A Quantitative Structure–Activity Relationship and Molecular Graphics Analysis of Hydrophobic Effects in the Interactions of Inhibitors with Alcohol Dehydrogenase

Corwin Hansch,*† Teri Klein,‡ Judith McClarin,§ Robert Langridge,‖ and Neal W. Cornell⊥

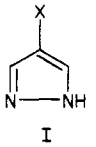
Department of Chemistry, Pomona College, Claremont, California 91711, Department of Medical Information Sciences, School of Medicine, University of California, San Francisco, California 94131, Department of Biological Sciences, University of Pittsburg, Pennsylvania 15260, Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143, and National Institute of Alcohol Abuse and Alcoholism, Laboratory of Metabolism, Rockville, Maryland. *Received February 21, 1985*

An analysis of the inhibition constants of pyrazoles, phenylacetamides, formylbenzylamines, and acetamides acting on liver alcohol dehydrogenase (ADH) yields quantitative structure–activity relationships (QSAR) having a linear dependency on octanol–water partition coefficients (log P). The average coefficient and standard deviation with the log P term for six different QSAR is 0.96 (±0.14). This suggests complete desolvation of the substituents (directly comparable to partitioning into octanol) on binding to the enzyme. Study of a molecular graphics model of ADH constructed from the X-ray crystallographic coordinates shows that the substituents are engulfed in a long hydrophobic channel which is so narrow that water of solvation must be removed from them in the binding process.

Understanding how organic compounds interact with macromolecules to affect all aspects of living organisms, including man, is of enormous interest to many areas of science from purely theoretical chemistry to the applied areas of drug development and toxicology. The problem can be factored into two main components: the movement of small molecules through macromolecular systems to the site of action and the reaction with a specific macromolecular bioreceptor.[1] While there are numerous ways to attack such problems, one of the most fruitful is that of quantitative structure–activity relationships (QSAR) which is an outgrowth of a kind of thinking initiated by Hammett about 1935.[2] To account for the electronic effect of substituents on organic reactions, Hammett used the effect of substituents on the ionization of benzoic acids as a model reference system.[3] The idea of using model systems was extended by Taft to steric effects and then, in the early sixties, to the hydrophobic effect of substituents on biochemical reactions.[2,3]

One of the best points of departure in the study of the horrendously complex problem of how a chemical might affect a whole organism, such as a mouse, is to first understand how the compound reacts with critical enzymes. Naturally those enzymes whose structures have been established by X-ray crystallography are the ideal starting points to learn about the means by which receptors recognize their substrates or inhibitors.

Alcohol dehydrogenase (ADH) is an excellent candidate for such work and in a recent report we derived QSAR (eq 1–3) for the inhibition of ADH from several sources by 4-substituted pyrazoles (I).[4] In these expressions K_i is the



I

Inhibition of Rat Liver ADH by Pyrazoles

$$\log 1/K_i = 1.22 \log P - 1.80\sigma_{meta} + 4.87 \quad (1)$$

$$n = 14,\ r = 0.985,\ s = 0.316$$

Inhibition of Human Liver ADH

$$\log 1/K_i = 0.87 \log P - 2.06\sigma_{meta} + 4.60 \quad (2)$$

$$n = 13,\ r = 0.977,\ s = 0.303$$

inhibition constant, P is the octanol–water partition coefficient, σ is the Hammett constant for X, n represents the number of data points used to derive the equation, r is the correlation coefficient, and s is the standard deviation from the regression equation. The parameters of eq 1 and 2 are similar and show that the greater the hydrophobicity of X the more potent the pyrazole is as an ADH inhibitor. The negative coefficient with σ indicates that electron release by X to nitrogen makes for better inhibitors. Since it is known that the pyrazole nitrogen binds to a Zn atom of ADH, this electronic effect of σ is to be expected. In fact, it also has been found for other ligands interacting with ADH.[5]

A point of increasing interest is: what information about the interaction of ligands with receptors can be gained from a study of the coefficients of such correlation equations? In the case of eq 1 and 2 we find an average coefficient with log P of essentially 1. One could interpret this to mean that partitioning of X between water and enzyme parallels that between water and octanol. That is, one might expect that complete desolvation of X by the enzyme occurs paralleling that occurring in the reference system, octanol/water.

At the time eq 1 and 2 were formulated, data on other pyrazoles binding to horse ADH were available[6,7] from which we derived eq 3.[4] Although qualitatively eq 3 is

Inhibition of Horse Liver ADH by Pyrazoles (I)

$$\log 1/K_i = 0.56 \log P - 1.11\sigma_{meta} + 6.99 \quad (3)$$

$$n = 16,\ r = 0.881,\ s = 0.404.$$

† Pomona College.
‡ School of Medicine, University of California.
§ University of Pittsburg.
‖ School of Pharmacy, University of California.
⊥ National Institute of Alcohol Abuse and Alcoholism.

