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Point of view

Carbonic anhydrase inhibitors. Part 86. A QSAR study on some sulfonamide drugs which lower intra-ocular pressure, using the ACE non-linear statistical method

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Abstract – Quantum chemical QSAR expressions have been developed for a heterogeneous group of 36 sulfonamides which have been shown to lower intraocular pressure in in vivo tests on animals. It was found, using the ACE statistical technique, that the lowering of intraocular pressure correlated non-linearly with K_I for carbonic anhydrase inhibition and with solubility. Non-linear transformations had to be applied to both the response variable and to solubility. Chemical variables found to be relevant to CA inhibition included the dipole moment vector, the frontier orbital energies, the solvation energy determined by the COSMO model, the electrostatic potential based charges on the atoms of the sulfonamide group, and the size and polarizability of the molecule. © 2000 Éditions scientifiques et médicales Elsevier SAS

carbonic anhydrase inhibitors / charge / dipole moment / intraocular pressure / QSAR / solvation energy

1. Introduction

Sulfonamides inhibiting the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) are widely used pharmacological agents for the treatment of glaucoma [1–7]. CA inhibition in ocular tissues (mainly the ciliary processes) with systemically or topically administered sulfonamide CA inhibitors, is followed by an effective reduction of intraocular pressure (IOP) due to the reduced rate of bicarbonate secretion within the aqueous humour [8–11]. Since the systemic inhibitors generally produce undesired side-effects due to inhibition of many of the 14 CA isozymes presently known, in other tissues than the eye, many efforts have been made in the last 15 years for the development of water-soluble sulfonamide CA inhibitors that should be administered via the topical route [12–20]. Solubility in general correlates negatively with lipophilicity, and in this data set, log P correlates negatively with log solubility with $R^2 = 0.27$. In seeking topically active drugs we have the conflicting requirements of a solubility large enough to give adequate concentrations, and a lipophilicity large enough to give good penetration of the ciliary body of the eye. Two such drugs are presently available, dorzolamide **A** [13, 17] and brinzolamide **B**, [21] both used as hydrochloride salts. The use of such hydrochloride salts is imposed by the need of assuring a good water solubility to the drugs, but in some cases this represents an undesired complication, since the pH of such solutions becomes acidic enough, and consequently produces eye irritation after the topical administration, as already reported for many patients treated with dorzolamide [22–24]. Moreover, the duration of action of these drugs is generally short (2–3 h) and they must be administered several times a day. It is thus of critical importance to design novel types of topically acting sulfonamide CA inhibitors in order to obtain third generation such drugs with less undesired side effects as compared to the classical drugs. Understanding the factors that govern SAR for this class of pharmacological agents is also of critical importance for the above-mentioned purpose. In a series of papers [25–29], we explored the principal factors governing SAR for several series of aromatic and heterocyclic sulfonamide CA inhibitors, which generally did not show topical antiglaucoma action. Correlations

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with the in vivo properties of such compounds had never been performed up to now. In the present paper we report a QSAR study correlating the IOP (intraocular pressure) lowering properties in a series of sulfonamides that have been investigated for the in vitro CA inhibition as well as in vivo IOP lowering in rabbits.

2. Calculations

The 36 molecules of interest (*figure 1*) were set up with the program Hyperchem [30] and optimized with molecular dynamics, then molecular mechanics using the MMX force field, and finally with the AM1 Hamiltonian [31]

