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Lipophilicity measurements of benzenesulfonamide inhibitors of carbonic anhydrase by reversed-phase HPLC

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Summary

The lipophilicity of 33 *meta* and *para* substituted benzenesulfonamides has been studied by reversed-phase high-performance liquid chromatography (RPLC) using methanol/water or acetonitrile/water as the mobile phases and μ-bondapak C_{18} as the stationary phase. A linear relationship between the capacity factor (log k') and the volume fraction of the organic modifier (ϕ) has been established for each solute. The extrapolation of the retention to $\phi = 0$ (0% of organic modifier) permitted the elimination of any selective and specific effects of organic modifier, to calculate log k_w and finally to derive τ_w for each substituent. The Hansch's π hydrophobic parameter was linearly correlated to τ_w ($r = 0.953$), but no improvement in the correlation equation was observed when τ_w values obtained by adding silanol masking amines to the mobile phase were used. By introducing an indicator variable which takes into account the possibility of the substituent to make hydrogen bondings, a slight but significant improvement was instead observed. The replacement of π with τ_w in the regression equation obtained in our recent quantitative structure-activity relationship (QSAR) study on carbonic anhydrase inhibition by benzenesulfonamides gives rise to a correlation equation with comparable statistical significance.

Introduction

The well recognized important role of hydrophobicity in determining the pharmacokinetics and pharmacodynamic properties of several drugs (Topliss, 1983; Hansch et al., 1987), the distribution, accumulation and biodegradation of pollutants and pesticides in the environment (McKinney, 1980), prompted many researchers to study deeper its "intimate" physicochemical character and composition (Guang-zhong et al., 1986; Pearlman, 1986; Dunn et al., 1987) and to develop fast and accurate methods for the determination of reliable hydrophobic descriptors. Partition coefficients are at the moment the most used indices of hydrophobicity and they can be easily obtained from the study of the distribution of a solute between two immiscible liquid phases, generally *n*-octanol and water, through the so called "shake-flask" technique (Leo et al., 1971; Martin, 1978).

Several drawbacks, like being time consuming, and its limited range of applicability, solute instability and intermolecular associations, are linked to this technique and very often the precision and accuracy of partition coefficient measurements

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markedly depend on a careful and severe control of several experimental factors (Van de Waterbeemd and Testa, 1987). For these reasons chromatographic retention data such as R_M from thin layer chromatography and log k' or log k_w from reversed-phase liquid chromatography (RPLC) have been proposed as alternative and significant hydrophobic indices (Van de Waterbeemd and Testa, 1987). Our recent quantitative structure-activity relationship (QSAR) study on the carbonic anhydrase inhibition by a large series of *meta* and *para* substituted benzenesulfonamides (BzSA) (Carotti et al., 1989), prompts us to investigate on the chromatographic retention properties of this set of congeners by RPLC. The group of BzSA in our hand presented a good variation in terms of hydrophobic, electronic, steric and hydrogen bonding properties and constituted a challenging set to study. However, a straightforward interpretation of retention data could not be expected since the very important physicochemical properties of the common SO_2NH_2 group could play a strong and dominant role in the chromatographic partition process, as recently observed (El Tayar et al., 1988).

Materials and Methods

Meta and *para* substituted benzenesulfonamides (BzSA) were synthesized according to known methods (Carotti et al., 1989) when they were not commercially available; their identity and purity were checked by ^{1}H-NMR, IR, elemental analysis, TLC (silica gel) and RPLC. Methanol, acetonitrile, triethylamine (TEA), *n*-decylamine (DA), and *N,N*-dimethyloctylamine (DMOA) (Aldrich, F.R.G.) were of analytical grade.

Chromatographic procedure

All the experiments were carried out on a Waters HPLC Model 600 multisolvent delivery system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Waters 481 variable wavelength detector (operating at 254 nm) and a Rheodyne Model 7125 injection valve (with a 10 μl loop).

