thermodynamics (25), there are some practical difficulties. The calculation requires the evaluation of partition function, which in turn requires the conformational energy distribution in the entire conformational space. Generation of such information for even medium-sized ligand molecules is prohibitively expensive, with the present state of computation.

During the application of this method, we faced several problems. 1) Often, the number of conformations having energy lower than a preassigned value was extremely large. Selection of a finite number of lowest lying conformations may miss the physicochemically optimum superposition, simply because the appropriate conformation was neglected. 2) At present, only the choice of the reference structure is based on the binding data; superposition is based on unweighted scaled physicochemical parameters. However, the relative importance of various physicochemical properties is not the same at different regions and is very much dependent on the surrounding receptor atoms.

To overcome the first problem, a finite number of geometrically diverse conformations of energy less than a preassigned value should be used. The sum of the differences in the interatomic distances may be used to select the geometrically diverse conformations. Alternatively, one can focus attention on a few physicochemically best superpositions by using atomic physicochemical property matching (4) as follows. If a superposition is better than the already accepted superpositions, use distance range matching (26) for initial acceptance and use distance geometry embedding (27) for the final acceptance of the molecular superposition. In order to make the superposition physically more realistic, an iterative approach should be used. Start with a superposition using unweighted scaled physicochemical parameter to evaluate the weight (step 8). Use the evaluated weight to reevaluate the superposition, and continue until the process converges.

Results and Discussion

Twenty-nine benzodiazepine receptor ligands were used to develop the present model. Their IC_{50} values (16) for [^{3}H]

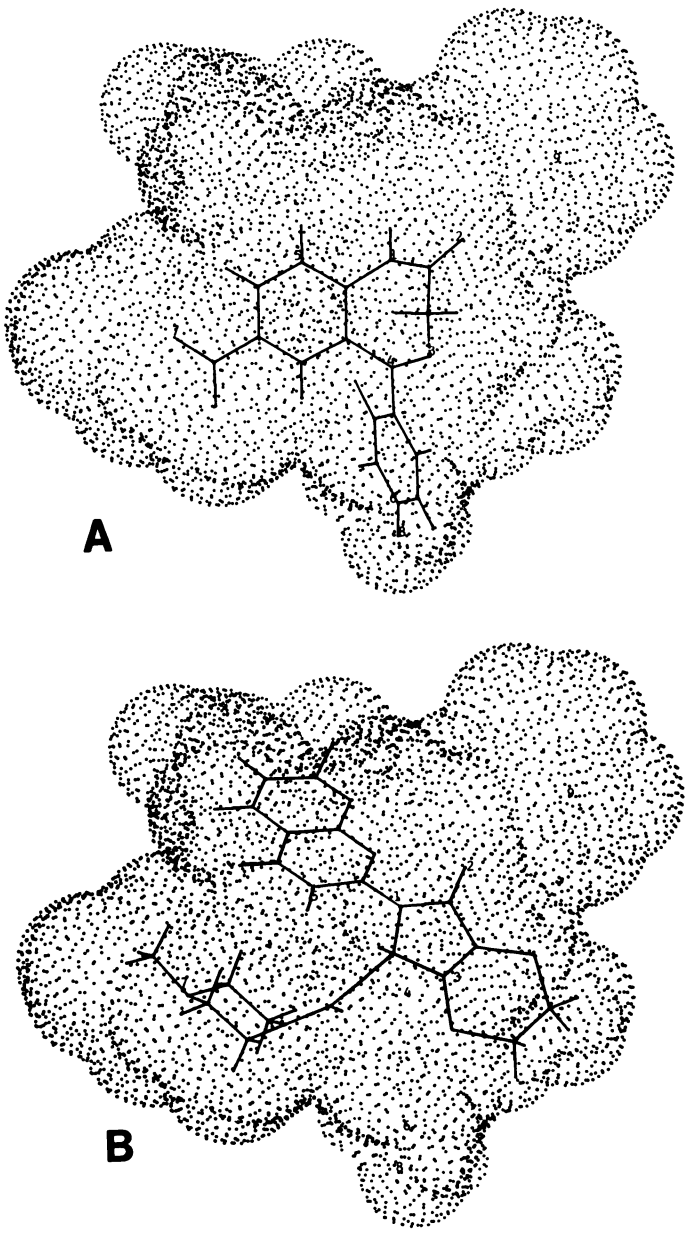

Fig. 3. Surface of the hypothetical active site cavity and the approximate location of the center of the different types of pockets. A, Reference compound **1**; B, compound **29** inside the cavity.