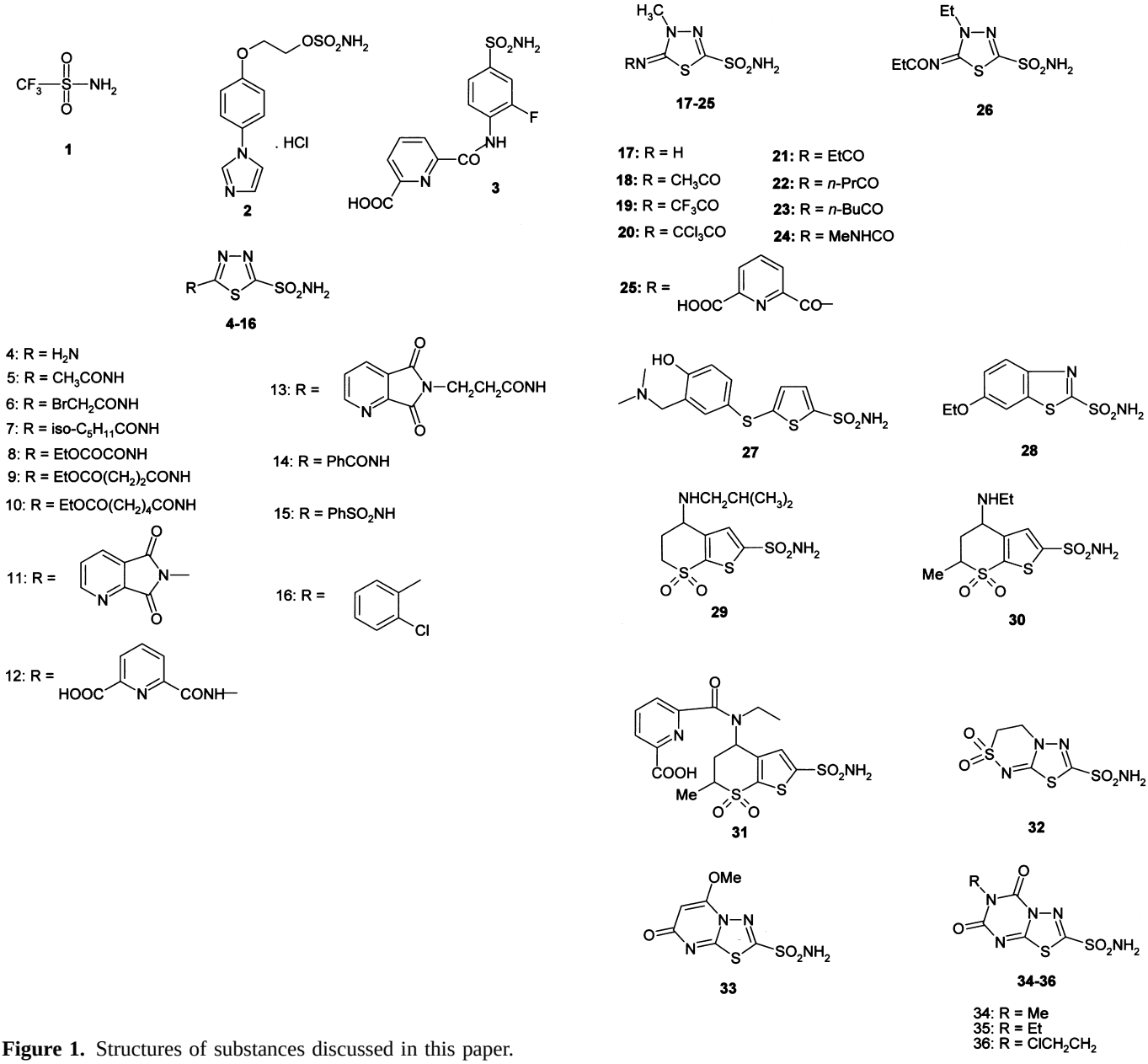

Figure 1. Structures of substances discussed in this paper.

using the program MOPAC, version 6 [32]. The components of the first order polarizability tensor and the heat of formation in vacuum were obtained at this stage. A single-point calculation on the molecule at this geometry using MOPAC 93 [33] and the COSMO [34] approximation, to allow for solvation, was run to obtain the electrostatic potential based charges [35–37] Q_A (A is the atom symbol), the frontier orbital energies E_H and E_L, and the dipole moment. As there is no natural set of Cartesian axes for these molecules, the Cartesian components of the dipole moment were not used in the correlations. From the X, Y and Z components of the dipole moment, the components μ_{S-N} and μ_{S-C} along the S–N and S–C bonds of the primary sulfonamide group were calculated. Also from the results of the single point calculation the electrophilic and nucleophilic superdelocalizabilities S^E_A and S^N_A [38] of the atoms of the primary sulfonamide group (A is the atom symbol) were computed, and also the mean absolute Mulliken charge Q_m and the local dipole index D_l [39] were obtained. The overall dimensions of the molecules, A_x, A_y and A_z were calculated from the principal moments of inertia [40]. The solvation energy of the molecule ΔH_S was estimated as the difference between the heat of formation calculated in solution (dielectric constant 72.7) and in vacuum. The surface area was obtained from the MOPAC 93 calculation. The compound descriptors $E_{L-H} = E_L - E_H$ and $\Pi = (\Pi_{xx} + \Pi_{yy} + \Pi_{zz})/3$ (Π is the mean polarizability) were also used in the statistics. No other compound descriptors were used. The observed K_I and k_{in} values, along with the measured solubility S and the logarithm of the experimentally determined chloroform–water partition coefficient log P are given in *table II*. Octanol values, either measured or calculated, were not available for some of the compounds. Experimental values if available are preferable in some ways.