A Waters 745 integrator was used for peak registration and calculation of the retention times. A μ-bondapak C_{18} (10 μm) column (150 × 3.9 mm i.d.) was used as the stationary phase. The mobile phases consisted of different volume fractions of methanol or acetonitrile in water (H_3PO_4, pH = 3.10). The estimated pK_a (Perrin et al., 1981) values of most BzSA are such that the compounds exist practically exclusively in their neutral form at pH = 3.10 with the exception of the amino-BzSA for which a higher pH (pH = 5.50, phosphate buffer 0.05 M) was required. In the above conditions according to the Henderson–Hasselbach equation the % of ionized form was always $< 1\%$.

In order to mask the silanol sites on the stationary phase 3 amine modifiers, namely TEA, DA and DMOA, were added to the eluent up to 20 mM concentration and the pH was adjusted to the appropriate values (3.10 and 5.50) by the addition of small volumes of concentrated phosphoric acid. Solutions of 0.1–1.0 mg/ml of BzSA were prepared in acetonitrile and injected onto chromatographic column. The flow-rate was 1 ml/min. Each chromatographic run was repeated at least 3 times; the retention times, t_R, measured at room temperature in replicate runs, had a relative S.D. less than 1.5%. The column dead time t_0 was determined by the method of Knox and Kaliszan (1985) by using D_2O as t_0 marker. The average of the retention times of D_2O peaks measured by a refractive index detector (Waters 401) at several mobile phase compositions was taken as t_0. The capacity factors, k', were calculated from the relationship $k' = (t_R - t_0)/t_0$. The log k' values were determined for at least 5 different concentrations of organic modifier; the extrapolation to 100% water eluent yielded the log k_w values reported in Table 1.

Results and Discussion

Retention parameters

For reversed-phase chromatography, Schoenmakers et al. (1978) suggested that the relationship between solute retention, expressed by log k', and the composition of the mobile phase can be described by

$$\log k' = \log k_w + A\phi^2 - S\phi \qquad (1)$$

where A and S are constants for a given solute–eluent combination, ϕ is the volume fraction of the organic modifier in the aqueous solvent and $\log k_w$ is the logarithm of the capacity factor in 100% water eluent. Snyder et al. (1979) showed that over a limited range of compositions of the binary mobile phase, a linear relationship can be used as a good approximation of Eqn. 1:

$$\log k' = \log k_w - S\phi \qquad (2)$$

Log k' values for a large series of *meta* and *para* substituted BzSA at various methanol–water ratios (ϕ_M) have been determined and a linear relationship between $\log k'$ and ϕ_M has been derived for each solute. The results reported in Table 1 indicate that linear Eqn. 2 excellently describes the retention over the whole volume fraction range studied ($0.05 \leq \phi_M \leq 0.70$). The squared correlation coefficient for linear regression, r^2, was always greater than 0.97.

However, $\log k_w$ values were experimentally determined only on a limited set of BzSA (6 compounds) for which the retention could be conveniently measured at $\phi_M = 0$, and as expected they resulted slightly greater than those obtained by the extrapolation from linear regression equations. The two data sets were however strongly interrelated ($r = 0.995$).

The curvature in the plot of $\log k'$ vs ϕ_M was mainly observed at very low ϕ_M values ($\phi_M < 0.05$). When linear regressions were restricted to $\phi_M \geq 0.05$, a good fit of the data to linear Eqn. 2 was obtained for the whole analyzed set.

These findings are in good agreement with those obtained by others (Braumann et al., 1983; Braumann and Jastorff, 1985; Reymond et al., 1987) and indicate that $\log k_w$ derived by linear extrapolation of retention data at several volume fractions of methanol-water mobile phase ($0.05 \leq \phi_M \leq 0.70$) can be used as a reliable hydrophobic parameter.

The chromatographic behaviour of another limited set of BzSA (20 compounds) was studied in aqueous eluents containing acetonitrile, which is a weak hydrogen-bonding acceptor only, in contrast to methanol which provides both hydrogen-bonding donor and acceptor capabilities.

