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Biological Activities of Drugs. VIII.[1] Structure-Activity Relationship of Sulfonamide Carbonic Anhydrase Inhibitors. (3)[2]

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Hansch–Fujita's method was applied to a structure–activity analysis of 22 sulfonamide derivatives with an inhibitory activity against carbonic anhydrase. Electronic parameters such as Hammett's σ factor, ΔpK_a, Δppm and Δf_r, and hydrophobic parameters such as π, π_c and β were employed. ΔpK_a, Δppm, Δf_r π, π_c and β were derived from dissociation constant, NMR chemical shift of sulfamoyl protons, S=O valence–force constant, *n*-octyl alcohol–water partition coefficient, chloroform–water partition coefficient and association constant to albumin, respectively. This analysis proved to be useful in predicting not only the activity of substituted benzenesulfonamides except *o*-substituted derivatives, but also that of 1,3,4-thiadiazole-5-sulfonamide derivatives. The activity of *o*-substituted derivatives was satisfactorily predicted with the use of polar parameter σ^* and steric constant E_S.

Recently, Fujita and Hansch have introduced a method for the analysis of correlating biological activity of a series of variously substituted compounds with substituent constant π, which is a free–energy–related parameter used in evaluating the hydrophobic binding power of a substituent, and electronic constant such as the Hammett's σ factor.[4–10] They found that the contribution of hydrophobic and electronic characters of a substituent to a specific biological activity could be expressed by Eq. 1 They also emphasized that Eq. 2, a simplified form of Eq. 1, was capable of rationalizing physiological actions including highly specific enzymatic reactions[7] as well as the action of enzyme inhibitors[9]

$$\log 1/C = -a\pi^2 + b\pi + \rho\sigma + c \quad (1)$$

$$\log 1/C = a\pi + \rho\sigma + c \quad (2)$$

where C is the equieffective molar concentration of compounds (the concentration for a standard response such as LD_{50}, ED_{50}, isotoxic concentration, isonarcotic concentration, minimum inhibitory concentration, *etc.*), and a, b and c are constants. π is defined as $\pi = \log P_X - \log P_H$. P_X and P_H are the partition coefficients determined in a *n*-octyl alcohol-water system of the substituted and unsubstituted compounds, respectively. ρ is a constant related to the reac tion the compounds are undergoing.

1) Part VII: N. Kakeya, N. Yata, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 2000 (1969).
2) Part of this work was reported at the 89th Annual Meeting of the Pharmaceutical Society of Japan at Nagoya, April 1969.
3) Location: *Toneyama, Toyonaka, Osaka.*
4) C. Hansch, R.M. Muir, T. Fujita, P.P. Maloney, F. Geiger, M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963).
5) C. Hansch, T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
6) C. Hansch, E.W. Deutsch, R.N. Smith, *J. Am. Chem. Soc.*, **87**, 2738 (1965).
7) T. Fujita, *J. Med. Chem.*, **9**, 797 (1966).
8) C. Hansch, E.W. Deutsch, *Biochim. Biophys. Acta.*, **112**, 381 (1966).
9) C. Hansch, S.M. Anderson, *J. Med. Chem.*, **10**, 745 (1967).
10) T. Fujita, C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).

Previously, we have reported the inhibitory activity of sulfonamide derivatives for carbonic anhydrase in reference to their physicochemical properties.[11] In the present study with sulfonamide derivatives, the Hansch–Fujita method was applied in analyzing a possible structure–activity relationship between their inhibitory activity for carbonic anhydrase and physicochemical parameters, electronic and hydrophobic parameters.

Results

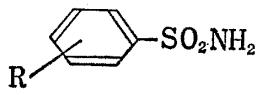
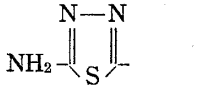
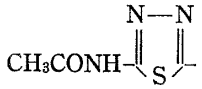
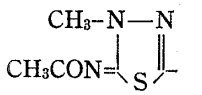
Electronic parameters (Hammett's σ factor, ΔpK_a, Δppm and Δf_r) and hydrophobic parameters (π, π_c and β) are presented in Table I. ΔpK_a, Δppm and Δf_r are defined as follows:

$$\Delta pK_a = (pK_a)_X - (pK_a)_H$$

$$\Delta \text{ppm} = (\text{ppm})_X - (\text{ppm})_H$$

$$\Delta f_r = (f_r)_X - (f_r)_H$$

Table I. Electronic and Hydrophobic Parameters of Sulfonamide Derivatives

Compd. No.	R	σ	ΔpK_a	Δppm	Δf_r	π	π_c	β
								
1	*p*-CH_3NH	−0.840	−1.05	−0.34	−0.33	−0.231	−0.354	0.114
2	*p*-NH_2	−0.660	0.53	−0.27	−0.24	−1.137	−1.447	−0.306
3	*p*-CH_3O	−0.268	0.22	−0.11	−0.18	0.163	0.390	0.399
4	*p*-CH_3	−0.170	0.16	−0.06	−0.08	0.505	0.572	0.426
5	*m*-CH_3	−0.069	0.09	−0.05	−0.06	0.540	0.561	0.194
6	H	0.000	0.00	0.00	0.00	0.000	0.000	0.000
7	*p*-Cl	0.227	−0.07	0.14	−0.06	0.531	0.382	0.751
8	*p*-Br	0.232	−0.08	0.13	−0.07	1.053	0.628	0.760
9	*m*-Cl	0.373	−0.15	0.18	0.05	0.981	0.500	0.551
10	*p*-CH_3CO	0.502	−0.29	0.22	0.12	−0.105	−0.121	0.083
11	*p*-CN	0.660	−0.69	0.30	0.15	−0.083	−0.369	0.173
12	*m*-NO_2	0.710	−0.53	0.39	0.27	0.242	−0.121	0.335
13	*p*-NO_2	0.778	−0.91	0.36	0.21	0.328	−0.356	0.253
14	3,4-di-Cl	0.600	−0.35	0.25	0.08	1.134	0.764	1.323
15	3-NO_2-4-Cl	0.937	−0.61	0.38	0.14	1.603	0.270	0.947
16	3-CF_3-4-NO_2	1.208	−0.81	0.50	0.11	1.420	0.433	1.082
17	*o*-CH_3	—	−0.02	0.18	−0.22	0.526	0.704	0.155
18	*o*-Cl	—	−0.37	0.27	0.00	0.427	0.704	0.360
19	*o*-NO_2	—	−1.28	0.47	0.27	0.033	0.382	—
				$R\text{-}SO_2NH_2$				
20		—	−2.15	—	0.19	−1.214	−3.553	—
21		—	−2.55	—	0.40	−0.557	−2.146	—
22		—	−2.75	0.93	0.36	−0.177	−1.157	—

11) N. Kakeya, N. Yata, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1010 (1969).

where the subscripts of H and X designate benzenesulfonamide and its substituted derivative, respectively. Dissociation constant pK_a was measured spectrophotometrically at 20±2°; chemical shift of the sulfamoyl protons ppm at 20° using tetramethyl silane as an internal standard; S=O valence-force constant f_r infrared-spectrophotometrically with a compressed disc method of KBr.[11] π and π_c are obtained from *n*-octyl alcohol–water and chloroform–water partition coefficients, respectively, following Hansch and Fujita's method. β is a logarithmic ratio of the association constant of substituted benzenesulfonamide for bovine serum albumin against that of benzensulfonamide. The association constant was measured at pH 7.4. Most sulfonamides studied have pK_a around 9—11 except for *o*-NO_2 compound and 1,3,4-thiadiazole-5-sulfonamide derivatives. Thus, they are present as unionized molecules at pH 7.4, a physiologic pH of living body fluids.

Analysis of structure–activity relationship for 16 sulfonamide derivatives (compd. No. 1—16) except for *o*-substituted compounds and 1,3,4-thiadiazole-5-sulfonamide derivatives was made following the Hansch–Fujita method. Using the method of least square analysis of linear combinations of physicochemical parameters, equations 3a—17b were obtained. K_I represents carbonic anhydrase inhibition constant.[11] The value of $1/K_I$ is proportional to the magnitude of carbonic anhydrase inhibitory activity. r is a correlation coefficient and s, standard deviation.