diazepam binding are shown in Table 1 and the general structures and atom numbering used in the present discussion are shown in Fig. 1. Except for compound **14** (28), these compounds were arbitrarily selected from the work of Haefely *et al.* (16), so that the number of compounds is at a manageable level and the structures are sufficiently varied. The structural variations made during the selection of the ligands are summarized below: 1) the fused benzene ring has been replaced by heterocyclic rings of diverse polarities (see Fig. 1, **V**, **VI**, and **X**); 2) the diazepine ring was saturated (**XII**) to check the conformational effect; 3) the 4-N was moved to the 5-position (**IX**); 4) the polar C═O group of the 2-position was altered by introducing different kinds of heterocyclic rings (**III** and **IV**); 5) the negative charge center at the 4-position was moved, in both the *N*-oxide (**XI**) and the amide (**IX**); 6) the 5-phenyl substituent has been replaced by a saturated ring (**VIII**) or a heterocyclic ring (**VII**); and 7) two very different structures, ethyl-β-carboline (**II**) and suriclone (**XIV**), were included, in addition to the usual alterations of the substituents among the benzodiazepines. Among these 29 benzodiazepine agonists, **1–14** can be classified as classical 1,4-benzodiazepines. It is, however, not the intention of the authors to create an universal model.

Molecule generation and conformational analysis. The initial structures of the various compounds studied in the present work were generated from a crystallographic fragment library (29–32). Because the crystal structure of compounds **16–21** were not available, these compounds were generated by fusing the necessary heterocyclic ring (32) with the unchanged diazepine ring. The conformational calculation was done using our CONFOR (2) program, details of which have been described recently (33). The rotation of the phenyl ring attached at the 5-position was of interest. Due to delocalization, this ring was presumed to be in the same plane as the C_5═N_4 bond. However, to avoid some steric repulsion, the phenyl ring went out of plane by 40°. The substituent at the 2′-position remained *anti* to the C_5═N_4 bond, possibly due to electrostatic repulsion between the negative substituent and the nitrogen. The minimum energy conformations of the various molecules, as obtained from the molecular mechanics calculation, are shown in Table 2.

Molecular superposition. The receptor site model presented here has been developed on the basis of the minimum energy conformation of compound **1**, the second most active compound in the data set. Use of the four lowest lying conformations of compound **15** failed as appropriate reference structures. The automated molecular superposition procedure, as described before (33), had no problem getting the expected superpositions for most of the molecules. By the term "expected superposition," we mean the superposition where the diazepine ring had the identical orientation. In this method, the atom-based physicochemical properties are used along with some property-matching functions for the quantification of the goodness of the molecular superposition. The puckering of the diazepine ring in a few molecules (compounds **17**, **24**, **27**, and **28**) was opposite to that in the reference compound. Initially, the program had problems in getting the expected superposition for these molecules. Because CONFOR does a fixed valence structure conformational calculation, the benzodiazepine rings were kept rigid throughout this calculation. However, when these rings were given comparable pucker, using the distance-constrained energy minimization (34), the program was able to find the desired superposition for **24**, **27**, and **28**. Although **15** and **29** had totally different structures and **17** had an enantiomeric structure, the program gave the best possible superposition. Some of these difficult superpositions are shown in Fig. 2. The conformational descriptions of the superposed structures are given in Table 3. Most of the molecules superposed most effectively at their minimum energy conformation. For **5**, the bulky chlorine is responsible for the moderately high energy conformation. Its minimum energy conformation is 60° from the planar structure. The active conformation of molecule **10** needs some explanation. Because in the reference structure the 7-position had a nitro group, which is physicochemically very different from NH_2, the physicochemically best superposition was obtained by the minimum contact of these two groups. During the quantification of the molecular superposi-

TABLE 4

Description of the site pockets

The coefficient for conformational energy is −0.6637. The values within parentheses represent the homogeneity of the corresponding physicochemical properties of the ligand molecules used in the present data set.