TABLE 1

Log k_w values of X-BzSA derived from linear relationships between the volume fraction of organic modifier (ϕ_M, methanol and ϕ_A, acetonitrile) and log k'

Substituent X	Methanol		Acetonitrile		log P [a]
	log $k_{w(M)}$	r	log $k_{w(A)}$	r	
H	0.88	0.993	0.69	0.988	0.35
4-CH_3	1.45	0.997	1.28	0.985	0.80
3-CH_3	1.49	0.999			0.90
4-Cl	1.55	0.998	1.49	0.995	1.10
3-Cl	1.55	1.000			1.20
4-Br	1.71	0.999	1.66	0.995	1.38
3-Br	1.70	1.000			1.39
4-I	1.97	1.000	1.72	0.991	1.59
3-I	1.93	1.000			1.62
4-$CH(CH_3)_2$	2.33	1.000	1.78	0.996	1.75
3-$CH(CH_3)_2$	2.29	0.999			1.70
4-C_6H_5	2.89	0.999	2.19	0.993	2.28
4-CN	1.09	0.998	1.09	0.989	0.22
3-CN	1.09	0.998			0.28
4-$COCH_3$	1.38	0.996	1.15	0.977	0.24
3-$COCH_3$	1.41	0.997			0.25
4-OCH_3	1.34	0.998	1.03	0.979	0.45
3-OCH_3	1.45	0.997			0.57
4-NO_2	1.19	0.998	1.20	0.986	0.75
3-NO_2	1.19	0.999			0.56
4-OC_4H_9	2.82	1.000	2.16	0.994	2.09
3-OC_4H_9	2.81	1.000			2.10
4-OC_6H_{13}	3.87	0.999	2.96	0.994	2.90
3-NO_2,4-OC_6H_{13}	4.06	1.000	3.34	0.999	3.03
4-SO_2NH_2	0.38	0.990	0.47	0.949	−0.96
3-SO_2NH_2	0.57	0.999			−0.46
4-$CONH_2$	0.59	0.994	0.30	0.932	−0.79
3-$CONH_2$	0.74	0.989			−0.80
4-NH_2	0.30	0.989	0.10	0.948	−0.62
3-NH_2	0.46	0.991			−0.28
4-$NHSO_2CH_3$	0.83	0.997	0.68	0.963	−0.36
4-$NHCOCH_3$	1.17	0.998	0.93	0.978	0
4-OH	0.55	0.985	0.47	0.926	−0.06

[a] The measured log P values, taken from Carotti et al. (1989), are reported for comparison.

The retention data ($\log k'$) for several acetonitrile/water mixtures indicate that the replacement of methanol by acetonitrile gives rise to a significant drop of the correlation coefficient in the linear relationships between $\log k'$ and ϕ (see Table 1). Log $k_{w(M)}$ values are, with only few exceptions, always greater than $\log k_{w(A)}$ values and the difference between them is more evident for the most lipophilic compounds.

However, the log $k_{w(A)}$ values are strongly correlated with log $k_{w(M)}$, as shown by the following equation:

$$\log k_{w(M)} = 1.25(\pm 0.10) \log k_{w(A)} - 0.05(\pm 0.16) \quad (3)$$

$$n = 20 \quad s = 0.186 \quad r = 0.986$$

Even if slight deviations from linearity in the log $k' - \phi_A$ relationship can not be completely explained, the different behaviour of BzSA in the two different eluent systems could be interpreted in the light of the "mixed" retention mechanism, for which both silanophilic and hydrophobic interactions are simultaneously operating (Nahum and Horvâth, 1981; Bij et al., 1981; El Tayar et al., 1985a).

By excluding solute conformational changes (Melander et al., 1979a), complex formation between solute and buffer (Melander et al., 1979b), solubility factors (Locke, 1974; Tijssen et al., 1976) and changes in solute ionization (El Tayar et al., 1985a) the deviations from linearity could be tentatively interpreted in terms of polar (presumably silanophilic) interactions which should be more marked in acetonitrile than in methanol (Hoffmann and Liao, 1978) as it was evidenced also from our data. In fact, by comparing the data of 4 X-substituted BzSA (X = H, 4-I, 4-OCH_3, 4-$NHSO_2CH_3$), for which a greater number of capacity factors was determined, a much more marked curvature in the log k'/ϕ plot was observed when acetonitrile was used in place of methanol as organic modifier as can be seen in the two examples reported in Fig. 1.