The measured inhibition constants were correlated with the calculated indices and the measured log P values using the 'all possible subsets' algorithm of Furnival and Wilson [41], implemented in the BMDP statistics package [42]. Control on the influence of collinearity was maintained by principal components analysis, using the Λ statistic. [43] An estimate of the likelihood of chance correlation was obtained by the random reassignment of the dependent variable.

The dependence of lowering of intraocular pressure on K_I, log P, k_{in} and solubility was studied using the alternating conditional expectations technique (ACE) [44] of Breiman and Friedman.

3. Results

1. Log K_I: after exclusion of some terms because of their contributions to collinearities, the most satisfactory equation obtained for K_I was *(table I)*:

Table I.

$$\log K_I = C_1 Q_c + C_2 S^E_N + C_3 D_1 + C_4 \mu_{S-N} + C_5 \Delta H_S + C_6 \Pi + C_7 E_{L-H} + C_8 \quad (1)$$

i	1	2	3	4	5	6	7	8
C_i	–3.51	41.7	–1.17	0.0827	–0.0157	–0.0205	0.968	7.03
σ	0.62	17.2	0.25	0.0120	0.0032	0.00315	0.161	5.43
α	0.00000	0.02196	0.00008	0.00000	0.00004	0.00000	0.00000	0.20627

N = 36, R^2 = 0.817, Q^2 = 0.658, s = 0.25, F = 17.92, P = 7×10^{-10}, Λ = 2.84.

Here, R^2 is the square of the multiple correlation coefficient, Q^2 is the same quantity based on predicted errors (the leave-one-out technique [45]), s is the standard error of estimate of the equation, F is the Fisher variance ratio, P is the probability (statistical significance) based on this F, and the α are the individual statistical significance of each of the coefficients of the equation, based on a Student's *t*-test. The σ are standard errors of estimate for each coefficient in the equation. The diagnostic Λ is defined as $\Lambda = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{\lambda_i}$, where *n* is the number of descriptors and the λ_i are the eigenvalues of the correlation matrix of descriptors [43]. A value of $\Lambda > 5$ is taken to indicate that a collinearity problem exists in the equation.

Less negative charge on the carbon bound to the primary sulfonamide, a large local dipole index, a high solvation energy, and a large polarizability favoured strong inhibitor properties, as did a low electrophilic

Table II. Buffer solubility, Log P, inhibition constants (K_I), rate constants for penetration across the cornea (k_{in}) and ΔIOP (after 1 h in normotensive rabbits) data for compounds **1–36**. All data are experimentally determined.