	r	s	
$\log 1/K_I(0.2°)=0.509\pi_c+0.324$	0.462	0.581	(3a)
$\log 1/K_I(15°)=0.494\pi_c+0.329$	0.450	0.621	(3b)
$\log 1/K_I(0.2°)=0.702\pi+0.439$	0.789	0.435	(4a)
$\log 1/K_I(15°)=0.670\pi+0.508$	0.758	0.462	(4b)
$\log 1/K_I(0.2°)=1.093\beta+0.315$	0.769	0.395	(5a)
$\log 1/K_I(15°)=1.109\beta+0.253$	0.775	0.382	(5b)
$\log 1/K_I(0.2°)=1.021\sigma+0.474$	0.938	0.208	(6a)
$\log 1/K_I(15°)=1.018\sigma+0.551$	0.939	0.212	(6b)
$\log 1/K_I(0.2°)=-1.025\Delta pK_a+0.587$	0.849	0.313	(7a)
$\log 1/K_I(15°)=-1.038\Delta pK_a+0.640$	0.862	0.296	(7b)
$\log 1/K_I(0.2°)=2.320\Delta ppm+0.451$	0.914	0.249	(8a)
$\log 1/K_I(15°)=2.360\Delta ppm+0.500$	0.936	0.205	(8b)
$\log 1/K_I(0.2°)=2.756\Delta f_r+0.804$	0.733	0.414	(9a)
$\log 1/K_I(15°)=2.906\Delta f_r+0.862$	0.777	0.375	(9b)
$\log 1/K_I(0.2°)=0.276\pi+0.800\sigma+0.413$	0.965	0.160	(10a)
$\log 1/K_I(15°)=0.223\pi+0.839\sigma+0.481$	0.958	0.176	(10b)
$\log 1/K_I(0.2°)=0.455\beta+0.820\sigma+0.324$	0.980	0.128	(11a)
$\log 1/K_I(15°)=0.471\beta+0.807\sigma+0.377$	0.978	0.132	(11b)
$\log 1/K_I(0.2°)=0.429\pi-0.726\Delta pK_a+0.447$	0.942	0.206	(12a)
$\log 1/K_I(15°)=0.379\pi-0.774\Delta pK_a+0.516$	0.938	0.216	(12b)
$\log 1/K_I(0.2°)=0.718\beta-0.761\Delta pK_a+0.310$	0.960	0.170	(13a)
$\log 1/K_I(15°)=0.690\beta-0.784\Delta pK_a+0.373$	0.968	0.158	(13b)
$\log 1/K_I(0.2°)=0.321\pi+1.752\Delta ppm+0.383$	0.956	0.180	(14a)
$\log 1/K_I(15°)=0.255\pi+1.913\Delta ppm+0.447$	0.963	0.168	(14b)
$\log 1/K_I(0.2°)=0.546\beta+1.770\Delta ppm+0.278$	0.966	0.156	(15a)

$\log 1/K_I(15°)=0.503\beta+1.860\Delta\text{ppm}+0.341$	0.981	0.121	(15b)
$\log 1/K_I(0.2°)=0.513\pi+1.906\Delta f_r+0.508$	0.917	0.248	(16a)
$\log 1/K_I(15°)=0.457\pi+2.160\Delta f_r+0.584$	0.924	0.236	(16b)
$\log 1/K_I(0.2°)=0.877\beta+2.079\Delta f_r+0.401$	0.938	0.206	(17a)
$\log 1/K_I(15°)=0.840\beta+2.257\Delta f_r+0.476$	0.961	0.170	(17b)

Relations of carbonic anhydrase inhibitory activity with hydrophobic parameter, π, π_c or β, were studied (Eq. 3a—5b). A correlation of the inhibitory activity with π_c was not significant unlike the other two parameters.

The most significant correlation was observed for electronic parameters of σ, ΔpK_a and Δppm presented in Eq. 6a—8b. A significant correlation was observed for electronic parameter Δf_r (Eq. 9a and b).

Combination of electronic parameter with hydrophobic parameter (Eq. 10a—17b) resulted in more significant correlation than either of the two parameters (Eq. 3a—9b). The positive sign of coefficients for π and β in Eq. 3a—5b suggests that the stronger the hydrophobicity of sulfonamides, the higher the potency of the carbonic anhydrase inhibition. The positive signs of coefficients of terms σ (Eq. 6a and b), Δppm (Eq. 8a and b), and Δf_r (Eq. 9a and b), and the negative sign of the coefficient of ΔpK_a (Eq. 7a and b) suggests that the more electronegativity in the sulfamoyl group, the stronger the inhibitory activity.