Site pocket	Coefficients of physicochemical parameters			Description (geometrical[d] and physicochemical[e])
	Hydrophobic[a]	Dispersive[b]	Electrostatic[c]	
1	−0.7028 (69.3)	−0.1234 (41.5)		Binds N1 and the attached H; favors small hydrophilic group
2		0.1239 (56.7)	1.1167 (56.1)	Binds C2 and O2; favors dispersive group and positive charge
3	1.6338 (48.1)	0.3806 (49.3)	4.3338 (62.3)	Binds C3 and the attached H and N4; favors hydrophobic, dispersive, and positive atoms
4	0.3287 (65.1)	−0.1351 (76.7)		Binds C5, C11, C6, C1′, and C12; favors small hydrophobic groups
5	−2.4006 (53.5)	0.3177 (27.0)		Binds C8, C9, their substitutents, and C10; favors both hydrophilic and dispersive groups
6	−5.5182 (40.9)	0.4551 (19.4)		Binds C3′, C5′, their substitutents, C2′, C4′, and C6′; favors both hydrophilic and dispersive groups
7	−0.8672 (69.2)	0.0633 (37.1)	2.6807 (58.7)	Binds C7 and its attached substituent; favors hydrophilic groups with positive charge
8		−0.6643 (28.8)		Binds the substituent attached to C4′; favors small atom
9				Binds some of the distant atoms of N1 substituents; interaction dormant

[a] Octanol-water partition coefficient was used to model the hydrophobic interaction.
[b] Molar refractivity was used to model the dispersive and steric interactions.
[c] CNDO/2 atomic charge density was used to model the electrostatic interaction.
[d] Geometrical description is provided with respect to the reference molecule, which is generally applicable for many other molecules.
[e] The physicochemical properties of the preferred atoms suggested here do not consider the effect on the conformational properties or the physicochemical properties of the other parts of the molecule. Those effects can be estimated by the actual generation of the molecule.

TABLE 5

Statistics of the study

No. of compounds	No. of site pockets	No. of parameters	SD	Correlation coefficient	Explained variance	*F* test
						%
29	9	18	0.223	0.980	0.898	99.998

tion, conformational energy was neglected, except that it was constrained to be less than 5 kcal/mol.

Binding site cavity. The hypothetical binding site cavity[1] was generated from the superposed structure of 29 molecules. The program divided the binding site cavity into nine pockets; the surface of the cavity and the approximate location of the centers of the site pockets are presented in Fig. 3. The physicochemical and geometrical descriptions of these site pockets are presented in Table 4. This table also contains the weight of the various physicochemical properties towards the binding affinity and the homogeneity (see Appendix) of the physicochemical property used to determine the weights (coefficients). Although at present it is not very obvious what the lower acceptable limit of homogeneity function is, we prefer values greater than 50%. The model suggests that N1 and its substituent should be small and hydrophilic in nature; the C7 substituent may be dispersive as well as hydrophilic. Although the substituent of the 4′-position is subject to strong steric repulsion, most other atoms of this ring are favored to be dispersive atoms. It may, therefore, be worth trying to replace the 5-phenyl ring with a dispersive thiophene ring. The statistics of fit for the study are given in Table 5. The SD of the study was 0.223, which is only 4.4% of the total range of 5.05 (4.0–9.05). An illustration of the calculation of the binding affinity of the ligands, using model compound **1**, is given in Tables 6 and 7. Table 6 gives the atomic physicochemical properties and the site occupancy by the atoms, and Table 7 gives the energy contribution. The physicochemical properties at various site pockets, as given in Table 7, can be easily calculated from the information given in Table 6. Let us, for example, consider site pocket 1. Two atoms of compound **1** occupy this site pocket, namely N1 and H1 (see Table 6). The octanol-water partition coefficient or the molar refractivity at site pocket 1 is simply the sum of the two atomic properties, as given in Table 6. The energy contribution is obtained by multiplying the physicochemical property by the corresponding coefficient, as given in Table 4. The dormant physicochemical properties (with the corresponding weight factor of zero) are omitted in Table 7. When a physicochemical property or a site cavity is dormant, it may be due to one of two reasons, the property is unimportant due to the site environment or it was not varied sufficiently. The total binding energy is the sum of the energy contributions of the individual site pockets and the contribution from the conformational energy.

Returning to the problem of the superposed conformation of molecule **5** (see Molecular Superposition), because only one site pocket (pocket 5) was used for the substituent at position 7, attaining a high energy conformation to avoid contact with the nitro group of the reference structure was unnecessary. This, in turn, indicates the necessity of using iterative superposition techniques, as discussed in Methods.