Relationship between capacity factors and partition coefficients from the n-octanol/water system

From the log k' values, the best statistical correlations between the retention data and the classical hydrophobic parameter log P were obtained using methanol/water mixtures with $\phi_M = 0.2 - 0.4$. For $\phi_M = 0.2$, the mobile phase composition at which log k' of several BzSA of different hydrophobicity (log P from -0.96 to 2.09) can be conveniently and correctly measured, the following correlation equation was formulated:

$$\log k' = 0.72\ (\pm 0.06) \log P + 0.15(\pm 0.07) \quad (4)$$

$$n = 31 \quad s = 0.152 \quad r = 0.976$$

However, the estimation of hydrophobicity based on isocratic capacity factors (log k') could be often misleading because of the strong influence of the organic modifier concentration, and thus several authors (Braumann et al., 1983; Braumann, 1986; El Tayar et al., 1985b and 1985c) suggested the use of log k_w as a better descriptor of hydrophobicity for QSAR studies in addition to, or in place of, the classical log P parameter. Log k_w and log P are usually strongly correlated

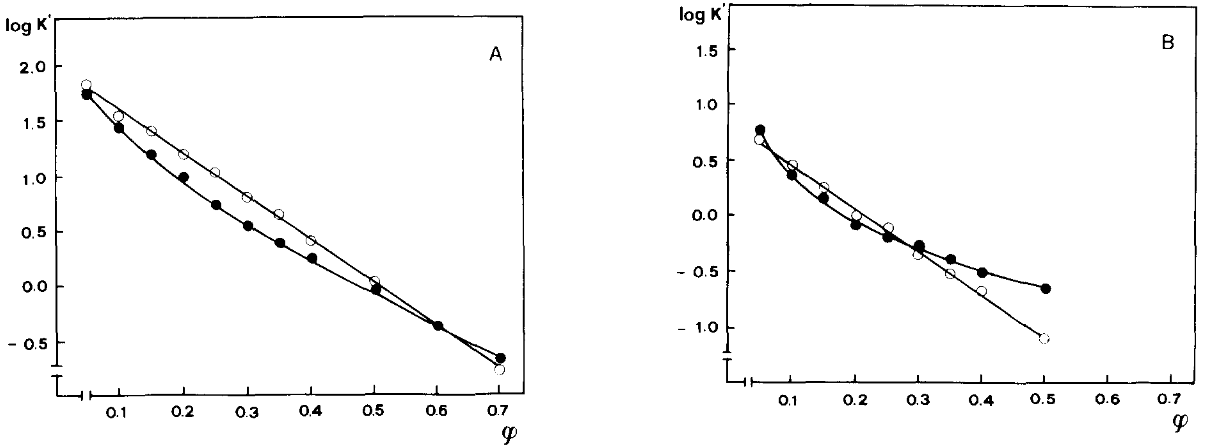

Fig. 1. Relationships between ϕ and log k' for 4-I-BzSA (A) and 4-CH_3SO_2NH-BzSA (B). ●, Acetonitrile/water; ○, methanol/water.

and this was confirmed also in the following regression equations obtained from data in Table 1 collected from the methanol/water system:

$$\log k_{\mathrm{w(M)}} = 0.83\ (\pm 0.10)\ \log P + 0.91\ (\pm 0.13)$$
$$n = 33 \quad s = 0.288 \quad r = 0.953 \qquad (5)$$

and from the acetonitrile system

$$\log k_{\mathrm{w(A)}} = 0.70\ (\pm 0.09)\ \log P + 0.77\ (\pm 0.12)$$
$$n = 20 \quad s = 0.218 \quad r = 0.969 \qquad (6)$$

Hydrophobic substituent constant

In order to investigate the capability of the chromatographic parameters to measure the hydrophobic nature of the substituents in BzSA inhibitors of carbonic anhydrase, the RPLC derived hydrophobic substituent constant τ was taken into account. τ may be defined (Chen and Horvâth, 1979) as

$$\tau = \log(k'_{\mathrm{j}}/k'_{\mathrm{i}}) \qquad (7)$$

where k' is the capacity factor of solutes j and i which differ by a substituent. τ_{w} is thus the substituent hydrophobic constant derived from $\log k_{\mathrm{w}}$. The values of τ_{w} calculated on our set of BzSA are reported in Table 2.