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Table I. Parameters for the Inhibition of Horse Liver ADH by 4-X-Phenylacetamides II Used To Derive Equation 4

no.	X	obsd log $1/K_i$	calcd log $1/K_i$	Δ log $1/K_i$	log P
1	H	3.85	3.96	0.11	0.45
2	4-Cl	4.70	4.59	0.11	1.16
3	4-F	4.19	4.08	0.11	0.59
4	4-Br	4.70	4.73	0.03	1.31
5	4-I	5.10	4.96	0.14	1.57
6	4-OMe	4.15	3.94	0.21	0.43
7	4-OC_2H_5	4.10	4.30	0.20	0.83
8	4-$OCH(CH_3)_2$	4.10[a]	4.72	0.62	1.30
9	4-OC_3H_7	4.60	4.90	0.30	1.50
10	4-OC_4H_9	5.15	5.38	0.23	2.04
11	4-$OCH_2CH_2CH(CH_3)_2$	5.22[a]	5.69	0.47	2.38
12	4-OC_5H_{11}	5.92	5.86	0.06	2.58
13	4-$OCH_2C_6H_5$	5.70	5.44	0.26	2.11
14	4-$OCH_2C_6H_4$-4'-Br	5.70[a]	6.21	0.51	2.97
15	4-$OCH_2C_6H_{11}$	6.00[a]	6.62	0.62	3.42

[a] These points not used in the derivation of eq 4.

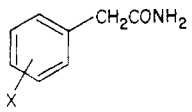
Table II. Parameters for the Inhibition of Horse Liver ADH by 4-X-Formylbenzylamines III Used To Derive Equation 5

X	obsd log $1/K_i$	calcd log $1/K_i$	Δ log $1/K_i$	π
H	5.52	6.01	0.49	0.00
OH	5.75	5.45	0.30	-0.67
$O(CH_2)_3CH_3$	7.40	7.34	0.06	1.59
$O(CH_2)_4CH_3$	7.52	7.79	0.27	2.13
$(CH_2)_5CH_3$	7.58[a]	8.66	1.08	3.17
$OCH_2C_6H_5$	7.82	7.40	0.42	1.66

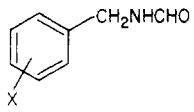
[a] This data point not used in the derivation of eq 5.

similar to eq 1 and 2, the difference between the coefficients and intercepts is large enough to lead one to expect a different mechanism of binding for horse ADH compared with rat and human. The discrepancy between the coefficients of eq 3 and 1 and 2 prevented us from attempting to draw conclusions about the desolvation of X from the coefficients with the log P terms of eq 1–3.

Recently Freudenreich et al.[8] have published K_i values for a large number of inhibitors of horse liver ADH. Sufficient data is available from their studies on two families of amide inhibitors (II and III) for QSAR analysis.



II



III

Derivation of QSAR for these two classes of inhibitors show that indeed the coefficients like those of eq 1 and 2 and not that of eq 3 are what can be expected with ADH from all three sources when steric factors can be eliminated.

These results encouraged us to test a set of pyrazoles (I) on horse ADH. The QSAR from this study is in agreement with eq 1 and 2.

Results

The amide inhibitors II and III are different from the pyrazoles in that substituents are insulated by saturated CH_2 units from the carbonyl group which binds to the Zn atom. In addition, only substituents with weak electronic effects were studied. Therefore it is not surprising that the electronic effects of substituents on K_i were found to be absent. From the data in Tables I and II, eq 4 and 5 have been derived using only the hydrophobic parameter log P. Equations 4a and 5a include all data points.

Table III. Parameters for the Inhibition of Horse Liver ADH by 4-X-Pyrazoles I Used in the Derivation of Equation 6

X	obsd log $1/K_i$	calcd log $1/K_i$	Δ log $1/K_i$	log P
H	5.73	5.95	0.22	0.26
CH_3	6.85	6.62	0.23	0.96
$(CH_2)_2CH_3$	7.65	7.58	0.07	1.96
$(CH_2)_4CH_3$	8.60	8.54	0.06	2.96
$(CH_2)_5CH_3$	8.88	9.02	0.14	3.46

