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A quantitative structure–activity relationship study on some aromatic/heterocyclic sulfonamides and their charged derivatives acting as carbonic anhydrase inhibitors

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Abstract
A quantitative structure–activity relationship (QSAR) study is made on a series of aromatic/heterocyclic sulfonamides and their charged derivatives acting as carbonic anhydrase (CA) inhibitors. These compounds were studied by Scozzafava et al. (J. Med. Chem. 2000; 43: 292) for the selective inhibition of CAs—sulfonamides generally do not discriminate between different CA isozymes and hence exhibit many undesirable side effects when used as drugs against a particular disease. In this communication, an attempt has been made to investigate the physicochemical and structural properties that can make them selective for a given CA isozyme. Based on *in vitro* data reported by Scozzafava et al. against two cytosolic isozymes and one membrane-bound isozyme, the QSAR study has shown that uncharged compounds cannot be made selective for cytosolic or membrane-bound isozyme since in both the cases the compounds appear to follow the same mechanism of inhibition. However, for the charged compounds the polarizability of the molecule seems to greatly favor the inhibition of the membrane-bound enzyme, and hence they can be made selective for this enzyme by enhancing their polarizability, which is found to play no role in the inhibition of cytosolic enzymes.

Keywords: *Carbonic anhydrase inhibitors, QSAR, sulfonamides*

Introduction

Among the different classes of carbonic anhydrase (CA) inhibitors, the most prominent class has been that of sulfonamides, which have been found to act against all the different CA isozymes. So far 14 different isozymes have been reported and their physiological functions studied [1], the most important being to catalyze the reversible hydration of carbon dioxide to bicarbonate ion. Because of their strong CA inhibitory properties, aromatic/heterocyclic sulfonamides have been studied for a long time for the development of drugs against a variety of diseases such as diuresis, gastric and duodenal ulcers, mountain sickness, glaucoma, epilepsy, congestive heart failure

[1–4]. However, these sulfonamides generally do not discriminate between different isozymes and hence exhibit many undesirable side effects when used as drugs against a particular disease. Therefore, attempts have been made to investigate compounds that may have selective inhibition of the CAs.

Out of the 14 isozymes (CA I–CA XIV) described so far, some are cytosolic (such as CA I–CA III and CA VII) and some are membrane bound (CA IV, CA IX, CA XII, and CA XIV). Many of the remaining are acatalytic (CA VIII, CA X, CA XI), whereas CA V is found only in mitochondria and CA VI is secreted in saliva [1]. For the selective *in vivo* inhibition of membrane-bound versus cytosolic

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isozymes, Scozzafava et al. [5] studied a new class of sulfonamides that were positively charged and membrane-impermeant. The compounds were obtained by attaching trisubstituted-pyridinium-methylcarboxy moieties to the molecules of classical aromatic/heterocyclic sulfonamides possessing free amino, imino, hydrazino, or hydroxyl groups in their molecules [5]. They were then studied for their CA inhibitory properties against three isozymes—two cytosolic human isozymes, hCA I and hCA II, and one membrane-bound bovine isozymes, bCA IV. In *in vitro* studies, all the compounds were found to have high affinity for all the three isozymes, but in *in vivo* studies they were found to discriminate between membrane-bound and cytosolic enzymes, selectively inhibiting only bCA IV. Our objective here is to carry out a quantitative structure–activity relationship (QSAR) study on these compounds to determine the physicochemical and structural properties of these molecules that can make them selective for a given CA isozyme. Since *in vivo* data are not sufficient for a QSAR study, we have proceeded with only *in vitro* data to see if any light can be thrown on CA selectivity.