Calculation was made of $\log 1/K_I$ with Eq. 10a—17b (Table IIA and B). The inhibitory activity of 1,3,4-thiadiazole-5-sulfonamide derivatives for carbonic anhydrase was reasonably predicted with Eq. 12a and b which were derived from π and ΔpK_a for 16 benzenesulfonamides (Table IIA and B). The *o*-substituted derivatives studied showed weaker inhibitory activity

Table IIA. Observed and Calculated Carbonic Anhydrase Inhibitory Activities of Sulfonamide Derivatives at 0.2°

Compd. No.	$\log 1/K_I$[a] (0.2°)								
	Obsd.	Calcd.							
		Eq. 10a	Eq. 11a	Eq. 12a	Eq. 13a	Eq. 14a	Eq. 15a	Eq. 16a	Eq. 17a
1	−0.176	−0.323	−0.313	−0.414	−0.407	−0.287	−0.261	−0.239	−0.248
2	−0.363	−0.428	−0.356	−0.425	−0.313	−0.455	−0.367	−0.533	−0.419
3	0.347	0.244	0.285	0.357	0.428	0.243	0.301	0.249	0.312
4	0.420	0.416	0.378	0.547	0.493	0.440	0.404	0.614	0.546
5	0.301	0.506	0.355	0.613	0.380	0.469	0.295	0.671	0.389
6	0.215	0.413	0.324	0.447	0.310	0.383	0.278	0.508	0.349
7	0.721	0.741	0.851	0.725	0.902	0.798	0.936	0.666	0.867
8	0.921	0.889	0.860	0.956	0.916	0.949	0.923	0.915	0.854
9	0.638	0.982	0.880	0.976	0.816	1.013	0.897	1.107	0.927
10	0.959	0.786	0.773	0.612	0.590	0.735	0.713	0.683	0.673
11	0.959	0.918	0.944	0.912	0.959	0.882	0.903	0.751	0.814
12	0.886	1.048	1.058	0.935	0.953	1.144	1.151	1.147	1.206
13	1.046	1.126	1.077	1.248	1.183	1.119	1.053	1.076	1.009
14	1.400	1.206	1.417	1.188	1.525	1.185	1.443	1.242	1.653
15	1.769	1.604	1.523	1.577	1.453	1.563	1.468	1.597	1.457
16	1.854	1.771	1.807	1.643	1.702	1.715	1.754	1.446	1.510
17	−0.204	—	—	0.687	0.436	0.867	0.681	0.359	0.018
18	0.496	—	—	0.898	0.850	0.992	0.952	0.727	0.658
19	0.331	—	—	1.390	—	1.217	—	1.039	—
20	1.602	—	—	1.487	—	—	—	0.247	—
21	2.180	—	—	2.059	—	—	—	0.984	—
22	2.236	—	—	2.367	—	1.956	—	1.103	—

a) K_I is carbonic anhydrase inhibition constant ($\times 10^{-5}$M).

Table IIB. Observed and Calculated Carbonic Anhydrase Inhibitory Activities of Sulfonamide Derivatives at 15°

Compd. No.	log $1/K_I$[a] (15°) Obsd.	Calcd. Eq. 10b	Eq. 11b	Eq. 12b	Eq. 13b	Eq. 14b	Eq. 15b	Eq. 16b	Eq. 17b
1	−0.046	−0.275	−0.247	−0.384	−0.372	−0.262	−0.234	−0.234	−0.243
2	−0.398	−0.326	−0.301	−0.325	−0.254	−0.359	−0.315	−0.454	−0.382
3	0.301	0.292	0.348	0.407	0.475	0.278	0.337	0.270	0.334
4	0.496	0.451	0.441	0.583	0.541	0.461	0.444	0.642	0.586
5	0.223	0.543	0.411	0.651	0.436	0.489	0.345	0.701	0.441
6	0.124	0.481	0.377	0.517	0.373	0.447	0.341	0.584	0.419
7	0.959	0.790	0.914	0.771	0.946	0.850	0.979	0.697	0.899
8	0.959	0.910	0.925	0.977	0.960	0.964	0.965	0.914	0.883
9	0.921	1.012	0.938	1.004	0.871	1.041	0.953	1.140	0.986
10	0.886	0.879	0.828	0.701	0.658	0.841	0.792	0.795	0.763
11	1.187	1.016	0.992	1.019	1.033	1.000	0.986	0.870	0.905
12	1.125	1.131	1.109	1.018	1.021	1.254	1.234	1.278	1.313
13	1.260	1.207	1.125	1.344	1.261	1.219	1.138	1.187	1.108
14	1.522	1.237	1.486	1.217	1.561	1.214	1.471	1.275	1.689
15	1.602	1.625	1.581	1.596	1.505	1.583	1.524	1.619	1.518
16	1.658	1.811	1.860	1.681	1.755	1.766	1.815	1.471	1.560
17	−0.080	—	—	0.731	0.496	0.925	0.753	0.349	0.042
18	0.620	—	—	0.964	0.911	1.072	1.024	0.779	0.715
19	0.455	—	—	1.519	—	1.355	—	1.182	—
20	1.267	—	—	1.719	—	—	—	0.440	—
21	1.919	—	—	2.278	—	—	—	1.193	—
22	2.000	—	—	2.577	—	2.181	—	1.281	—