Inhibition of Horse ADH by Phenylacetamides II

$$\log 1/K_i = 0.89(\pm 0.20) \log P + 3.56(\pm 0.29) \quad (4)$$

$$n = 11,\ r = 0.960,\ s = 0.197,\ F_{1,9} = 107$$

$$\log 1/K_i = 0.62(\pm 0.20) \log P + 3.81(\pm 0.40) \quad (4a)$$

$$n = 15,\ r = 0.878,\ s = 0.360,\ F_{1,13} = 43.6$$

Inhibition of Horse ADH by Formylbenzylamines III

$$\log 1/K_i = 0.84(\pm 0.58)\pi + 6.01(\pm 0.83) \quad (5)$$

$$n = 5,\ r = 0.935,\ s = 0.442,\ F_{1,3} = 20.9$$

$$\log 1/K_i = 0.63(\pm 0.47) \log P + 6.10(\pm 0.87) \quad (5a)$$

$$n = 6,\ r = 0.881,\ s = 0.538,\ F_{1,4} = 13.8$$

Graphics analysis clearly accounts for the aberrant behavior of the four compounds dropped from eq 4 (see section on Molecular Graphics).

For eq 4 a coefficient near 1, as with eq 1 and 2, is found with the hydrophobic term. The correlation is good. Note, however, that four data points in Table I have not been included: 4-$OCH(CH_3)_2$, 4-$OCH_2CH(CH_3)_2$, 4-$OCH_2C_6H_4$-4'-Br, and 4-$OCH_2C_6H_{11}$.

To obtain log P values for eq 4, we added π constants[3] from the benzene system to log P of 0.45 for phenylacetamide.[9] Although there can be an electronic effect of X on log P, in this mode of calculation it is not significant for substituents with low values of σ since the functional $CONH_2$ group is insulated by a CH_2.[10]

Equation 5 for congeners III is much less significant than eq 4 partly because of fewer data points and partly because of the lower quality of fit. Here the hydrophobic parameter π has been employed since log P for the parent compound has not been determined. Therefore the intercept of eq 5 cannot be compared with the others. The slope is in reasonable agreement with that of eq 1, 2, and 4.

From the data in Table III on pyrazoles (I), eq 6 has been derived. In developing eq 6 only pyrazole and its alkyl derivatives were tested. Since these have for practical purposes a constant electronic effect, it is not necessary to include an electronic term in eq 6. Hydrogen, the substituent of the parent compound has a σ value of 0 while the alkyl groups have a σ of -0.05 to -0.07. The ρ value of 1.80 of eq 1 indicates that a correlation of 0.13 should be subtracted from the calculated value of pyrazole itself. This small correction has been neglected.

Inhibition of Horse ADH by Pyrazoles (I)

$$\log 1/K_i = 0.96(\pm 0.25) \log P + 5.70(\pm 0.56) \quad (6)$$

$$n = 5,\ r = 0.990,\ s = 0.207,\ F_{1,3} = 153$$

Equation 6 is probably the best test of the hydrophobic effect on binding because no polar or branched substitu-

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ents have been used in its development. Its slope of 0.96 is in excellent agreement with the average value of 0.955 for eq 1, 2, 4, and 5.

Molecular Graphics

The coordinates of the pyrazole (I) and amide (II and III) inhibitors and the enzyme horse liver alcohol dehydrogenase were displayed and manipulated in real time with the program MIDAS[11] on an Evans and Sutherland picture system 2.[12] Surfaces for the protein were generated as solvent-accessible surfaces with the program MS[13] and interactively as van der Waals surfaces[14] for the ligands.

The structures of the enzyme alcohol dehydrogenase (ADH) were determined from X-ray crystallography studies by Eklund et al.[15-19] The coordinates for the apoenzyme, published in the Brookhaven Protein Data Bank[20] at 2.4-Å resolution, were used to build the binary complex. The structure of the holoenzyme has been solved from the ADH–NADH–dimethyl sulfoxide triclinic complex and refined at 2.9 Å.[16] Eklund et al. have shown that the ternary pyrazole and oxidized coenzyme complex and the H2NADH–DACA enzyme complex crystallize with triclinic symmetry isomorphous to the ADH–NADH–dimethyl sulfoxide complex.[16-18] In addition, Eklund et al. have found that the coenzyme/analogue is identically bound to the protein in all triclinic complexes in which the enzyme undergoes conformational change and that the inhibitors bind to the catalytic zinc atom in the substrate binding site.[21] Coordinates of the holoenzyme for the molecular modeling experiments were retrieved from the Brookhaven Protein Data Bank.[22]

The binding modes for the pyrazoles and amides are similar to those of imidazole in that they are bound in the narrow hydrophobic channel and coordinated to the zinc.[17] Preliminary coordinates for the imidazole complex[23] and selected protein atoms, including the zinc atom, were used to build the pyrazole compounds and position the compounds in the protein. The pyrazole ring was repositioned slightly in accordance with recent X-ray data[17] so that the ring occupied a narrow slit between the side chains of Phe-93 and Ser-48. The N2 nitrogen atom of the pyrazole is within binding distance of the catalytic zinc atom (2.0–2.2 Å) and the other nitrogen atom is 2.0 Å from the C4 carbon of the nicotinamide ring.[17] Models of the substituted pyrazoles were built by adding the substituents to the ring in an extended conformation, using standard geometries. Only small torsion angle changes were required in order to fit the substituents to the active-site cavity, as previously reported.[17]

