MOLECULAR PHARMACOLOGY, 27:493–498

A Quantitative Structure-Activity Relationship and Molecular Graphics Study of Carbonic Anhydrase Inhibitors

CORWIN HANSCH, JUDITH MCCLARIN,[1] TERI KLEIN, AND ROBERT LANGRIDGE

Department of Chemistry, Pomona College, Claremont, California 91711 (C.H.) and the Department of Pharmaceutical Chemistry, School of Pharmacy (J.M., R.L.) and Department of Medical Information Sciences, School of Medicine (T.K), University of California, San Francisco, California 94143

Received July 20, 1984; Accepted February 19, 1985

SUMMARY

A quantitative structure-activity relationship (QSAR) (log $K = 1.55\alpha + 0.64 \log P - 2.07I_1 - 3.28I_2 + 6.94$) has been formulated for the binding of a set of substituted benzenesulfonamides to human carbonic anhydrase. The binding constant (K) are from the studies of King and Burgen [*Proc. R. Soc. Lond. B.* **193**:107–125 (1976)], σ is the Hammett electronic substituent constant, P is the octanol/water partition coefficient, and I_1 and I_2 are indicator variables for *meta* and *ortho* substituents, respectively. The negative coefficients with the indicator variables suggest steric hindrance by these substituents in contrast to *para* substituents. Qualitative features of the QSAR are correlated with a color stereomolecular graphics model of the enzyme-inhibitor complex which was constructed from the X-ray crystallographic coordinates of the enzyme.

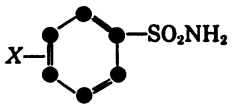
INTRODUCTION

In the study of drug structure-activity relationships via quantitative techniques, one would like a system of precisely known structure on which values of various physicochemical parameters and mathematical techniques can be tested. While X-ray crystallographic techniques have not been used to establish structures of drug receptors in general, structures of a number of enzymes, some of which are important in medicine, have been established. Recently, the use of computer graphics has enormously facilitated the study of macromolecules. We can now readily visualize from graphics what kind of (polar or hydrophobic) surfaces are involved in the interactions of ligands with enzymes. We can also appreciate what parts of the macromolecule are causing steric effects. This report shows how quantitative correlation equations based on substituent constants can be compared with the qualitative molecular graphics models. These comparisons, using the enzyme carbonic anhydrase, provide direct evidence that the terms in our QSAR[2] equations do bear a relationship to reality as seen from X-ray crystallography.

Carbonic anhydrase (EC 4.2.2.2) is a zinc-containing enzyme with a molecular weight of about 30,000. The enzyme catalyzes the reaction $CO_2 + H_2O \rightleftarrows HCO_3^-$, the hydration of certain aldehydes (1), and the hydrolysis of some esters (2–5). Carbonic anhydrase is a well characterized enzyme whose three-dimensional structure has been established by X-ray crystallography (6–8). The structure-activity relationships of various sulfonamide drugs have been studied by X-ray crystallography and molecular modeling (8, 10–13). Sulfonamide inhibitors of CA have found wide application as diuretics (14), have shown promise as antiepileptic agents (15), and are a possible treatment of glaucoma (16). There have also been extensive studies of its mechanism of action (8).

The great practical as well as theoretical interest in CA has spurred many kinetic studies on the action of various inhibitors. Unfortunately, most of these studies have been made with sets of congeners not well suited for QSAR analysis. Two important exceptions come from the studies of Kakeya *et al.* (17, 18) and King and Burgen (19). Their work has provided data sets to analyze further enzyme-ligand interactions by combining QSAR and molecular graphics (20, 21).

Kakeya *et al.* and King and Burgen both studied the interactions of variations of I with CA; however, each group used different types of X substituents.



STRUCTURE I

The Japanese study was concerned with relatively small substituents containing rather good variation in electronic and hydrophobic characteristics of X. The QSAR equations derived from their data are shown in Eqs. 1 and 2. These equations were reformulated using log P values instead of the π values used by Kakeya *et al.*

[1] Present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

[2] The abbreviation used is QSAR, quantitative structure-activity relationship.