Since the methanol/water mobile phase has been recognized as the best RPLC reference system to determine the hydrophobic parameters (Braumann, 1986) we attempted to gain more insight in the relationship between $\tau_{\mathrm{w(M)}}$ and π hydrophobic substituent constant by carefully analyzing the data from this eluent system. First the data have been divided into two groups, *para* and *meta* substituted congeners, for separate analysis aiming at the detection of eventual position-dependent effects.

The following correlations have been formulated for *meta* congeners:

$$\tau_{\mathrm{w(M)}} = 0.71\ (\pm 0.17)\ \pi + 0.29\ (\pm 0.15) \qquad (8)$$
$$n = 14 \quad s = 0.247 \quad r = 0.932$$

TABLE 2

Hydrophobic substituent constants, τ_{w}, derived from Table 1 [a]

Substituent X	Methanol/water $\tau_{\mathrm{w(M)}}$	Acetonitrile/water $\tau_{\mathrm{w(A)}}$	π [b]
No-HB [c]			
H	0	0	0
4-CH_3	0.57	0.59	0.45
3-CH_3	0.61		0.55
4-Cl	0.67	0.80	0.75
3-Cl	0.67		0.85
4-Br	0.83	0.97	1.03
3-Br	0.82		1.04
4-I	1.09	1.03	1.24
3-I	1.05		1.27
4-$CH(CH_3)_2$	1.45	1.09	1.40
3-$CH(CH_3)_2$	1.41		1.35
4-C_6H_5	2.01	1.50	1.93
HB acceptors			
4-CN	0.21	0.40	−0.13
3-CN	0.21		−0.07
4-$COCH_3$	0.50	0.46	−0.11
3-$COCH_3$	0.53		−0.10
4-OCH_3	0.46	0.34	0.10
3-OCH_3	0.57		0.22
4-NO_2	0.31	0.51	0.40
3-NO_2	0.31		0.21
4-OC_4H_9	1.94	1.47	1.74
3-OC_4H_9	1.93		1.75
4-OC_6H_{13}	2.99	2.27	2.55
3-NO_2,4-OC_6H_{13}	3.18	2.65	2.68
HB acceptors and donors			
4-SO_2NH_2	−0.50	−0.22	−1.31
3-SO_2NH_2	−0.31		−0.81
4-$CONH_2$	−0.29	−0.39	−1.14
3-$CONH_2$	−0.14		−1.15
4-NH_2	−0.58	−0.59	−0.97
3-NH_2	−0.42		−0.63
4-$NHSO_2CH_3$	0.05	0.01	−0.71
4-$NHCOCH_3$	0.29	0.24	−0.35
4-OH	−0.33	−0.22	−0.41

[a] Log k_{w} for unsubstituted benzenesulfonamide is taken as reference.

[b] π values, taken from Carotti et al. (1989), are reported for comparison.

[c] HB = hydrogen bonding.

and for *para* congeners:

$$\tau_{\mathrm{w(M)}} = 0.83\ (\pm 0.13)\ \pi + 0.33 (\pm 0.14) \qquad (9)$$
$$n = 19 \quad s = 0.279 \quad r = 0.958$$