Compound	Solubility[a] (mM)	Log P[b] ($CHCl_3$/buffer)	K_I[c] (nM)	k_{in}[d] $\times 10^3$ (h^{-1})	ΔIOP[e] (mm Hg)
1	500	0.006	2	12	6.0
2.HCl	70	0.5	7	2.7	2.6
3.HCl	66	0.117	8	1.6	4.2
4.HCl	100	?	46	0.30	0
5	3.2	0.001	6	0.37	0
6	1.0	3.10^{-4}	20	0.29	0
7	1.1	0.05	10	0.40	0
8	91	0.0014	4	3.2	3.0
9	5.8	0.024	13	15.2	2.2
10	0.5	0.0292	21	48.4	1.0
11.HCl	72	0.315	8	2.5	9.0
12[f]	83	0.085	6	1.1	9.3
13	81	0.449	3	3.8	9.2
14	0.4	0.03	2	0.43	0
15	1.2	0.0001	1	0.1	0
16	0.4	10	1	13	0
17.HCl	117	0.014	100	0.56	0
18	12	0.06	8	1.90	0.6
19	60	0.3	10	14	3.1
20	1	4	20	78	1.0
21	30	0.62	15	24	2.2
22	8	0.60	12	72	1.7
23	5.5	4.3	10	96	1.3
24	20	0.02	18	3	2.1
25[f]	79	0.428	7	3.6	8.0
26	3.5	1.0	35	24	1.7
27.HCl	50	0.50	0.3	7.0	3.0
28	0.04	25	1	40	0
29.HCl	60	0.3	4	3.0	2.6
30.HCl	60	2.0	8	5.0	3.5
31[f]	64	1.736	5	5.9	7.2
32	2.0	0.006	14	1.5	0
33	5.0	0.010	50	0.8	0
34	5.6	0.02	20	1.5	1.0
35	4.8	0.01	20	1.8	0
36	3.5	0.02	18	3.0	0

[a] Solubility in pH 7.40 buffer, at 25 °C; [b] chloroform–buffer partition coefficient; [c] against purified human CA II; [d] determined as described by Maren et al. (1983); [e] $\Delta IOP = IOP_{control\ eye} - IOP_{treated\ eye}$, at 1 h after topical administration in normotensive rabbits; [f] as sodium carboxylate salt.

superdelocalizability on the sulfonamide N, a strong negative component of dipole moment vector along the S–N direction and a small separation of HOMO and LUMO energy. Negative charge on the sulfonamide N could substitute for the superdelocalizability term, and charge on the sulfonamide S and O could enter with a positive coefficient. Quantitatively, in terms of contribution to R^2, and also in order of statistical significance α, the dipole moment component term μ_{S-N} was the most important, followed closely by the frontier orbital energy term, then to a lesser degree the polarizability. Unlike some of our previous results with CA inhibitors, the dependence of log K_I on polarizability showed no sign of anisotropy. A plot of observed log K_I against that predicted by equation 1 is shown in *figure 2*.

2. Log k_{in}, the forward rate constant for formation of the enzyme–inhibitor complex.

Log k_{in} is uncorrelated with log K_I in this data set ($r = 0.004$). A regression of log k_{in} on the calculated descriptors gives, after elimination of collinearities *(table III)*:

Table III.

$$\log k_{in} = C_1 Q_C + C_2 S_S^E + C_3 A_x + C_4 A_y + C_5 S + C_6 \log P + C_7 \quad (2)$$

i	1	2	3	4	5	6	7
C_i	5.16	724.8	0.169	0.341	0.00645	0.0882	93.03
σ	1.06	154.0	0.040	0.065	0.00181	0.0199	19.65
α	0.00004	0.00006	0.00026	0.00001	0.00136	0.00013	0.00006

N = 35, R^2 = 0.637, Q^2 = 0.362, s = 0.50, F = 8.18, P = 0.00004, Λ = 2.42.

This is a result distinctly less satisfactory than equation 1, especially in Q^2. It implies that the rate increases with increasing values of all six descriptors, and that the intermediate linear dimension A_y is the most important, followed by the charge on the carbon bound to the primary sulfonamide and the electrophilic superdelocalizability of the S of that group.

3. The intra-ocular pressure lowering effect:

Attempts to relate ΔIOP to K_I, k_{in}, solubility and log P using multiple regression were not successful. Only solubility proved statistically significant, and this yielded a negative Q^2. The dependence was therefore studied by means of the ACE technique of Breiman and Friedman [46, 47]. This procedure finds optimal smooth univariate non-linear functions g and f_i such that $g(y) = \Sigma f_i(x_i) + \varepsilon$, y being the response variable, x_i the predictors and ε the residuals. The functions are returned in graphical form, with one point for each data point. Unlike multiple regression or partial least squares, ACE is a non-linear procedure, but it is not a general non-linear fitting method. It is restricted to fitting a sum of univariate non-linear functions of the response and predictor variables such that R^2 is maximized.