The method we have developed is capable of giving a three-dimensional physical interpretation of binding affinity data. Such an interpretation helps the medicinal chemists to develop hypotheses for future synthetic work. The method has already

[1] The model consisted of 145 small spheres of nine types. The ninth type was dummy, involving no interaction with the receptor. The coordinates of the center of the spheres, their radii, and types may be obtained from the authors on request. Superposition of a molecule in this cavity can predict the biological activity data.

TABLE 6

Illustration of the evaluation of the biological activity using model compound 1: site occupancy and atomic physicochemical properties

Atom	Atom type[a]	Site pocket occupied	Octanol-H_2O partition coefficient	Molar refractivity	Formal charge density
N1	72	1	−0.0528	2.9645	−0.2000
C2	40	2	0.0709	3.1093	0.3500
O2	58	2	−0.3514	1.4001	−0.3300
C3	6	3	−0.8370	3.3267	0.0000
N4	74	3	0.1461	2.8308	−0.1300
C5	39	4	−0.1116	4.3795	0.1000
C6	24	4	0.0068	3.4759	0.0000
C7	26	7	−0.1033	3.8245	0.0000
C8	24	5	0.0068	3.4759	0.0000
C9	24	5	0.0068	3.4759	0.0000
C10	26	5	−0.1033	3.8245	0.1500
C11	25	4	0.1600	4.1100	0.0000
C1′	25	4	0.1600	4.1100	0.0000
C2′	26	6	−0.1033	3.8245	0.2000
C3′	24	6	0.0068	3.4759	0.0000
C4′	24	6	0.0068	3.4759	0.0000
C5′	24	6	0.0068	3.4759	0.0000
C6′	24	6	0.0068	3.4759	0.0000
N7	76	7	−2.7640	4.6755	0.4800
O7	61	7	1.5810	1.6228	−0.3300
O7	61	7	1.5810	1.6228	−0.3300
F2′	84	4	0.5839	0.8000	−0.2000
H1	50	1	−0.3260	0.8001	0.0000
H3	51	3	0.2099	0.8434	0.0000
H3	51	3	0.2099	0.8434	0.0000
H6	47	4	0.3343	0.8000	0.0000
H8	47	5	0.3343	0.8000	0.0000
H9	47	5	0.3343	0.8000	0.0000
H3′	47	6	0.3343	0.8000	0.0000
H4′	47	8	0.3343	0.8000	0.0000
H5′	47	6	0.3343	0.8000	0.0000
H6′	47	3	0.3343	0.8000	0.0000

[a] See Ref. 33 for the atom classifications and their hydrophobicity and molar refractivity. The charge densities were obtained from the CNDO/2 calculation.

TABLE 7

Illustration of the evaluation of the biological activity: local physicochemical properties in different site types and the corresponding interaction energy for molecule 1

The interaction energy is simply the product of the physicochemical property and the corresponding site-dependent coefficient, as shown in Table 5. The contribution from conformational energy is zero, because the binding conformation is at its minimum energy conformation.

Site	Octanol-H_2O partition (hydrophobic contribution)	Molar refractivity (dispersive contribution)	Charge density (electrostatic contribution)
1	−0.3788	3.7646	
	(0.266)	(−0.464)	
2		4.5094	0.0200
		(0.559)	(0.022)
3	0.0632	8.6443	−0.1300
	(0.103)	(3.290)	(−0.563)
4	1.1334	17.6754	
	(0.373)	(−2.389)	
5	0.5789	12.3763	
	(−1.390)	(3.932)	
6	0.5925	19.3281	
	(−3.270)	(8.797)	
7	0.2947	11.7456	−0.1800
	(−0.256)	(0.743)	(−0.483)
8		0.8000	
		(−0.531)	

been found to be a useful tool in nucleoside chemistry (4, 35).[2] We show here that it may be equally effective for other classes of compounds.

Prediction. The model was used to predict the binding affinity of five compounds, shown in Fig. 4. The structures of some of these compounds varied considerably from those of the training compounds. The predicted binding affinities are given in Table 8. Except for compound **30**, the prediction was excellent. It is, however, necessary to describe briefly the algorithm for the prediction module. One requirement of the process is that it should predict the binding affinity of a member of the training set exactly as it calculated during the model-building process. In other words, the process of determining the optimal superposition in the binding site cavity should be the same. For prediction, therefore, we first superposed the test molecules on the reference compound **1**, using the criteria followed for the training set, and then the site pocket occupancy of the atoms was determined, to evaluate the interaction energies. While correlating a relatively large number of parameters by regression analysis, one may often face the problem of chance correlation (36). A good prediction often helps to screen the chance correlations, although cross-validation within the training set, as introduced by Wold (37) in his partial least-squarse (PLS) technique, followed by predictions on a training set, may be an even better approach.