Materials and methods

The parent uncharged sulfonamides studied by Scozzafava et al. [5] are shown in Figure 1 and their *in vitro* CA inhibition activity data and the physicochemical and structural variables are listed in Table I. The charged derivatives of these sulfonamides are listed in Table II along with the physicochemical parameters that could be correlated with their activity. In these Tables, K_i refers to the enzyme inhibition constant, $^1\chi^v$ is Kier's first-order valence molecular connectivity index [6], and $\log P$ and Pol are calculated hydrophobicity and polarizability parameters, respectively, which were calculated using software (http://www.daylight.com and www.acdlabs.com) freely available on the internet. The molecular connectivity index $^1\chi^v$ was calculated as described below.

According to Kier and Hall [6], $^1\chi^v$ is defined as:

$$^1\chi^v = \Sigma(\delta_i^v \delta_j^v)^{-1/2} \tag{1}$$

where δ_i^v and δ_j^v are the vertex connectivity indices of atoms i and j, respectively, and the summation extends to all bonded pairs of non-hydrogenic atoms in the group or molecule. For the second and third rows of atoms, a unified definition of δ_i^v, as expressed by Equation 2 [7], is used. In this Equation, Z_i^v is the number of valence electrons of the atom i, h_i is the number of hydrogen atoms attached to it, and Z_i is its atomic number. The connectivity index $^1\chi^v$ signifies the degree of branching, connectivity of atoms, and

the unsaturation in the molecule and is simple to calculate. It is a good structural parameter to account for the variation in the activity, particularly, when no experimental data for any physicochemical properties of the molecules are available.

$$\delta_i^v = (Z_i^v - h_i)/(Z_i - Z_i^v - 1) \tag{2}$$

Results and discussion

We first analyzed the first 26 parent uncharged compounds (**1**–**26**) with the use of the data as given in Table I and found the correlations as:

hCA I (compound **22** excluded)

$$\log(1/K_i) = 0.699(\pm 0.346)\log P + 1.122(\pm 0.436)$$
$$\times (\log P)^2 - 3.032(\pm 1.754)^1\chi^v$$
$$+ 0.284(\pm 0.133)(^1\chi^v)^2$$
$$+ 12.538(\pm 5.399)$$

$$n = 25, \quad r = 0.940, \quad r_{cv}^2 = 0.80, \quad s = 0.50,$$
$$F_{4,20} = 38.06(4.43), \quad (^1\chi^v)_{opt} = 5.34 \tag{3}$$

hCA II (compounds **15**, **16**, **23** excluded)

$$\log(1/K_i) = 0.530(\pm 0.311)\log P + 0.911(\pm 0.576)$$
$$\times (\log P)^2 - 1.193(\pm 1.187)^1\chi^v$$
$$+ 0.120(\pm 0.090)(^1\chi^v)^2 + 9.663(\pm 3.645)$$

$$n = 23, \quad r = 0.921, \quad r_{cv}^2 = 0.71, \quad s = 0.32,$$
$$F_{4,18} = 24.98(4.58), \quad (^1\chi^v)_{opt} = 4.97 \tag{4}$$

bCA IV (compounds **19**, **20** excluded)

$$\log(1/K_i) = 0.603(\pm 0.301)\log P + 0.590(\pm 0.378)$$
$$\times (\log P)^2 - 1.591(\pm 1.589)^1\chi^v$$
$$+ 0.164(\pm 0.123)(^1\chi^v)^2 + 9.946(\pm 4.859)$$

$$n = 24, \quad r = 0.907, \quad r_{cv}^2 = 0.70, \quad s = 0.43,$$
$$F_{4,19} = 22.11(4.50), \quad (^1\chi^v)_{opt} = 4.85 \tag{5}$$