The amide inhibitors were built such that the electron-donating carboxy oxygen was within binding distance of the catalytic zinc atom[16-18] and with no contacts closer than 3 Å (except for hydrogen bonds).[8] There are two binding modes for the phenylacetamides. In both modes, the carboxy oxygen is placed 2.1 Å from the zinc atom. The NH_2 moiety of the amide can be positioned toward the nitrogen of the amide or the ring nitrogen of the dihydronicotinamide ring of the coenzyme NADH.[13] As previously reported,[8] the methylene group of the phenylacetamide residues between the side chains of Phe-93 and Ser-48 in either binding mode. The phenyl ring is positioned such that the hydrophobic interactions can be maximized with the active site's cavity wall which is formed by the residues Leu-116, Leu-141, Val-294, and Ile-318. The substituents were placed on the phenyl ring of phenylacetamide using standard geometries. The position of the various substituted phenylacetamides was kept constant for all models. The formylbenzylamines binding mode is similar to that of the phenylacetamide except that there is a possibility of hydrogen bond formation between the formylbenzylamine nitrogen and the oxygen of Ser-48 (distance: 2.7 Å).[8] Models of the substituted formylbenzylamines were built by adding the substituents to the phenyl ring using standard geometries.

The active site of the enzyme is characterized by a channel ca. 20 Å long from solution to the catalytic zinc atom.[15] The narrowest part of the channel is essentially hydrophobic except near the catalytic zinc atom and Ser-48. Ligands of the zinc atom include His-67 and Cys-46.[15] The active-site cavity is further characterized by a widening of the substrate channel and then a narrowing of the channel by residues Leu-57 and Leu-116. The channel then widens again near the outer surface of the enzyme and becomes more polar.

As previously reported, the pyrazole compounds lie in the channel between the residues Phe-93 and Ser-48. Hydrophobic alkyl substituents in the 4-position will increase the binding affinity of the inhibitors. The binding affinity of the substituents will increase as the alkyl chains increase in length up to six carbons. Substituents in the 5-position will sterically interfere with the coenzyme and substituents in the 3-position will make close contacts with Ser-48 and His-67.[17]

The two classes of amide inhibitors, like the pyrazole inhibitors, show increased binding affinity for para-substituted compounds. Substitions on the phenyl ring in the ortho or meta position of the phenyl ring amide will make close, unfavorable contacts with the active site's cavity wall.

A great advance in the ease of visualization of enzyme–ligand complexes constructed from X-ray crystallographic data has been programs for constructing models containing van der Waals surfaces on the active site and/or the ligands. These surfaces enable one to immediately recognize the character of the surface and the quality of fit between receptor and substrate. Such models have been of much help in interpreting QSAR studies of enzymes of known structure.[24-27]

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Stereo views (Figures 1 and 2 show pyrazole (I) with X = hexyl fit to the active site of horse liver ADH. The colored dots code for surface character with red for hydrophobic (C) and blue for hydrophilic (O and N). The 4-*n*-hexylpyrazole wire (Figure 1) model with its van der Waals surface is shown in green partially surrounded by red hydrophobic surface. The red surface on the near side of the model has been deleted for the sake of clarity. On the right side is the Zn atom to which one of the pyrazole nitrogens binds and above in blue is part of the cofactor with which the other nitrogen interacts. On the lower right in purple is displayed, on the same scale, a water molecule with its van der Waals surface. The relatively large size of the water molecule clearly illustrates that all water would be shaved off in the binding of the inhibitor in the rather tight pocket. Hence if octanol–water partitioning is a good model for enzyme–water partitioning, one would expect a coefficient of 1 with the hydrophobic terms in eq 1, 2, and 4–6 (assuming of course, that no unfavorable steric interactions of substituents occur with the surface).

In Figure 2 is shown an end-on view of the hexylpyrazole which shows the close fit to the hydrophobic hole, again illustrating that water would perforce be removed in the binding process.

A point of particular interest is that the branched 4-$OCH(CH_3)_2$ group is more poorly fit by the amides II than for the pyrazoles. A graphics study of this point reveals that on the parent structure of the pyrazole the 4-$OCH(CH_3)_2$ group is free of unfavorable surface contacts. In the case of the phenylacetamide the branched OR groups are placed much further out from the Zn where they encounter steric effects by being squeezed between Leu-166 on the left (view I) and Leu-57 on the right.