Comparison with previous studies. There are quite a few excellent works on the structure-activity relationships of the benzodiazepine receptor ligands, which deserve some discussion and comparison with the present work. Borea *et al.* (38) attacked the problem from two directions. They superimposed different kinds of benzodiazepine receptor ligands, divided the superimposed regions into four smaller zones, and suggested the type of groups in these zones favoring the various types of pharmacological activities. Their superimposition seems to be guided by the hypothesis of Camerman and Camerman (29), in which they attributed the pharmacological activity to two electron-donating groups, O_2 and N_4, and two hydrophobic phenyl rings. Although the work is very qualitative and places major emphasis on the pharmacological activity, some superimpositions are worth comparison. The relative superimpositions of zopiclone and classical benzodiazepine in their work closely resembled our automated superposition. However, the β-carboline derivatives display a full spectrum of agonist, inverse agonist, and antagonist properties, according to their molecular substitution (39). Our program tried to get the maximum similarity with the benzodiazepines to explain their agonist properties; Borea *et al.* (39) tried to depict them as antagonists. Borea *et al.* (39) also made a Hansch type QSAR with a series of β-carbolines.

Loew *et al.* (40, 41), on the other hand, studied the protonation energies and molecular electrostatic potentials of the various regions of the molecule. Some of their findings contradicted our results, because they considered the potential at the surface of a particular atom and our charge density is the sum over a few atoms in the neighborhood. This, in turn, suggests the

[2] V. N. Viswanadhan, A. K. Ghose, N. B. Hanna, S. S. Matsumoto, T. L. Avery, G. R. Revankar, and R. K. Robins. Analysis of the antitumor activity of purine-6-sulfenamide, sulfinamide and sulfonamide nucleosides and certain related compounds using a novel computer aided receptor modeling procedure (REMOTEDISC). Submitted for publication.

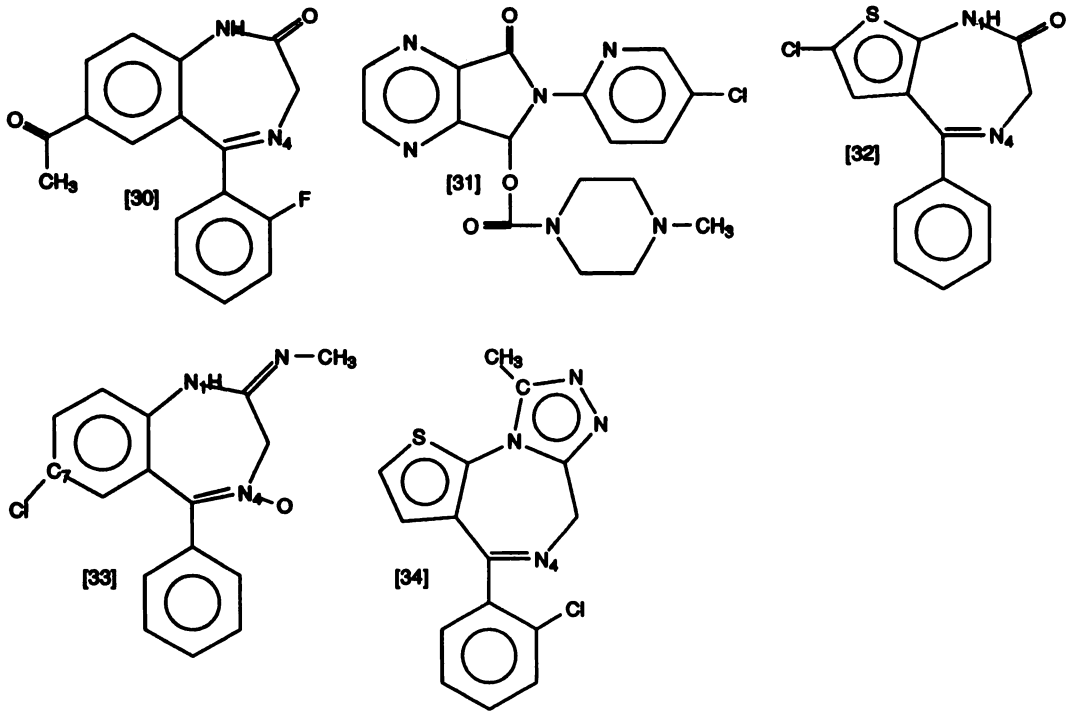

Fig. 4. Structures of the five compounds used to test the predicting power of the model.