In these equations, n is the number of data points, r is the correlation coefficient, r_{cv}^2 is the square of the cross-validated correlation coefficient obtained from leave-one-out jackknife procedure,
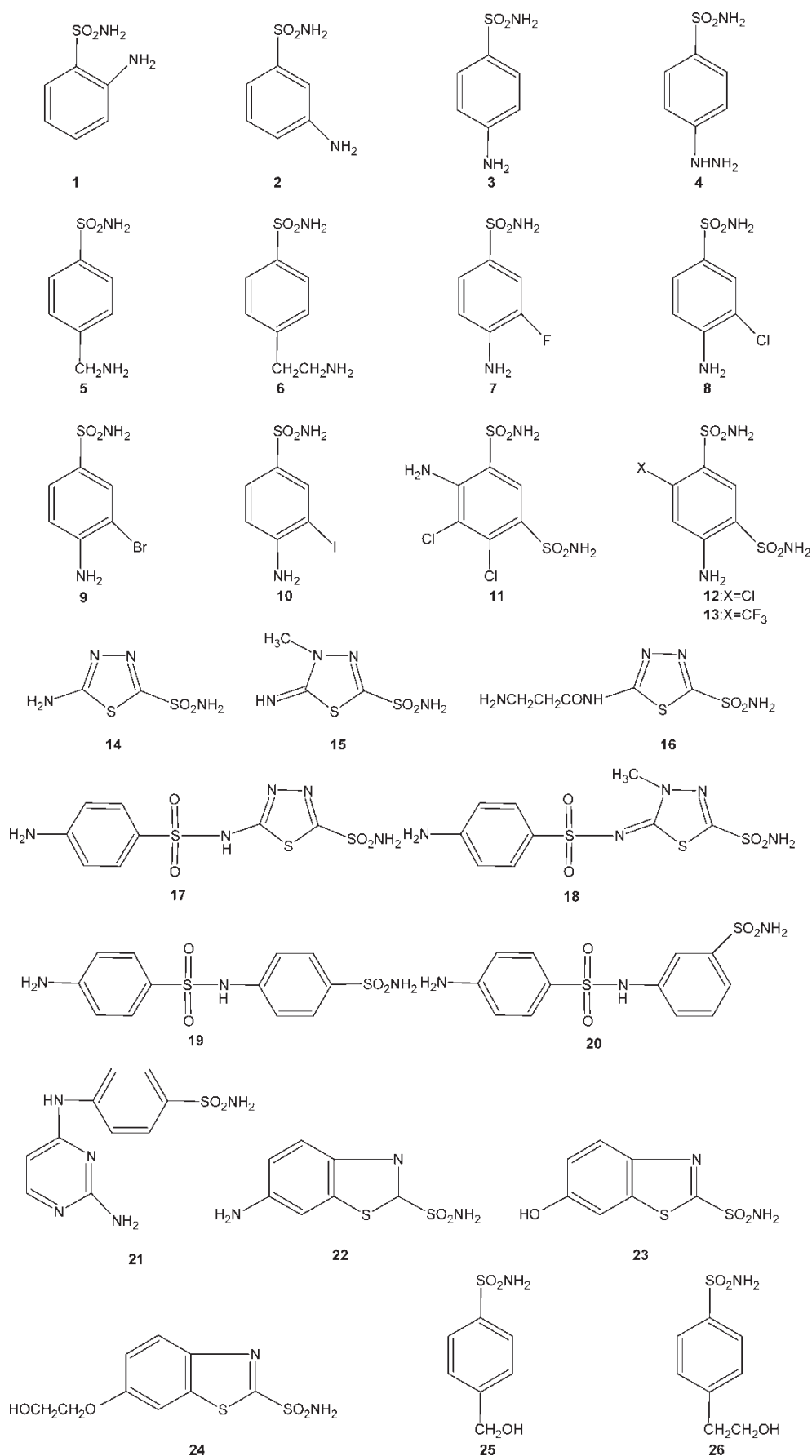
Figure 1.   The series of parent uncharged sulfonamides.

Table I.   A series of parent uncharged sulfonamides (Figure 1) with different structural variables and their observed and calculated potencies.