Two other compounds in Table I which are not well fit are 4-$OCH_2C_6H_4$-4′-Br and 4-$OCH_2C_6H_{11}$. An analysis of the fit of these compounds shows that part of the Br projects beyond the surface and part of its runs into Met-1306. The bulky 4-$OCH_2C_6H_{11}$ group of about the same length as 4-$OCH_2C_6H_5$ is as expected more active than the benzyloxy group but falls below its predicted activity because of unfavorable contacts.

An example in Table II which does not fit our model is the 4-*n*-hexyl derivative. Despite the fact that it is no longer than the well-predicted 4-*O*-*n*-amyl analogue it is less active than expected.

Discussion

The above results lend credence to the hypothesis that, when a substituent is engulfed in hydrophobic space in a macromolecule and steric effects are absent, one might expect to find a coefficient near 1 with a log P or π term. Or, vice versa, finding a coefficient near 1 with a hydrophobic term suggests binding of substituents with complete desolvation of the type encountered with octanol-water partitioning. Another limiting case would be the binding of a substituent to a flat surface where one would expect a coefficient of about 0.5 due to half-desolvation. There would of course be intermediate cases where binding in a shallow trough might result in desolvation somewhere between 50% and 100%. While examples of the two extremes have been uncovered,[24,26,27] there are complicating factors which could obscure such simple conclusions.

The most obvious example is that of one or a few substituents interacting hydrophobically while at the same time experiencing steric inhibition. This can be illustrated with the data in Table I. If one includes all of the data points in one equation, the correlation is quite poor; however, dropping the worst point (11) yields eq 7. While

$$\log 1/K_i = 0.75 \log P + 3.67$$

$$n = 14,\ r = 0.935,\ s = 0.276 \qquad (7)$$

the correlation is not bad in terms of r, the slope is out of line with our other equations as well as with what one expects from the molecular graphics model. More serious steric effects could yield QSQR with even lower coefficients.

Another problem is that in a few instances coefficients with hydrophobic terms distinctly larger than 1 have been found. These examples suggest that in addition to simple partitioning the substituent in some way produces an additional amplification of the biochemical effect. This might be the result of the production of a conformational change in the receptor.

Another possibility for confusion is ambiguity in binding. The assumption that all members of a similar nature bind in the same way may not hold.[26]

Problems with the lack of correspondence between QSAR and graphics models built from X-ray crystallographic data can be illustrated with data from one of our earlier studies.[5] From the data in Table IV for aliphatic amides of the type X-CH_2CONH_2, eq 8 has been formulated. Equation 8a includes all data points.

$$\log 1/K_i = 0.98(\pm0.39) \log P - 0.83(\pm0.21)\sigma^* + 3.69(\pm0.38) \qquad (8)$$

$$n = 14,\ r = 0.937,\ s = 0.28,\ F_{2,11} = 39.8$$

$$\log 1/K_i = 0.56(\pm0.48) \log P - 0.58(\pm0.25)\sigma^* + 3.15(\pm0.39) \qquad (8a)$$

$$n = 16,\ r = 0.830,\ s = 0.446,\ F_{2,13} = 14.4$$

In these expressions σ^* is Taft's electronic parameter for aliphatic systems.[3] It is satisfying that a coefficient near 1 is found with the log P term (eq 3) and that a negative coefficient is found with σ^*. Two data points in Table IV (10 and 11) have not been used in this derivation. While it is clear from the graphics analysis that one of these [X = $(C_2H_5)_2$] would encounter steric hindrance from Phe-93 and Ser-48, there is no apparent reason for the poor fit of $(CH_3)_3$. An analogue of essentially the same size (Cl_3) is well fit. In our earlier analysis[5] of this data we included the congener X = C_6H_5. Under this condition the coefficient with log P was only 0.81. This analysis was not

Table IV. Parameters for the Inhibition of Horse Liver ADH by X-CH_2CONH_2 Used in the Derivation of Equation 8

no.	X	obsd[a] log $1/K_i$	calcd log $1/K_i$	Δ log $1/K_i$	log P	σ^*
1	F	1.41	1.77	0.36	−1.03	1.10
2	F_2	1.12	1.24	0.12	−0.76	2.05
3	F_3	1.33	1.09	0.24	−0.11	3.00
4	Cl	2.12	2.24	0.12	−0.59	1.05
5	Cl_2	2.19	2.05	0.14	−0.03	1.94
6	Cl_3	2.07	2.26	0.19	0.79	2.65
7	Br	2.24	2.35	0.11	−0.52	1.00
8	Br_2	2.49	2.20	0.29	0.18	2.00
9	I	2.72	3.13	0.41	0.15	0.85
10	$(C_2H_5)_2$	3.41[b]	4.39	0.98	0.53	−0.22
11	$(CH_3)_3$	2.46[b]	4.08	1.62	0.15	−0.30
12	CH_2=	2.11	2.16	0.05	−1.01	0.65
13	CH_2=, CH_3	3.02	2.54	0.48	−0.71	0.55
14	CH_3CH=	2.89	2.89	0.00	−0.51	0.36
15	CH_3CH=, CH_3	3.15	3.27	0.12	−0.21	0.26
16	$(CH_3)_2$	3.77	3.46	0.31	−0.39	−0.19

[a] Parameters from ref 5. [b] These data points not used in the derivation of eq 8.