0026-895X/85/050493-06$02.00/0

$$\log 1/K_i = 1.02(\pm 0.21)\sigma + 4.75(\pm 0.13) \quad (1)$$

$$n = 16,\ r = 0.939,\ r = 0.220,\ F_{1,14} = 105$$

$$\log 1/K_i = 0.80(\pm 0.22)\sigma + 0.27(\pm 0.18)\log P + 0.33(\pm 0.14) \quad (2)$$

$$n = 16,\ r = 0.968,\ s = 0.168,\ F_{1,13} = 11.0$$

In these expressions, K_i is the inhibition constant of bovine carbonic anhydrase,[3] σ is the Hammett constant (22), P is the octanol/water partition coefficient of the un-ionized form of the sulfonamide, n represents the number of congeners upon which the equation is based, r is the correlation coefficient, s is the standard deviation from the regression equation, and F is the F statistic. In Eqs. 1 and 2, the parameter of greatest importance is σ. The positive coefficient on σ indicates that electron withdrawal by X increases inhibitory potency. The log P term reveals a less important but direct relationship between hydrophobicity and inhibition. The most negative σ was for 4-NCH_3 ($\sigma = -0.84$) while the most positive was for 3-CF_3, 4-NO_2 ($\sigma = 1.21$). Log P varied from −0.83 (4-NH_2) to 1.91 (3-NO_2, 4-*d*1). Although the substituents were small in size, there was excellent variation in both σ and log P. The derivatives were selected so that there was good coverage of data space between the extremes.

King and Burgen did not do a formal QSAR treatment of their data, although they did point out the importance of the hydrophobic effect of substituents. A QSAR treatment was performed by Testa and Purcell (23) but with novel parameters which cannot be compared with those of Kakeya *et al.* We have treated King and Burgen's data with the same parameters used by the Japanese group.

RESULTS

The King and Burgen data, shown in Table 1, were used to derive Eqs. 3–5 for the 4-substituted congeners I.

$$\log K = 0.65(\pm 0.22)\log P + 7.30(\pm 0.40) \quad (3)$$

$$n = 19,\ r = 0.834,\ s = 0.457,\ F_{1,17} = 39.1$$

$$\log K = 1.55(\pm 0.39)\sigma + 0.65(\pm 0.10)\log P + 6.93(\pm 0.20) \quad (4)$$

$$n = 19,\ r = 0.971,\ s = 0.204,\ F_{1,16} = 69.4$$

K in these equations is not an inhibition constant as in Eqs. 1 and 2. It is instead a binding constant for absorption of the sulfonamides to human carbonic anhydrase isoenzyme C prepared from erythrocytes.

The compounds in Table 1 show a large variation in log P and a smaller variation in σ. For this reason, Eq. 3 has log P as the most important single variable rather than σ as seen in Eq. 1. Only 5-*meta* substituents were studied by King and Burgen and they were obviously less active than the corresponding *para* analogues. These *meta* compounds were omitted in deriving Eqs. 3 and 4. Assuming the action of 3- and 4-substituents to be parallel, except for a position-dependent steric effect, the two types of congeners can be combined by using an indicator variable given the value of 1 for 3-substituents and 0 for 4-substituents:

$$\log K = 1.55(\pm 0.40)\sigma + 0.62(\pm 0.09)\log P - 2.07(\pm 0.23)I + 6.98(\pm 0.20) \quad (5)$$

$$n = 24,\ r = 0.982,\ s = 0.210,\ F_{3,20} = 177$$

The coefficients with σ and log P and intercept of Eq. 5 are essentially identical to those of Eq. 4. The standard deviations are also quite close. Hence, within the limits of the standard deviation, the 3- and 4-substituents seem to be acting in the same fashion once correction is made for some sort of steric effect which lowers the activity of the isolipophilic 3-substituents by a factor of 100.

The same treatment can be carried out with 2-substituents as shown in Eq. 6:

$$\log K = 155(\pm 0.38)\ \sigma + 0.64(\pm 0.08)\log P - 2.07(\pm 0.22)I_1 - 3.28(\pm 0.23)I_2 + 6.94(\pm 0.18) \quad (6)$$

$$n = 29,\ r = 0.991,\ s = 0.204,\ F_{4,24} = 324$$

Note that the parameters in Eq. 6, equivalent to those in Eq. 5, have not changed significantly and the standard deviations for the two equations are essentially identical. The coefficient with I_2 shows that 2-substituents have 2000 times lower affinity than the equivalent 4-substit-

TABLE 1

Parameters used to derive Eq. 3–8 for the binding of X-$C_6H_4SO_2NH_2$ to human carbonic anhydrase