			$\log (1/K_i)$								
			hCA I			hCA II			bCA IV		
Compd No.	$^1\chi^v$	Log P	Obsd[a]	Calcd Equation 3	Loo	Obsd[a]	Calcd Equation 4	Loo	Obsd[a]	Calcd Equation 5	Loo
1	4.435	0.168	4.34	4.83	4.94	6.53	6.85	6.93	5.88	6.23	5.85
2	4.429	−0.572	4.60	4.65	4.65	6.62	6.73	6.75	5.66	5.96	5.39
3	4.429	−0.572	4.55	4.65	4.67	6.52	6.73	6.76	5.52	5.96	5.42
4	4.679	−0.194	4.11	4.48	4.52	6.49	6.64	6.66	5.49	6.00	5.83
5	4.902	−0.743	4.60	4.60	4.60	6.77	6.81	6.82	5.55	5.97	5.90
6	5.402	−0.404	4.68	4.35	4.30	6.80	6.66	6.63	5.61	5.99	6.56
7	4.535	−0.337	5.08	4.52	4.44	7.22	6.65	6.56	6.74	5.97	6.43
8	4.912	0.313	5.01	4.83	4.81	6.96	6.96	6.95	6.49	6.33	6.95
9	5.328	0.513	5.19	5.10	5.09	7.40	7.23	7.21	7.18	6.59	6.94
10	5.612	0.723	5.22	5.56	5.60	7.15	7.61	7.74	6.90	6.93	6.85
11	7.638	−0.264	5.21	5.85	6.00	7.55	7.49	7.47	6.76	7.24	7.08
12	7.148	−0.881	5.08	5.64	5.80	7.12	7.52	7.67	6.80	6.88	7.23
13	7.398	−0.748	5.12	5.76	5.93	7.21	7.53	7.63	6.85	7.03	5.93
14	4.479	−1.115	5.07	5.27	5.34	7.22	7.27	7.36	6.27	6.17	6.51
15	4.888	−0.910	5.03	4.80	4.78	7.72[c]	6.97	–	6.45	6.03	6.31
16	6.156	−1.546	6.34	6.24	6.12	8.52[c]	8.22	–	6.90	6.85	7.64
17	8.525	−0.747	8.22	7.44	7.27	8.70	8.34	8.23	8.30	8.18	7.55
18	9.131	−0.623	9.00	8.54	8.20	9.22	8.82	8.56	9.10	8.95	7.55
19	8.724	0.111	7.38	7.80	7.91	8.22	8.48	8.59	7.30[d]	8.62	–
20	8.724	0.111	7.36	7.80	7.95	8.05	8.48	8.62	7.28[d]	8.62	–
21	6.480	0.676	6.16	5.81	5.71	7.92	7.76	7.73	6.81	7.20	6.30
22	6.017	0.749	7.15[b]	5.73	–	8.05	7.75	7.65	7.72	7.09	7.50
23	5.952	1.356	7.26	7.57	8.18	8.10[c]	9.21	–	7.77	8.19	8.31
24	7.038	0.645	7.30	6.19	5.98	8.15	7.94	7.88	7.82	7.51	7.16
25	4.810	−0.733	4.62	4.62	4.62	6.90	6.81	6.30	6.25	5.96	5.91
26	5.310	−0.504	4.74	4.38	4.33	6.96	6.68	6.64	6.35	5.97	6.51

[a] Taken from ref 5.
[b] Not used in the derivation of Equation 3.
[c] Not used in the derivation of Equation 4.
[d] Not used in the derivation of Equation 5.

s is the standard deviation, and F is the F-ratio between the variances of calculated and observed activities. Figures written with ± sign within parenthesis are 95% confidence intervals and those written within the parenthesis for the F-ratio is the standard statistical F-value as given in the literature.