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(27) Carotti, A.; Hansch, C.; Mueller, M. M.; Blaney, J. M. *J. Med. Chem.* 1984, *27*, 1401.

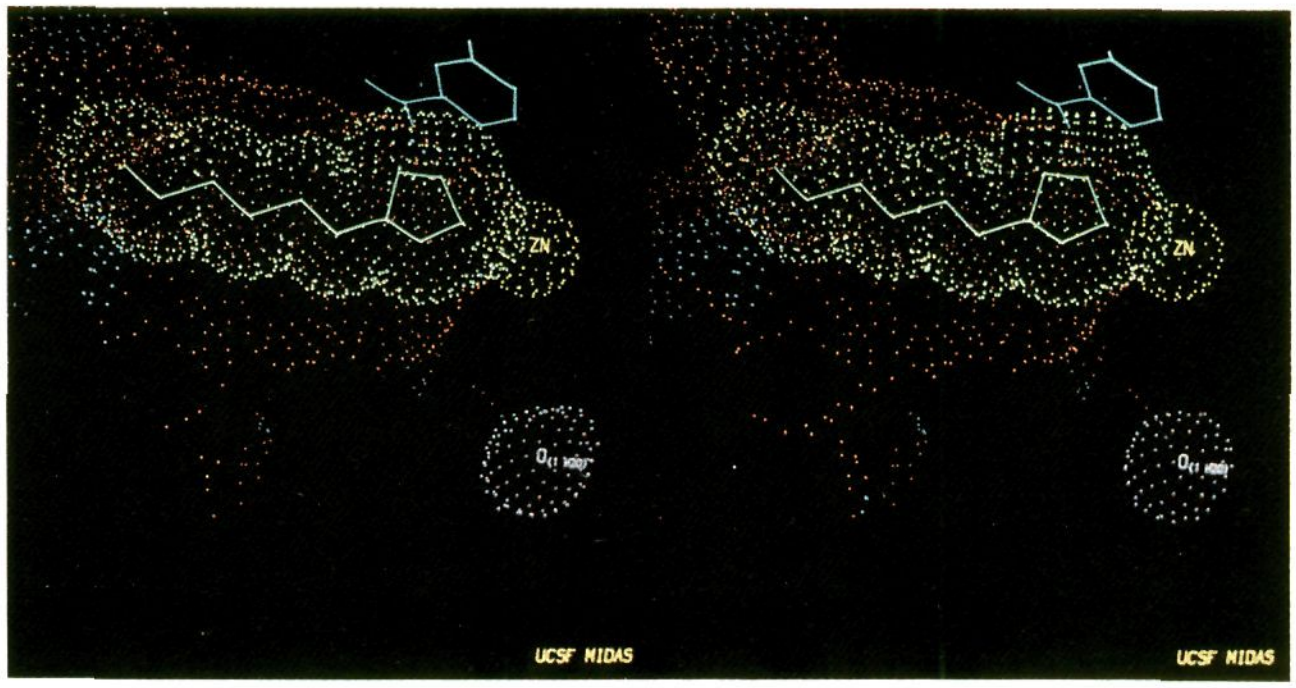

Figure 1.