	X	σ	log P	I_1	I_2	Observed log K	Calculated log K
1.	H	0.00	0.21	0	0	6.69	7.08
2.	4-CH_3	−0.17	0.69	0	0	7.09	7.12
3.	4-C_2H_5	−0.15	1.31	0	0	7.53	7.55
4.	4-C_3H_7	−0.15	1.64	0	0	7.77	7.76
5.	4-C_4H_9	−0.15	2.45	0	0	8.30	8.28
6.	4-C_5H_{11}	−0.15	2.97	0	0	8.86	8.62
7.	4-CO_2CH_3	0.45	0.64	0	0	7.98	8.05
8.	4-$CO_2C_2H_5$	0.45	1.17	0	0	8.50	8.39
9.	4-$CO_2C_3H_7$	0.45	1.75	0	0	8.77	8.76
10.	4-$CO_2C_4H_9$	0.45	2.34	0	0	9.11	9.14
11.	4-$CO_2C_5H_{11}$	0.45	2.71	0	0	9.39	9.38
12.	4-$CO_2C_6H_{13}$	0.45	3.23	0	0	9.39	9.71
13.	4-$CONHCH_3$	0.36	−0.31	0	0	7.08	7.30
14.	4-$CONHC_2H_5$	0.36	0.03	0	0	7.53	7.52
15.	4-$CONHC_3H_7$	0.36	0.51	0	0	8.08	7.83
16.	4-$CONHC_4H_9$	0.36	1.05	0	0	8.49	8.17
17.	4-$CONHC_5H_{11}$	0.36	1.54	0	0	8.75	8.49
18.	4-$CONHC_6H_{13}$	0.36	2.05	0	0	8.88	8.82
19.	4-$CONHC_7H_{15}$	0.36	2.57	0	0	8.93	9.15
20.	3-CO_2CH_3	0.37	0.62	1	0	5.87	5.84
21.	3-$CO_2C_2H_5$	0.37	1.11	1	0	6.21	6.16
22.	3-$CO_2C_3H_7$	0.37	1.72	1	0	6.44	6.55
23.	3-$CO_2C_4H_9$	0.37	2.24	1	0	6.95	6.88
24.	3-$CO_2C_5H_{11}$	0.37	2.71	1	0	6.86	7.19
25.	2-CO_2CH_3	0.45	0.45	0	1	4.41	4.65
26.	2-$CO_2C_2H_5$	0.45	0.72	0	1	4.80	4.82
27.	2-$CO_2C_3H_7$	0.45	1.49	0	1	5.28	5.32
28.	2-$CO_2C_4H_9$	0.45	2.01	0	1	5.76	5.65
29.	2-$CO_2C_5H_{11}$	0.45	2.55	0	1	6.18	6.00

[3] N. Kakeya, private communication.

uents. The interactions of the substituents with the protein appear to be parallel, since the coefficients in Eqs. 4–6 are essentially the same.

From an inspection of the data in Table 1, it seems clear that King and Burgen attained the maximum chain length which could make hydrophobic contact with the enzyme. Hydrophobic contact appears to be roughly the same at the 3- and 4-positions. Activity has not yet leveled off with the 2-$CO_2C_5H_{11}$ derivatives, however, so that the size of the hydrophobic region for 2-substituents is less well defined. The bilinear model (24) of Eq. 7 has been derived to account for what appears to be lack of contact by the longer alkyl groups with hydrophobic space.

$$\log K = 1.59(\pm0.36)\sigma + 1.07(\pm0.39)\log P - 0.59(\pm0.52)\log(\beta + 10^{\log P} + 1) - 2.11(\pm0.21)I_1 - 3.33(\pm0.22)I_2 + 6.83(\pm0.20) \quad (7)$$

$$n = 29,\ r = 0.993,\ s = 0.190,\ \log \beta = -0.846,\ F_{2,22} = 2.74$$

The F statistic shows that Eq. 7 is not significant ($F_{2,22,\alpha 0.1} = 2.95$).

Another approach to evaluating this problem is to delete four data points which appear to have reached the limit for hydrophobic interaction. Dropping compounds 12, 18, 19, and 24 yields Eq. 8.

$$\log K = 1.69(\pm0.34)\sigma + 0.72(\pm0.08)\log P - 2.05(\pm0.21)I_1 - 3.36(\pm0.20)I_2 + 5.85(\pm0.16) \quad (8)$$

$$n = 25,\ r = 0.994,\ s = 0.171$$

The coefficient for log P in Eq. 8 is close to that of Eq. 6, but not enough superoptimal log P data points are available in Table 1 to justify the use of Eq. 8.

The variables of Eq. 6 are reasonably orthogonal as shown in the following squared correlation matrix.

	σ	I_1	log P	I_2
σ	1	0.02	0.00	0.10
I_1		1	0.01	0.04
log P			1	0.00
I_2				1

MOLECULAR GRAPHICS

The coordinates from human carbonic anhydrase C determined from X-ray crystallography at 2.0-Å resolution (7, 9, 11) were obtained from the Brookhaven Protein Data Bank (29). Coordinates for the benzenesulfonamide and its associated Zn atom, but not the protein, were available from the crystallographic analysis of the benzenesulfonamide-carbonic anhydrase C complex published by Kannan *et al.* (11). (There was an error in the published coordinates of the phenyl ring of the benzenesulfonamide. The coordinates of C6 of the phenyl ring were corrected to allow the ring to have standard geometry.) A complete data set including enzyme, Zn, and inhibitor was generated by merging the set coordinates, and a rigid body optimization procedure was used to refine the fit. Vedani and Meyer (13) doing a similar model-building experiment noted apparent close contacts between the benzenesulfonamide and the active site which they relieve by reorienting the protein side chains 196–200°. In the current work, these apparently unfavorable contacts were not observed after the fit was optimized.