a) K_I is carbonic anhydrase inhibition constant ($\times 10^{-5}$M).

than expected from Eq. 12a—17b (Table IIA and B). Their inhibitory activity was expressed by Eq. 18a and b employing polar parameter σ^*[12] and steric parameter E_s[12] presented in Table III.

$$\log 1/K_I(0.2°) = 1.388\sigma^* + 1.190E_s - 0.204 \quad (18a)$$

$$\log 1/K_I(15°) = 1.231\sigma^* + 0.975E_s - 0.080 \quad (18b)$$

Here, it must be admitted that only three *o*-substituted derivatives have been employed for the analysis, so that further study is necessary for generalization.

Table III. Polar and Steric Substituent Constants[12]

Substituents	σ^*	E_s
o-CH_3	0.00	0.00
o-Cl	0.37	0.18
o-NO_2	0.95	−0.71

Discussion

Carbonic anhydrase inhibitory activity of 22 sulfonamide derivatives at 0.2° and 15° was significantly correlated.

12) R.W. Taft, *J. Am. Chem. Soc.*, **75**, 4538 (1953).

$$\log 1/K_I(0.2°) = 1.084 \log 1/K_I(15°) - 0.091 \quad (19)$$
$$(n=22,\ r=0.974,\ s=0.030)$$

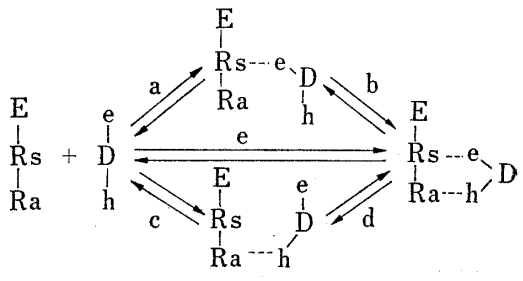
Thus, temperature for the measurements of the inhibitory activity slightly influenced the monoparameter equations (3a—9b). A good correlation was obtained in analyzing structure–activity correlations employing any of σ, ΔpK_a and Δppm. It was interesting that a correlation coefficient for π_c was much smaller than that for π. Chloroform for π_c has been recommended for the study of drug absorption as lipid barrier in the living body. And *n*-octyl alcohol was recommended by Hansch, *et al.* for a structure–activity analysis of drugs. It is to be noted that chloroform is one of lipophilic solvents which donate hydrogen while *n*-octyl alcohol is both a hydrogen donor and an electron donor. Correlation coefficients in the monoparameter equations of β, π and Δf_r were similar. The best correlation was obtained in σ, ΔpK_a and Δppm. A comparison of Eq. 3a—5b with Eq. 6a—8b revealed that the inhibitory activity of sulfonamide derivatives was more influenced by their electronic property than their hydrophobic property. Thus, for the inhibitory activity of sulfonamides, the electronic property in the sulfamoyl group was important with the hydrophobic property apparently playing supplementary role.

An analysis of structure–activity correlationship with two parameters proved to be better than that with monoparameter. *o*-Substituted derivatives were excluded from the analysis because of their poor correlationship. A structure–activity analysis with ΔpK_a and π (Eq. 12a and b) was found to be useful for the prediction of the activity of benzenesulfonamide derivatives and 1,3,4-thiadiazole-5-sulfonamide derivatives.

The activity of *o*-substituted benzenesulfonamides was not predicted with two parameters, the electronic and hydrophobic parameters, becasue of the intramolecular steric effect of *o*-substituted derivatives (Eq. 12a—17b). But an analysis with E_s and σ^* (eq 18a and b) was found to be useful for the prediction of the activity of *o*-substituted derivatives.