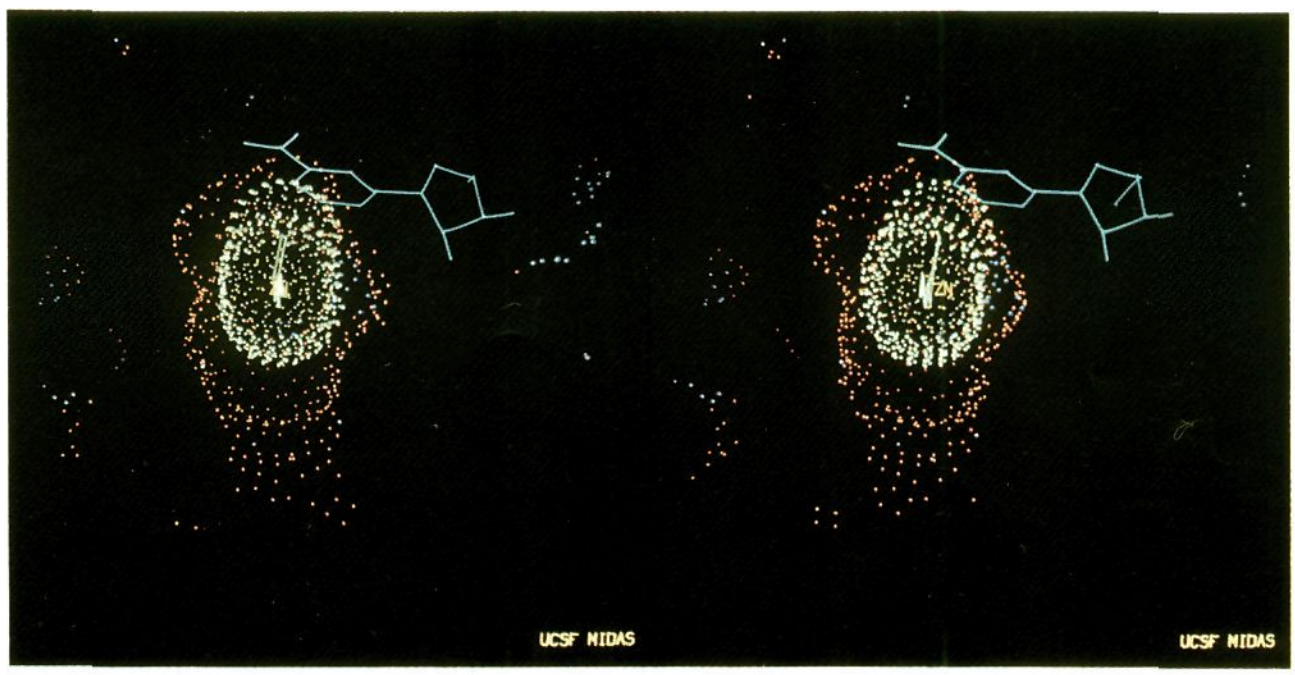

Figure 2.

strictly proper since we did not have a σ^* for phenyl comparable to the values used for the aliphatic substituents.

Another group of inhibitors which did not yield a correlation equation with a coefficient of 1 for the log P term is that of a set of carboxylates ($RCOO^-$).[5] No obvious explanation is available.

A set of benzamides studied by Sarma et al. has yielded a confusing result in that meta substituents do not appear to exhibit a hydrophobic effect.[28,29] This, however, may be the result of compensation by a concurrent, negative, steric effect. There are not enough congeners in this data set to disentangle the complex of steric, electronic, and hydrophobic interactions of enzyme and ligands; hence, we are in the process of extending this study.

In summary the results in this report and others[24,27] show that the hydrophobic parameters π and log P can be used with correlation analysis to define the hydrophobic areas around the active site of a bioreceptor. Moreover, QSAR techniques can be used to draw conclusions about the shape of hydrophobic space. In addition, evidence is beginning to accumulate to show that the combination of hydrophobic parameters with those for the bulk of substituents (e.g. MR, E_s) can be used in diagnosing polar regions in bioreceptors as well.[30] As more work is done with enzymes whose X-ray crystallographic structures have been established, our understanding of the use of QSAR in the characterization of bioreceptors will be strengthened so that we believe that we can attack the problem of structure definition of unknown receptors with much more confidence. While it may be many years in the future before we have detailed X-ray structures of drug receptors, the QSAR studies with the well-characterized enzymes encourage us to believe that we can begin to more clearly define those drug receptors which can be more or less purified.

Experimental Section

Determination of Pyrazole K_i Values. The enzyme used to obtain the K_i values listed in Table III was horse liver alcohol dehydrogenase, no. 102733, purchased from Boehringer Mannheim Corp., Indianapolis, IN. That preparation contains crystalline enzyme suspended in phosphate buffer, pH 7, and stabilized with 10% ethanol. At the beginning of experiments on any day, 0.050 mL of the commercial preparation was centrifuged for 3 min at 12000*g* in an Eppendorf centrifuge. The supernatant was removed as completely as possible, the pellet was dissolved in 0.50 mL of 0.1 M potassium phosphate, pH 7.0, and the solution was stored on ice. As needed for kinetic assay, aliquots of the solution were diluted 1:10 with phosphate buffer. Control assays were run at

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(29) Hansch, C.; Kim, K. H.; Sarma, R. H. *J. Am. Chem. Soc.* **1973**, *95*, 6447.
(30) Carotti, A.; Casini, G.; Hansch, C. *J. Med. Chem.* **1984**, *27*, 1427.

2-h intervals to ascertain that the ethanol-free enzyme solution was not losing activity.