With all four descriptors, an R^2 of 0.975 was obtained. The terms k_{in} and log P made relatively little contribution to this, and without them an R^2 of 0.936 was obtained. Restriction of the K_I term to linearity reduced this to 0.930. Further restrictions resulted in severe loss of fit. Ordinal transformations were necessary for ΔIOP and solubility, and these are plotted in *figures 3* and *4*, respectively. The cross-validated R^2 was 0.31, and a plot of the predicted values is shown in *figure 5*. As was previously shown [48] cross-validation with ACE produces a small number of severe errors, corresponding to the extrapolation of the non-linear functions at extreme values of any of the variables. This is clearly shown by comparison of *figure 2* with *figure 5*. This is also the reason that ACE cross-validated R^2 values are apparently so poor. Because of this, predictions with ACE cannot be made if any of the variables is outside of the bounds of the values in the training set.

4. Randomization test.

As a test of the possibility of accounting for equation 1 as a chance effect of the type described by Topliss and Edwards [49], a randomization experiment was carried

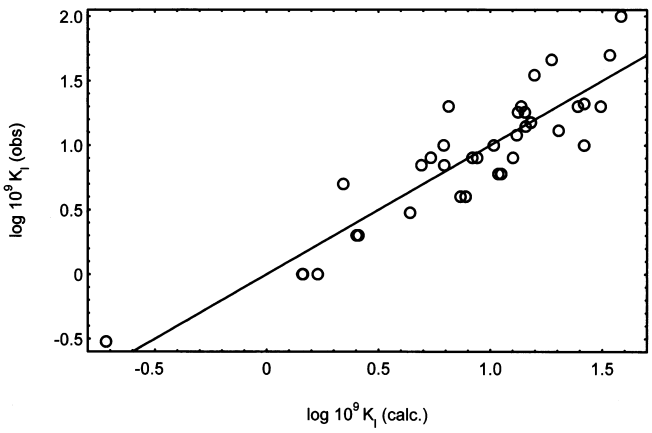

Figure 2. Plot of observed K_I against that calculated by equation 1.

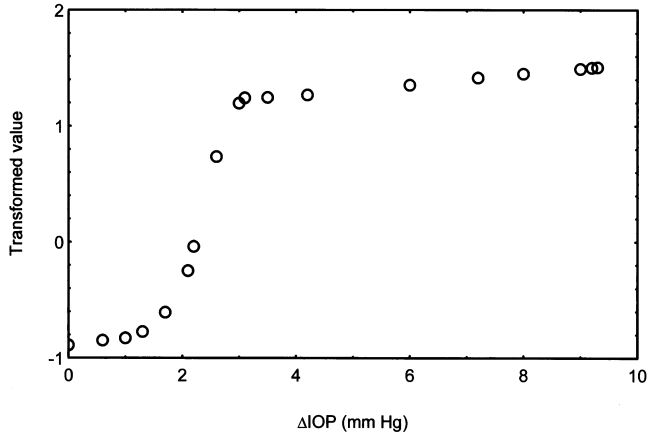

Figure 3. Plot of optimal non-linear transformation of the dependent variable, ΔIOP, in ACE regression of dependence on K_I and solubility for the compounds of *table I*.

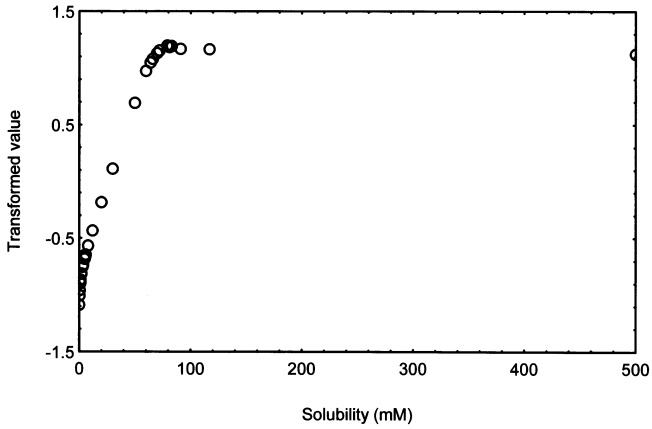

Figure 4. Plot of optimal non-linear transformation, calculated by ACE, of solubility as a descriptor for IOP lowering capability of the compounds in *table I*. The corresponding plot for K_I was linear.