It has been recognized that the inhibitory activity of drugs for enzyme is influenced by the electronic property of the specific group in the drug molecule. The activity was also influenced by the molecular size.[13] Ariens[13] suggested that an increased activity with an increase in molecular size of drugs was ascribed to an additional possibility for the binding of drugs on the enzyme surface near the active site with the enhancement of a specific interaction between the active site and the drug molecule. Such enhancement resulted in an additional interaction of the drug molecule with the subactive sites in enzyme by weak forces such as van der Waals' forces.

Thus, the affinity of a drug–receptor interaction is determined by the probability that the drug molecule and the receptor approach so that they may interact. The drug–receptor interaction may be interpreted in a two-step reaction as shown in chart I. Here, E–Rs–Ra is the enzyme molecule in which Rs designates the specific receptor for the enzymatic action and Ra is the additional group for the drug–receptor interaction; e–D–h is the drug molecule; e designates the active group for the drug activity being featured by electronic parameters; h is the subactive group represented by hydrophobic parameters.



The drug–receptor interaction was assumed to involve two paths of consecutive reactions, a—b and c—d. Specific interaction in the step a and d may be mainly ascribed to strong

13) E.J. Ariens, "Molecular Pharmacology," Academic Press, New York and London, 1964, Vol. 1, p. 119—269.

forces such as ionic forces. In the step b and c, van der Waals' forces which have a short range of action, may contribute to the additional binding between drug and enzyme. Electronic attraction of ions decreases with the square of the distance. For van der Waals' forces, there is a decrease to the sixth or seventh power of the distance. Ionic forces have a radius of action much larger than short-range van der Waals' forces. Thus, drug–enzyme interaction may progress mainly through the route a—b under equilibrium condition. The equilibrium constants at the steps a and b are K_1 and K_2, respectively; apparent over-all equilibrium constant at the route e, K_e. The three constants can be defined by Eq. 20.

$$K_e = K_1 K_2 \tag{20}$$

The inhibitory action of drugs for enzyme will be caused at an equieffective concentration of drug–receptor complex under a designated conditions. Thus, K_e is comparable with $1/K_I$.

$$1/K_I = cK_1K_2 \tag{21}$$

or

$$\log 1/K_I = \log K_1 + \log K_2 + c \tag{22}$$

where c is a constant.

The process a is the specific reaction for the inhibition of enzymatic action in which the sulfamoyl group of the sulfonamide molecule reacts with the active center of enzyme. Thus, the equilibrium constant K_1 may be highly dependent on the electronic characters of the sulfamoyl group.

$$\log K_1 = \rho\varepsilon + c \tag{23}$$

where ρ and c are constants; ε is electronic parameter at the sulfamoyl group, such as σ, ΔpK_a, Δppm and Δf_r.

The process b is assumed to be the additional binding of subactive group of sulfonamide molecule with enzyme around the active center. This binding may contribute to the stabilization of the specific reaction in process a. It is considered to be a weak bonding. Thus, K_2 may be highly dependent on the hydrophobic parameter π.

$$\log K_2 = a\pi + c \tag{24}$$

where a and c are constants. Substitution of Eq. 23 and 24 into Eq. 22 yields Eq. 25.

$$\log 1/K_I = a\pi + \rho\varepsilon + c \tag{25}$$

Thus, the two-parameter analysis with electronic and hydrophobic parameters seems recommendable for a structure–activity analysis of benzenesulfonamide derivatives and 1,3,4-thiadiazole-5-sulfonamide derivatives against carbonic anhydrase.

Experimental

Partition Coefficient: *n*-Octyl alcohol–water partition coefficient was spectrophotometrically obtained by shaking the same volume of aqueous solution of a drug (4 mM) and of *n*-octyl alcohol for 2 hr at 30°. The aqueous phase was adjusted to such pH that the drug might exist mainly in unionized form: pH 5.5 phosphate buffer (0.01M) for *p*-aminobenzenesulfonamide, *p*-methylaminobenzenesulfonamide and 2-amino-1,3,4-thiadiazole-5-sulfonamide; 0.005M H_2SO_4 for the other 19 sulfonamides.

Binding to Albumin; Five % of bovine serum albumin (Armour Pharmaceutical Company) was dissolved in a pH 7.4 phosphate buffer (0.1M). Sulfonamide was dissolved in a pH 7.4 phosphate buffer at the concentration of 0.1—10 mM. Binding was studied with an equilibrium dialysis method employing a cellophane membrane. After shaking for 7 hr at 15°, the amount of an unbound drug was measured spectrophotometrically. An association constant was calculated using the Scatchard equation.[13]

13) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).