Since pyrazoles are known to inhibit alcohol dehydrogenase in a manner that is competitive with ethanol,[31,32] it was decided to use the method of Dixon[33] for determining K_i values. Rate measuremnts were performed by adding 0.004 unit (10 μL) of centrifuged, diluted enzyme to reaction mixtures at 38 °C containing, in a final volume of 2.0 mL, 0.195 M Tris, 0.085 M H_3PO_4, 0.04 M KCl, and 1.0 mM NAD. That mixture gives the final assay solution an ionic strength of 0.25 M and a pH (at 38 °C) of 7.3 without titration.[34] Any pyrazole to be tested was added to the mixture after the enzyme, and the concentrations of each pyrazole were those that caused 10–60% inhibition of ethanol oxidation. Reaction was initiated with ethanol and was monitored at 340 nm in a Gilford spectrophotometer. Rates (ν) were measured with 5 and 10 mM ethanol, and plots of $1/\nu$ against inhibitor concentration were linear, intersecting at a point above the abscissa equal to $-K_i$ (see ref 4). The observed K_i values given in Table III are averages of at least three determinations with each pyrazole.

NAD was grade I from Boehringer Mannheim, and Tris was Trizma grade base from Sigma Chemical Co., St. Louis, MO. Other chemicals were reagent-grade commercial products.

Partition Coefficients. The log P values in Table I were calculated by adding π constants from the benzene system[3] to log P (0.45) for the parent compound phenylacetamide. A few measured π constants were not available and these were calculated from additivity principles[3] as follows. The values for the larger alkoxy groups were based on π of 1.05 for OC_3H_7. To this was added 0.54 for each additional CH_2 moiety. For branched compound 11 the branching factor[3] of 0.2 was subtracted from compound (log P_{parent} + $\pi_{OC_3H_7}$ + $2\pi_{CH_2}$ − 0.2 = 0.45 + 1.05 + 1.08 − 0.2 = 2.38). For compound **14**, π of 0.86 for Br was added to π of 1.66 for $OCH_2C_6H_5$. For compound **15**, log $P_{cyclohexane}$ − log $P_{benzene}$ (1.31) was added to π of $OCH_2C_6H_5$.

In Table II, π constants were employed since log P for the parent compound has not been reported.

The log P values of Table III were experimentall determined except for compounds CH_3 and C_3H_7, which were calculated via additivity principles.[4]

Most of the values in Table IV were estimated from similar amides.[5]

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Registry No. **I** (X = H), 288-13-1; I (X = Me), 7554-65-6; I (X = Pr), 21037-81-0; I (X = $(CH_2)_4Me$), 17072-39-8; I (X = $(CH_2)_5Me$), 73123-47-4; **II** (X = H), 103-81-1; **II** (X = 4-Cl), 20101-92-2; **II** (X = 4-F), 332-29-6; **II** (X = 4-Br), 74860-13-2; **II** (X = 4-I), 84863-81-0; **II** (X = 4-OMe), 6343-93-7; **II** (X = 4-OEt), 40784-91-6; **II** (X = 4-OPr-i), 89790-01-2; **II** (X = 4-OPr), 89790-02-3; **II** (X = 4-OBu), 3413-59-0; **II** (X = 4-$OCH_2CH_2CHMe_2$), 14442-83-2; **II** (X = 4-OC_5H_{11}), 5100-05-0; **II** (X = 4-OCH_2Ph), 84199-13-3; **II** (X = 4-$OCH_2C_6H_4$-4′-Br), 89790-04-5; **II** (X = 4-$OCH_2C_6H_{11}$), 89790-05-6; **III** (X = H), 6343-54-0; **III** (X = OH), 86386-69-8; **III** (X = $O(CH_2)_3Me$), 87578-63-0; **III** (X = $O(CH_2)_4Me$), 89790-09-0; **III** (X = $(CH_2)_5Me$), 89790-10-3; **III** (X = OCH_2Ph), 89790-11-4; ADH, 9031-72-5; CH_2FCONH_2, 640-19-7; CHF_2CONH_2, 359-38-6; CF_3CONH_2, 354-38-1; $CH_2ClCONH_2$, 79-07-2; $CHCl_2CONH_2$, 683-72-7; CCl_3CONH_2, 594-65-0; $CH_2BrCONH_2$, 683-57-8; $CHBr_2CONH_2$, 598-70-9; CH_2ICONH_2, 144-48-9; $Et_2CHCONH_2$, 1114-38-1; $Me_3CHCONH_2$, 754-10-9; $CH_2{=}CHCONH_2$, 79-06-1; $CH_2{=}C(Me)CONH_2$, 79-39-0; $MeCH{=}CHCONH_2$, 23350-58-5; $MeCH{=}C(Me)CONH_2$, 32793-37-6; $Me_2CHCONH_2$, 563-83-7.

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