Now all the three equations exhibit the parallel correlations indicating that a similar mechanism is involved in the inhibition of all three isozymes. The inhibitory activity of the compounds against all the three isozymes that include both the cytosolic ones and the membrane-bound bCA IV is shown to have a sharp dependence on the hydrophobic property of the molecules. However, with $^1\chi^v$ the activity is shown to have a parabolic dependence, where initially the activity decreases with $^1\chi^v$ till $^1\chi^v$ attains an optimum value, almost identical in all three cases (5.34, 4.97 and 4.85 respectively), after which the activity starts increasing with $^1\chi^v$. Thus the large size of the molecules, which can be accounted for by $^1\chi^v$, and their hydrophobic property appear to control their

inhibitory activity with no selectivity between cytosolic and membrane-bound enzymes. Thus for all the three isozymes, a strong hydrophobic interaction appears to take place between them and the inhibitors, greatly helped by the bulk of the molecule. In none of the three cases was there found any significant mutual correlation existing between log P and $^1\chi^v$ (r = −0.383, −0.518, −0.469, for Equations 4–6, respectively).

In deriving all the above correlations, however, some compounds were excluded as mentioned in the respective equations. These compounds were excluded simply because they were acting as outliers, but unfortunately these outliers were not common in all the equations. Therefore, their aberrant behavior cannot be easily attributed to the experimental error or some specific structural role played by them. The only explanation that can be offered is that because of some unpredictable specific interaction they might be changing the conformation of the receptor, or of their own, before interacting.

Table II.   Sulfonamides attached with trisubstituted-pyridinium-methylcarboxy (TPMC) moieties with different structural variables and their observed and calculated potencies. All sulfonamide molecules are attached to TPMC moieties through their free amino, imino, hydrazino, or hydroxyl goup.
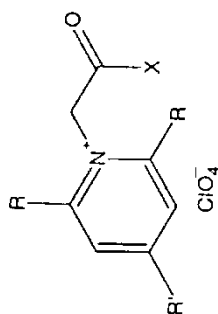
A: R = R' = Me
B: R = Me; R' = Ph
C: R = Et; R' = Ph
D: R = i-Pr; R' = Ph
X: a sulfonamide molecule attached through its free amino, imino, hydrazino, or hydroxyl group