The inhibitor-enzyme complex (11) has the inhibitor placed such that the sulfonamide group is bound in the fourth coordination site of the zinc atom replacing a water molecule. The distance between the sulfonamido moiety and the zinc atom is 2.9–3.0 Å and the sulfonamido group is oriented such that there is a hydrogen bound to the oxygen (OG1) of Thr 199 (11). This position also allows one of the oxygens of the sulfonamido group to occupy a distant fifth coordination site of the zinc atom (11). There are hydrophobic interactions between the phenyl ring of the inhibitor and Val 121 (13). Movement of the phenyl ring of the benzenesulfonamide appears to be restricted not only by the interactions of Val 121, but also by Gln 92 and Phe 131.

The active site of the enzyme is characterized by a conical cavity approximately 12 Å deep with a zinc atom located near the bottom of the cavity. The active site cavity is divided into two halves: a hydrophilic half consisting of the residues Tyr 7, Asn 61, His 64, Asn 67, Glu 69, Gln 92, His 94, His 96, Glu 106, His 119, and Thr 199, and Thr 200 and a hydrophobic half consisting of the residues Ala 65, Ile 91, Val 121, Phe 131, Leu 141, Val 143, Gly 145, Pro 201, (*cis*) Pro 202, Val 207, Trp 209, and Val 211 (10–12). The active site surface was extended to include the residues Asp 62, Val 135, and Leu 198 (13). The zinc atom is coordinated to the residues His 94 (at NE2), His 96 (at NE2), and His 119 (at NG) and the oxygen of a water molecule in a distorted tetrahedral geometry (11, 13).

Models of the substituted benzenesulfonamide inhibitors were built by adding substituents to benzenesulfonamide coordinates using standard geometries. The fit of the substituents on the sulfonamides in the active site was optimized by manipulating the substituent torsion angles. Unfavorable inter- and intramolecular contacts could be observed directly by use of the surface representations of the active site and the inhibitor. The position of the benzenesulfonamido ring was maintained in the position determined by the crystallographic data (11).

It was possible to orient the *para* substituents so that the alkyl chains lie along the hydrophobic wall near Phe 131 and Val 135 or so that they lie in a small channel between Asp 62 and Asn 67. Clearly, the area around Phe 131 and Val 135 is more hydrophobic than the channel and provides favorable interactions with the alkyl chain. In addition, there is the possibility of a hydrogen bond between the ester or amide groups and Gln 92 (NE2).

meta substituents were found to be able to occupy the same two sites in the active site cavity. A binding mode near Phe 131 was again chosen as the most likely location for the substituent due to the increased hydrophobocity. Binding of *meta* substituents differs only from *para* in that they make contact with the wall at Leu 198.

All *ortho* substituents display an unfavorable interaction with Pro 201. Attempts to improve the fit by rotation of the phenyl ring did not result in a better orientation

of the substituents so the benzenesulfonamide was kept constant as with the other inhibitors. The alkyl chain of the *ortho* substituents is therefore constrained to occupy the area along the hydrophobic wall near Leu 198. A hydrogen bond might exist between the ester or amide substituent and Thr 200 (OG1).

Fig. 1 shows the active site cavity with the surface calculated using the MS program (27, 28). The surface dots are color-coded red for hydrophobic surface (carbon) and blue for polar surface (nitrogen and oxygen). Sulfonamide I, with an ester group in the *para* position, is bound to the active site. The alkyl group falls outside the narrow active site cavity onto the slightly concave hydrophobic (red) surface. From this view, it seems likely that 60–70% of the surface of the alkyl group would be in contact with the hydrophobic surface (red) which would account for the coefficients in Eqs. 6 and 8 being 0.6–0.7 rather than 1, which is expected for complete desolvation (20).

Fig 2 shows a view of an ester moiety in the 2-position of the sulfonamide. Beginning with the third carbon, the ester alkyl group is interacting with the hydrophobic active site wall. In Table 1, this hydrophobic effect can be seen in the rise of the binding affinity values for the *ortho* substituents beginning with the propyl ester. Long alkyl groups from all three positions, *ortho*, *meta*, and *para*, can interact with the large hydrophobic field.