The quality of the fit in the above equations was acceptably good and slightly better in Eqn. 9 than in Eqn. 8. No very significant difference could, however, be observed both in the slopes and intercepts and this result could rule out any particular position-dependent interactions in the retention process. The combination of Eqns. 8 and 9 gives rise to the following regression equation for the whole set of X substituents:

$$\tau_{w(M)} = 0.83(\pm 0.10)\pi + 0.32(\pm 0.11)$$

$$n = 33 \quad s = 0.288 \quad r = 0.953 \quad F_{1,31} = 305 \tag{10}$$

With the purpose of pointing out any particular different behaviour in the partition process between BzSA containing X-substituents able or unable to do hydrogen bonds, the data set has been accordingly divided into two groups and the following correlation equations have been found:
no HB X-substituents

$$\tau_{w(M)} = 0.98\ (\pm 0.18)\ \pi - 0.04\ (\pm 0.20) \tag{11}$$
$$n = 12 \quad s = 0.138 \quad r = 0.967$$

HB X-substituents

$$\tau_{w(M)} = 0.90\ (\pm 0.10)\pi + 0.44(\pm 0.12) \tag{12}$$
$$n = 21 \quad s = 0.262 \quad r = 0.972$$

Note that in Eqn. 11 the slope is very close to 1 and the intercept to zero; these results indicate that for BzSA containing no HB X-substituents the chromatographic partition process between the C_{18} stationary phase and methanol/water mobile phase is almost equal to the partitioning in the octanol/water system.

The analysis of Eqn. 12 reveals instead that BzSA containing HB X-substituents present $\tau_{w(M)}$ values always greater than π as can be seen in Table 2 (note also the different intercepts in Eqns. 11 and 12).

Since the most striking differences in the $\tau_{w(M)}/\pi$ correlation equations are due to the presence or absence in the data set under study of HB X-substituents, a general equation could be formulated for the whole series of BzSA by introducing an indicator variable which took the value of 1 when HB X-substituents are present and 0 when they are absent:

$$\tau_{w(M)} = 0.91\ (\pm 0.08)\ \pi + 0.41(\pm 0.18)\ I_1 + 0.03(\pm 0.16) \tag{13}$$

$$n = 33 \quad s = 0.224 \quad r = 0.972 \quad F_{1,30} = 21.0$$

The quality of the fit in Eqn. 13 is slightly better than in Eqn. 10 and the introduction of the indicator variable I_1 was highly significant ($P <$ 0.001). The coefficient with π approaches the value of 1 and the intercept is close to zero but with a very high 95% confidence interval. These results are in excellent agreement with those obtained by others (Braumann, 1986) and demonstrate that when the possibility of making hydrogen bondings is properly taken into account the chromatographic C_{18}/methanol/water and octanol/water partitioning is quite similar.

Several authors have previously shown that there are "imperfect" linear correlations between chromatographic parameters (log k', log k_w, τ_w) and log P (or π) in sets of compounds (or substituents) comprising HB donors and acceptors of different strength (Unger et al., 1978; Unger and Chiang, 1981; Terada, 1986).

The deviations of the correlations from linearity can be attributed to some differences in partitioning of HB groups because of the interaction with the silanol sites remained in the stationary phase even after persilation. According to previous works, we try to overcome this problem by adding to the mobile phase some silanol masking

TABLE 3

Regression equations between τ_w (determined in methanol/water mobile phases containing silanol masking amine modifiers) and π: $\tau_w = a\pi + b$

Mobile phase	*a*	*b*	*n*	*s*	*r*
Methanol/water [a]	0.70	0.28	13	0.260	0.929
+ TEA (20 mM)	0.71	0.26	13	0.275	0.925
+ DMOA (20 mM)	0.66	0.34	13	0.291	0.903
+ DA (20 mM)	0.35	0.26	5	0.214	0.830

[a] For comparison the regression equation derived from τ_w values measured in methanol/water mobile phases is reported.

agents such as TEA, DMOA and DA. A limited set of *para*-substituted BzSA, containing both no-HB and HB substituents was examined and the resulting regression equations $\pi - \tau_w$ are reported in Table 3. As can be seen, no significant improvement over the linear equations $\pi - \tau_w$ was obtained by using silanol masking agents.

These results further confirm that the masking process is not irreversible and it can lose some of its efficiency in the presence of strongly interacting functional groups such as the SO_2NH_2 in our solutes.