TABLE 8

Prediction of binding affinity of the benzodiazepine receptor ligands

See Fig. 4 for the structure of the compounds.

Compound	log(1/IC_{50})		
	Observed	Calculated	Δ_{c-o}
30	7.74	9.57	−1.83
31	7.51	7.71	0.20
32	7.43	7.42	−0.01
33	6.45	6.54	0.09
34	8.46	8.57	0.11

TABLE 9

Illustration of the homogeneity function for a few model data sets

Set of 10 data points	τ	τ_{max}	h
1, 10, 10, 10, 10, 10, 10, 10, 10, 10	4.000	4.000	0.000
1, 5, 5, 5, 5, 5, 5, 5, 5, 10	1.778	4.000	55.556
1, 1, 1, 5, 5, 5, 5, 10, 10, 10	1.111	4.000	72.222
1, 2, 3, 4, 5, 6, 7, 8, 9, 10	0.000	4.000	100.000

necessity of developing distance-dependent potentials, as indicated in Methods, in our approach.

Codding and Muir (42) suggested a site model to explain the activities of both antagonists and agonists. The model had four recognition features for the antagonists, an aromatic six-membered ring, a carbonyl oxygen, a hydrophobic side chain, and a hydrogen atom-donor group. The agonists utilized the first two and the fourth recognition sites, with three additional features. These are a site to bind the imine nitrogen of the diazepine ring, a site for the C5 phenyl ring, and a site for the C7 electronegative substituents. They assumed that the site to recognize the carbonyl oxygen was flexible in nature and that such a conformational change is responsible for the difference in the pharmacological responses. The model is qualitative in nature. These authors later determined the X-ray crystal structure of suriclone (43) and compared its structure with that of one of the benzodiazepines. During this superposition, they overlayed the pyrrolidone and dithiacyclohexane rings on the benzodiazepine portion. The superposition of suriclone differed by almost 180° rotation around the C=O bond from the present superposition. However, in the present superposition, we not only superposed C=O but also the amide nitrogen in the two systems. The site pockets in the present study were comparable, although the characterization here is quantitative and more definitive in terms of the three major physical interactions. Allen *et al.* (44) recently studied the structural requirement of the β-carbolines and suggested that the N9-H interaction is important. The replacement of the N2 nitrogen decreased the binding affinity greatly. The 3-carboxy ester group can be replaced by an ethoxy group without much alteration of the binding affinity. The present method supports most of these findings.

Appendix

First, rank the variables of a column of the data matrix according to the increasing order of their values. Suppose the ranked values are $x_1, x_2, \ldots x_n$, where n is the number of values. Scale (range scaling) the values so that they vary between 0 and 1 as follows:

$$y_i = (x_i - x_{min})/(x_{max} - x_{min}) \quad \text{(i)}$$

where x_{min} and x_{max} are the minimum and maximum values of the variables ($x_{min} < x_{max}$). The ideal increment of the scaled values is $1/(n-1)$. The ideal scale ith value of a variable is therefore:

$$v_i = (i-1)/(n-1) \quad \text{(ii)}$$

The sum of the difference between the ideal value and the actual value is given by:

$$\tau = \sum_{i=1}^{n} | (y_i - v_i) | \quad \text{(iii)}$$

The minimum value of τ is, of course, when $y_i = v_i$ for $i = 1, 2, \ldots n$.

$$\tau_{pmin} = 0 \quad \text{(iv)}$$

The maximum value of τ will be when only one value of the scaled variable is at its lower range and all other values are at their upper range ($y_1 = 0$ and $y_i = 1$ for $i = 2, \ldots n$) or vice versa:

$$\tau_{max} = \sum_{i=2}^{n} | 1 - [(i-1)/(n=1)] | = \sum_{i=2}^{n} | (n=i)/(n-1) | = (n-2)(n-1)/2(n-1) = (n=2)/2 \quad \text{(v)}$$

The homogeneity function h is, finally, defined as follows:

$$h = [1 - (\tau/\tau_{max})] \cdot 100 \quad \text{(vi)}$$

The values of h in a few model data sets are shown in Table 9.

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