All of the 3-substituents are of the type -COOR and rotation of the carboxylate groups places R so that it falls into the same groove as the 4-substituents. The 2-substituents show some unfavorable interaction with the enzyme but the R group falls into the same hydrophobic groove. The longest 2-alkyl group does not cover the hydrophobic wall of the cavity. Compound 25 (2-$COOCH_3$) in Table 1 is considerably less active than predicted, but the other 2-COOR have well predicted activities, indicating that the hydrophobic interaction of the alkyl moeity of 2-COOR is the same as for 4- and 3-alkyl groups. The large coefficient with I_2 in Eq. 6 suggests that there is about 4.5 kcal of steric hindrance in fitting 2-substituents into the active site, while the coefficient with I_1 suggests about 2.8 kcal of steric hindrance in fitting 3-substituents into the active site. Since one has no idea how much "give" there might be between inhibitor and receptor or how flexible the surface around the active site may be, meaningful energy calculations to check these values are not possible at this time.

The primary purpose of the molecular graphics models of the active site is to show the viewer in a qualitative way what the main features of the QSAR imply. There obviously is a large hydrophobic surface for binding of the alkyl chains. Steric hindrance by *ortho* and *meta* substituents is to be expected. It is by this kind of testing that QSAR modeling can be placed on a more firm foundation.

DISCUSSION

Although King and Burgen did not attempt a QSAR analysis of their results, it was clear to them that the hydrophic interaction of the substituents was important. Testa and Purcell (23) concluded that "the affinity constant of the sulfonamides for carbonic anhydrase is massively structure-dependent, and depends only to a very limited extent on the partition properties of the ligand." Their best equation is:

$$\log AC = 0.26(\pm 0.14)\ \log P + 0.0226\ (\pm 0.0061)\,V_s + 1.08(\pm 0.04)\mathrm{Dist} \quad (9)$$

$$n = 34,\ r^2 = 0.995,\ s = 0.536$$

In this expression, AC corresponds to K in our equations. The V_s is the molar volume of the substituents calculated according to Bondi. It is rather co-linear with log P (r = 0.72), as one might expect for the type of substituents in Table 1. The variable Dist is a measure in angstroms between the S atom in the sulfonamide and the first C atom falling into one of four possible sectors defined by the X, Y, and Z Cartesian coordinates.

Note that Eq. 9 is based on 34 data points while our study contains only 29. The additional five points are thiophene analogues which we have not attempted to

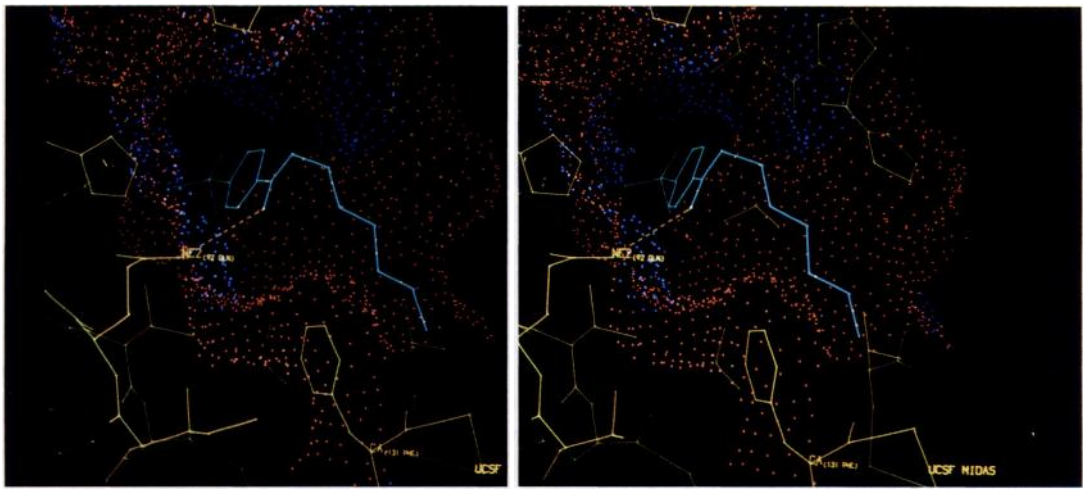


FIG. 1. *Carbonic anhydrase active site containing the inhibitor* $H_2NSO_2C_6H_4$-4-$COOC_6H_{13}$.

The van der Waals surface of the active site is colored red for hydrophobic surface (carbon) and blue for polar surface (nitrogen and oxygen). The "wire" model of the sulfonamide shows the nitrogen deep in the pocket bound to zinc while the long hydrophobic alkyl group falls onto a large hydrophobic surface.