QSAR studies on carbonic anhydrase inhibition by BzSA

Braumann (1986) recently observed, that in spite of the easy experimental methods and calculations, the high accuracy of measurements and the well documented correlation with other classical hydrophobic parameters, RPLC retention data have been used only in very few QSAR studies. The present work therefore, offers a good chance to test the capacity of τ_w to describe properly the hydrophobic nature of the interactions which take place in the formation of enzyme–inhibitor (or substrate) complexes.

In a recent paper (Carotti et al., 1989) we reported on the carbonic anhydrase inhibition by the series of BzSA listed in Table 1. The following correlation equation was formulated:

$$\log 1/K_i = 0.95(\pm 0.33)\sigma + 0.54(\pm 0.12)\pi - 0.35(\pm 0.11)B_{5,3} + 6.29(\pm 0.22)$$

$$n = 31 \quad s = 0.294 \quad r = 0.914 \quad F_{4,27} = 45.6 \tag{14}$$

where K_i is the inhibition constant, σ is the Hammett electronic constant, π is the hydrophobic parameter and $B_{5,3}$ is the STERIMOL steric parameter for the *meta* substituents.

As pointed out by Eqn. 14 and further confirmed by molecular graphics analysis (Hansch et al., 1985), *meta* and *para* substituents of BzSA interacted with an enzymic hydrophobic region. Furthermore, *meta* substituents suffered of a detrimental steric effect as indicated by the negative sign with the STERIMOL parameter $B_{5,3}$. The substitution of the π term with $\tau_{w(M)}$ led to the correlation equation reported below:

$$\log 1/K_i = 1.04(\pm 0.31)\sigma + 0.66(\pm 0.13)\tau_{w(M)} - 0.38(\pm 0.11)B_{5,3} + 6.14(\pm 0.21)$$

$$n = 31 \quad s = 0.274 \quad r = 0.926 \quad F_{4,27} = 54.1 \tag{15}$$

Eqn. 15 is almost superimposable with Eqn. 14 and carries even slightly improved statistics. The substitution of π with $\tau_{w(M)}$ has been thus completely successful leading to an equation with unaltered physicochemical meaning and prediction value. In this respect, the inhibitory activity of the 3-NO_2,4-OC_6H_{13}-BzSA, a compound which we successfully synthesized to maximize the inhibition potency of BzSA, was acceptably well predicted by Eqn. 15 ($\log 1/K_i$ pred. = 7.84 vs $\log 1/K_i$ obs. = 7.56).

Conclusions

The above results clearly pointed out the interchangeability of π and τ_w and support the belief (or the auspice?) that RPLC data will represent an alternative to classical hydrophobic parameters in the near future. It is important to note, however, that, unlike $\log k_w$, a large number of $\log P$ values have been measured and collected in a data base. Furthermore, several programs performing fully computerized $\log P$ calculations have been developed and are at present under continuous refinement and improvement (Leo, 1985; Van de Waterbeemd, 1986).

Several examples of combined applications of QSAR and molecular graphics analysis in the study of enzyme–ligand interactions recently led to the challenging hypothesis that coefficients associate with π (or $\log P$) close to 1 should be indicative of a hydrophobic interaction in a cleft (or hole) whereas those close to 0.5 should represent hydrophobic interaction on an enzymic surface (Hansch and Klein, 1986). With the sole exception of this paper, no applications have so far appeared in the literature on the use of RPLC-derived hydrophobic parameters in study of enzyme–ligand interactions by QSAR and molecular graphics. In our opinion, this research field should be explored in more detail, simulating the main enzyme surface properties with different stationary phases having diverse characteristics in terms, for instance, of hydrophobicity and capability to make hydrogen bondings. However, for all the above considerations and in agreement with the comments and the conclusions reached in a recent review by Van de Waterbeemd and Testa (1987) we believe that log P (π) and RPLC-derived hydrophobic parameters (log k_w and τ_w) will have to coexist as independent and not mutually exclusive lipophilicity descriptors for a long time in the future.

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