out on all of the calculated descriptors and the log K_I data. A stepwise regression using the Effroymson algorithm [50] and F-to-enter and F-to-leave both set at 3.0 gave an R^2 of 0.555 and included three variables, A_x, μ_{S-N} and E_{L-H} in the model. When the dependent variable was randomly scrambled and the stepwise regression repeated 10 000 times, allowing a maximum of seven variables to enter, the best R^2 obtained was 0.803. Based on the distribution of R [51] this gives a statistical significance of the actual regression of 0.032. While this is rather poor (the maximum acceptable being 0.05), it should be realized that the R^2 for equation 1 is 0.817, and none of the 10 000 R^2 values from the randomization test reached this. Neither the original Effroymson regression nor the 10 000 regressions with randomized dependent variable are finding optimal subsets of variables. Using a number of admitted variables in the randomization trials much larger than the number which entered the stepwise regression is a severe test, and more realistic tests give a statistical significance of 0.01. Because in the randomization trials no attempt was made to exclude models suffering from collinearity problems, even this would be unduly pessimistic. This test is based on the assumption that a data set based on the original, but with the dependent value randomized will have no valid correlations. Thus the results reported here are very unlikely to be ascribable to chance.

This is confirmed by the cross-validation test. If the model was based on chance effects it would have no predictive power. In fact, a regression of the values of the leave-one-out values on the observed values of log K_I gives Q^2 of 0.637, with a significance of 6×10^{-10}. This is very close to the value based on F found in equation 1.

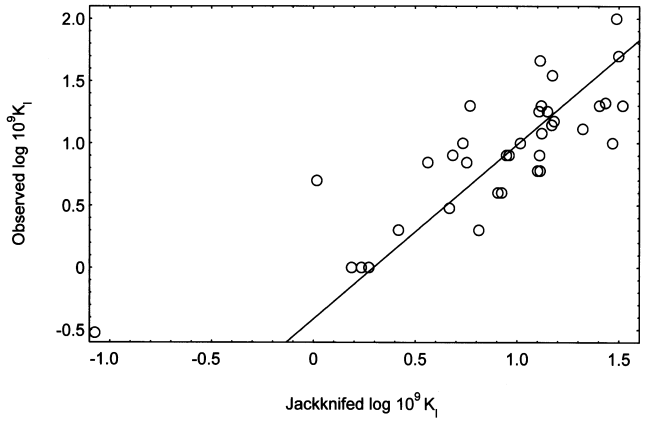

Figure 5. Plot of observed K_I against the jackknifed value using equation 1.

4. Discussion and conclusions

The principal factors influencing the intraocular pressure lowering effect of these drugs are the K_I value and the solubility. As may be seen from *figure 4* the effect of solubility rises sharply, but reaches a plateau beyond which an increase in solubility has little effect. The dependence is approximately logarithmic. It should be noted that this curve is strongly influenced by the very soluble compound **1**. The pressure response itself is non-linear, and may be thought of as the inverse function of that plotted in *figure 3*. The response increases at a diminishing rate until it reaches a threshold, beyond which it increases very sharply. The dependence on K_I is approximately linear, and as expected, decreasing K_I leads to increasing reduction of IOP.

The inhibition constant depends on multiple factors, many of which are properties of the sulfonamide moiety. Thus inhibitory activity depends most strongly on the component of the dipole moment in the direction of the S–N bond, but also on the frontier orbital energy difference and on the polarizability of the molecule. A number of these compounds are aromatic in nature, and the interaction of π electron systems on the drug with similar systems on the enzyme may account for the former of these and dispersion force interactions for the latter. We have previously noted the dependence of CA inhibitory activity on the charge of the atoms of the sulfonamide moiety. This effect is reflected in the present set in the dependence on Q_C, and in dependence on the dipole

moment. The effect of polarizability [28, 29] and solvation energy [29, 52] we have also previously observed in studies on this enzyme.

Finally, it should be stated that this series of drugs is not a congeneric series in the usual sense in which that term is used in QSAR. Use of our results for predictive purposes is possible only for drugs which resemble those on which the model is based, and that is rather ill defined, but certainly does not include all sulfonamides. Thus the results developed here should be used with caution.

Values of the calculated descriptors for each of the drugs are available via the Internet [53].

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