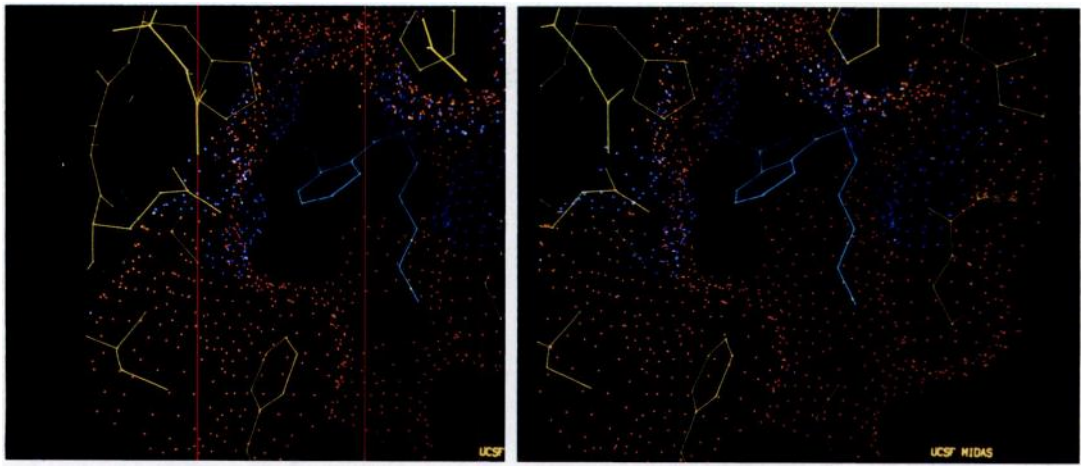


FIG. 2. *Carbonic anhydrase active site containing the inhibitor* $H_2NSO_2C_6H_4$-2-$COOC_5H_{11}$.

Color coding of the active site is the same as in Fig. 1. The ester portion of the *ortho* substituent makes unfavorable contact with the enzyme surface which is accounted for by I_2 in Eqs. 6–8. The alkyl group of the ester then falls onto the hydrophobic surface and exhibits the same kind of binding as the 3- and 4-substituted sulfonamides.

mix with the benzene derivatives. This could be done with an indicator variable.

Two features of the Testa and Purcell results are at odds with our study. We found, as did Kakeya *et al*, that the electronic effect of the substituents is important, as brought out by Eqs. 3 and 4. In Eq. 2, the electronic term is the most important term. Moreover, there is considerable evidence that it is the anionic form of the sulfonamide which is bound to the enzyme (30). Kakeya *et al.* reported ΔpK_a values for the sulfonamides upon which Eqs. 1 and 2 are based. We have derived Eq. 10 from their data.

$$\Delta pK_a = -0.86(\pm 0.14)\sigma + 0.08(\pm 0.09) \qquad (10)$$
$$n = 16,\ r = 0.962,\ s = 0.146$$

The ΔpK_a parameter is simply the difference between the pK_a of a derivative and the parent compound. To convert Eq. 10 into log *Ka*, one would multiply by −1. The conversion yields a slope of 0.86 which is extremely close to the coefficient with σ in Eq. 2; i.e., the electronic effect of X on the ionization of the sulfas parallels exactly the electronic effect of X on log $1/K_i$. Eq. 9 should be redone to reflect the incontrovertible evidence that the electronic effect of substituents on the binding of sulfonamide to CA is highly important.

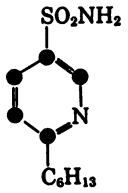
Our second disagreement with the conclusions of Testa and Purcell is that hydrophobicity is of great importance. This point is somewhat compromised, however, by the type of substituents used by King and Burgen because the co-linearity between hydrophobicity and volume of substituents is high; thus, it is possible that a combined steric and hydrophobic effect is involved. If the log P term in Eq. 7 represents only a hydrophobic effect, we must ask ourselves why the coefficient with log P in Eqs. 4 and 7 is higher than in Eq. 2. This could, of course, be caused by different experimental conditions and different sources of the enzymes. In Eq. 2, an inhibition constant was determined, while in Eqs. 4–7, affinity constants were used.

The QSAR data from two different laboratories support the importance of electronic and hydrophobic effects of substituents on the binding of sulfonamide inhibitors to carbonic anhydrase. The slope of the log P term of between 0.64 (Eq. 6) and 0.72 (Eq. 8) suggests that substituents are only partially desolvated. As we have noted with other enzymes (20), what appears to occur is binding to a surface rather than in a hydrophobic cleft or pocket. In addition, steric effects are observed for moderate size substituents in positions 2 and 3 of the sulfonamide ring.

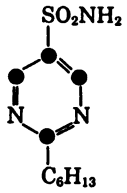
Although the role of the hydrophobic substituents in Eqs. 2 and 6 is different, this may be a result of the difference in the structures of bovine enzyme. The amino acid composition of carbonic anhydrase is known to vary with its source (10).

The correspondence between the graphics model and the mathematical model of Eq. 6 is gratifying. The models can be used for the design of tighter binding sulfonamides. Both Eqs. 2 and 6 show that potency can be gained by the use of electron-withdrawing substituents. It also appears that alkyl groups in the 4-position as large as hexyl can make hydrophobic contact with the carbonic anhydrase surface. An inhibitor can be designed which uses the 4-substituent to maximize the hydrophobic contact. Then one is left with the sterically sensitive 2- and 3-positions for placement of electron-withdrawing substituents. Due to steric restrictions, the best substituent would appear to be F. If we assume 4-C_6H_{13} has a log K of 9.00 (a modest increase over 4-C_5H_{11}) and we introduce an F into the *meta* position, the calculated log K (from Eq. 6) would be 9.5. With two *meta* fluorines, it would be 10.0. An alternative approach would be to use ring hetero atoms as in *II* or *III* to introduce electron-withdrawing characteristics.

Although a number of σ constants have been reported for 3-pyridyl (22), the value of 0.73 seems to be a reasonable, conservative figure. Using this figure and assuming that N can be oriented to allow the alkyl group to make maximum contact with no detrimental effect by N, we



STRUCTURE II



STRUCTURE III

estimate log K to be 10.0. If we assume the electronic effect of N to be additive, then a calculated value of 11.3 ensues for *III*.

Many of the best clinical carbonic anhydrase inhibitors contain heterocyclic rings. This is not surprising since our study brings out the importance of electron withdrawal on the SO_2NH_2 group and the low steric effect of such atoms. However, while *II* and *III* may have high binding constants for isolated enzymes, this does not mean that they would be ideal for clinical use. Their overall partition coefficient would have to be adjusted so that while maximum hydrophobic contact was obtained by the 4-alkyl group, overall hydrophobicity would be optimum for proper distribution in the body.

In conclusion, the combined use of QSAR and molecular graphics offers an effective means for increasing our understanding of the interaction of organic compounds with biological receptors. The insight gained from such studies will enable us to design more effective substrates and inhibitors for biological process and eventually will lead to better drugs.

REFERENCES

1. Pocker, Y., and J. E. Meany. The catalytic versatility of erythrocyte carbonic anhydrase, II. Kinetic studies of the enzyme-catalyzed hydration of pyridine aldehydes. *Biochemistry* **6**:239–246 (1967).
2. Schneider, F., and M. Liefländer. Über die Reaktion von Carbonat-Hydrolyase mit p-Nitrophenylacetat. *Hoppe-Seylers Z. Physiol. Chem.* **334**:279–282 (1963).
3. Tashian, R. E., D. P. Douglas, and Y. L. Yu. Esterase and hydrase activity of carbonic anhydrase-I from primate erythrocytes. *Biochem. Biophys. Res. Commun.* **14**:256–261 (1964).
4. Pocker, Y., and M. W. Bueg. Kinetic studies of bovine carbonic anhydrase catalyzed hydrolysis of para-substituted phenyl esters. *Biochemistry* **11**:698–707 (1972).
5. Pocker, Y., L. Bjorkquist, and D. W. Bjorkquist. Zinc II and cobalt II bovine carbonic anhydrase: comparative studies and esterase activity. *Biochemistry* **16**:3967–3973 (1977).
6. Kannan, K. K., B. Notstrand, K. Fridborg, S. Lövgren, A. Ohlsson A. and M. Petet. Crystal structure of human erythrocyte carbonic anhydrase B: three-dimensional structure at a nominal 2.2Å resolution. *Proc. Natl. Acad. Sci. USA* **72**:51–55 (1975).
7. Kannan, K. K., M. Petef, K. Fridborg, H. Cid-Dresdner, and S. Lövgren. Structure and function of carbonic anhydrases. *FEBS Lett.* **73**:115–119 (1977).
8. Lipscomb, W. N. Structure and catalysis of enzymes. *Annu. Rev. Biochem.* **52**:17–34 (1983).
9. Liljas, A., K. K. Kannan, P. C. Bergstén, I. Waara, K. Fridborg, B. Strandberg, U. Carlbom, L. Järup, S. Lövgren, and M. Petef. Crystal structure of human carbonic anhydrase C. *Nature New Biol.* **235**:131–136 (1972).
10. Lindskog, S., L. E. Henderson, K. K. Kannan, A. Liljas, P. O. Nyman, and B. Strandberg. Carbonic anhydrase, in *The Enzymes* (P. D. Boyer, ed.), 3rd ed., Vol. 5, Academic Press, New York, 587–661 (1971).
11. Kannan, K. K., I. Waara, B. Notstrand, S. Lövgren, A. Borell, K. Fridborg, and M. Petef. Structure and function of carbonic anhydrase: comparative studies of sulfonamide binding to human erythrocyte carbonic anhydrases B and C, in *Drug Action at the Molecular Level* (G. C. K. Roberts, ed.). University Park Press, Baltimore, 73–91 (1977).
12. Coleman, J. E. Chemical reactions of sulfonamides with carbonic anhydrase. *Annu. Rev. Pharm.* **15**:221–243 (1975).
13. Vedani, A., and E. F. Meyer, Jr. Structure-activity relationships of sulfonamide drugs and human carbonic anhydrase C: modeling of inhibitor molecules into the receptor site of the enzyme with an interactive computer graphics display. *J. Pharm. Sci.* **73**:352–358 (1984).
14. Werner, L. H. Diuretic and uricosuric agents, in *Burger's Medicinal Chemistry* (M. E. Wolff, ed.), 4th ed., Vol. III. Wiley, New York, 147–189 (1981).
15. Woodbury, D. M. Carbonic anhydrase inhibitors, in *Antiepileptic Drugs: Mechanism of Action* (G. H. Glaser, J. K. Penry, and D. M. Woodbury, eds.). Raven Press, New York, 617–633 (1980).
16. Maren, T. H. HCO_3 formation in aqueous humor and relation to the treatment of glaucoma. *Invest. Ophthalmol.* **13**:479–484 (1974).
17. Kakeya, N., N. Yata, A. Kamada, and M. Aoki. Biological activities of drugs. VII. Structure-activity relationship of sulfonamide carbonic anhydrase inhibitors. 2. *Chem. Pharm. Bull.* **17**:2000–2007 (1969).
18. Kakeya, N., N. Yata, A. Kamada, and A. Masaru. Biological activities of drugs. VIII. Structure-activity relationship of sulfonamide carbonic anhydrase inhibitors. 3. *Chem. Pharm. Bull.* **17**:2558–2564 (1969).
19. King, R. W., and A. S. V. Burgen. Kinetics aspects of structure-activity relations: the binding of sulphonamides by carbonic anhydrase. *Proc. R. Soc. Lond. B* **193**:107–125 (1976).
20. Carotti, A., R. N. Smith, S. Wong, C. Hansch, J. M. Blaney, and R. Landridge. Papain hydrolysis of X-phenyl-N-methanesulfonyl glycinates: a quantitative structure-activity relationship and molecular graphics analysis. *Arch. Biochem. Biophys.* **229**:112–125 (1984).
21. Hansch, C. Dihydrofolate reductase inhibition: a study in the use of X-ray crystallography, molecular graphics and quantitative structure-activity reactions in drug design. *Drug Intell. Clin. Pharm.* **16**:391–396 (1982).
22. Hansch, C., and A. Leo. Substituent constants for correlation analysis, in *Chemistry and Biology*. Wiley-Interscience, New York, (1979).
23. Testa, B., and W. P. Purcell. A QSAR study of sulfonamide binding to carbonic anhydrase as test of steric models. *Eur. J. Med. Chem.* **13**:509–514 (1978).
24. Kubinyi, H. Lipophilicity and drug activity. *Drug Res.* **23**:97–198 (1979).
25. Gallo, L., C. Huang, T. E. Ferrin, R. Langridge, *MIDAS: Molecular Interactive Display and Simulation.* Computer Graphics Laboratory, University of California, San Francisco.
26. Langridge, R., T. E. Ferrin, I. D. Kuntz, and M. L. Connolly. Real-time color graphics in studies of molecular interactions. *Science* **211**: 661–666 (1981).
27. Connolly, M. L. Solvent-accessible surfaces of proteins and nucleic acids. *Science* **221**:709–713 (1983).
28. Bash, P. A., N. Pattabiraman, C. Huang, T. E. Ferrin, and R. Langridge. Van der Waals surfaces in molecular modeling: implementation with real-time computer graphics. *Science* **222**: 1325–1327 (1983).
29. Brookhaven Protein Data Bank File: ICAC (May 22, 1976).
30. Kumar, K., R. W. King, and P. R. Carey. Carbonic anhydrase-aromatic sulfonamide complexes: a resonance Raman study. *FEBS Lett.* **48**:283–287 (1974).

Send reprint requests to: Corwin Hansch, Department of Chemistry, Pomona College, Claremont, CA 91711.