Compd No.	$^1\chi^v$	logP	Pol	I	I_1	I_A	hCA I Obsd^a	hCA I Calcd Equation 6	hCA I Loo	hCA II Obsd^a	hCA II Calcd Equation 7	hCA II Loo	bCA IV Obsd^a	bCA IV Calcd Equation 8	bCA IV Loo
1A	8.618	−4.354	3.585	0	0	0	4.49	5.10	5.19	6.55	6.94	6.95	6.37	6.81	6.84
1B	10.279	−2.965	4.381	0	0	0	4.60	5.52	5.60	6.67	7.15	7.13	6.54	6.94	6.96
1C	11.400	−2.307	4.748	0	0	0	4.56^e	5.76	–	6.62^f	7.31	–	6.47^g	6.06	–
1D	12.166	−1.109	5.113	0	0	0	4.52^e	5.88	–	6.56^f	7.36	–	6.46^g	6.48	–
2A	8.612	−4.354	3.585	0	0	0	4.62	5.10	5.14	6.60	6.93	6.96	6.39	6.81	6.84
2B	10.273	−2.965	4.381	0	0	0	4.71	5.52	5.59	6.70	7.15	7.12	6.55	6.94	6.96
2C	11.394	−2.307	4.748	0	0	0	4.66^e	5.76	–	6.69^f	7.31	–	6.51^g	6.06	–
2D	12.160	−1.109	5.113	0	0	0	4.64^e	5.88	–	6.63^f	7.36	–	6.46^g	6.48	–
3A	8.612	−4.354	3.585	0	0	0	4.80	5.10	5.12	6.84	6.93	6.94	6.72	6.81	6.81
3B	10.273	−2.965	4.381	0	0	0	4.89	5.52	5.58	6.96	7.15	7.11	6.81	6.94	6.95
3C	11.394	−1.109	4.748	0	0	0	4.82	5.73	5.74	6.90	7.25	7.23	6.77	6.82	6.83
3D	12.160	−2.307	5.113	0	0	0	4.82	5.84	5.91	6.89	7.29	7.35	6.74	7.08	7.09
4A	8.862	−4.983	3.730	0	0	0	4.46	5.17	4.88	6.49	6.97	6.99	6.35^g	4.86	–
4B	10.523	−3.594	4.526	0	0	0	4.59	5.57	5.59	6.64	7.17	7.18	6.51^g	5.59	–
4C	11.644	−3.153	4.893	0	0	0	4.55^e	5.80	–	6.54^f	7.33	–	6.47^g	5.84	–
4D	12.410	−1.738	5.258	0	0	0	4.47^e	5.91	–	6.52^f	7.38	–	6.44^g	6.35	–
5A	9.070	−4.717	3.768	0	0	0	5.82	5.23	5.19	7.37	7.00	6.94	7.10	6.86	6.83
5B	10.731	−3.328	4.565	0	0	0	5.90	5.61	5.59	7.51	7.19	7.20	7.30	7.02	7.01
5C	11.852	−2.670	4.932	0	0	0	5.85	5.80	5.81	7.41	7.28	7.31	7.17	7.06	7.06
5D	12.618	−1.472	5.297	0	0	0	5.84	5.90	5.96	7.38	7.31	7.37	7.12	7.02	7.02
6A	9.570	−4.542	3.952	0	0	0	6.09	5.36	5.35	7.44	7.07	7.05	7.15	6.91	6.90
6B	11.231	−3.153	4.748	0	0	0	6.14	5.70	5.63	7.52	7.24	7.23	7.34	7.06	7.05
6C	12.352	−2.495	5.115	0	0	0	6.12	5.87	5.82	7.49	7.30	7.32	7.21	7.10	7.09
6D	13.118	−1.297	5.481	0	0	0	6.11	5.95	5.94	7.46	7.33	7.38	7.15	7.05	7.04
7A	8.718	−4.647	3.589	0	1	0	6.12	5.63	5.60	7.72	7.44	7.38	7.36	7.06	7.05
7B	10.379	−3.258	4.385	0	1	0	6.15	6.04	6.06	7.92	7.65	7.67	7.44	7.22	7.21
7C	11.500	−2.600	4.753	0	1	0	6.12	6.25	6.32	7.80	7.74	7.79	7.41	7.26	7.25
7D	12.266	−1.402	5.118	0	1	0	6.12	6.36	6.32	7.74	7.79	7.73	7.41	7.22	7.20
8A	9.095	−4.327	3.776	0	1	0	6.17	5.74	5.65	7.68	7.49	7.58	7.15	7.11	7.11

Table II – *continued*

Compd No.	$^1\chi^v$	log P	Pol	I	I_1	I_A	hCA I Obsd[a]	hCA I Calcd Equation 6	hCA I Loo	hCA II Obsd[a]	hCA II Calcd Equation 7	hCA II Loo	bCA IV Obsd[a]	bCA IV Calcd Equation 8	bCA IV Loo
8B	10.756	−2.938	4.572	0	1	0	6.22	6.12	6.13	8.00	7.68	7.56	7.42	7.24	7.24
8C	11.877	−2.280	4.940	0	1	0	6.21	6.31	6.37	7.82	7.77	7.83	7.34	7.28	7.28
8D	12.643	−1.082	5.305	0	1	0	6.22	6.40	6.33	7.77	7.80	7.75	7.25	7.22	7.22
9A	9.511	−4.207	3.891	0	1	0	6.10	5.84	5.80	7.35	7.55	7.49	7.11	7.14	7.14
9B	11.172	−2.818	4.687	0	1	0	6.22	6.19	6.22	7.89	7.72	7.79	7.46	7.27	7.26
9C	12.293	−2.160	5.054	0	1	0	6.21	6.36	6.29	7.68	7.79	7.80	7.26	7.30	7.30
9D	13.059	−0.962	5.420	0	1	0	6.19	6.44	6.37	7.44	7.82	7.82	7.15	7.23	7.24
10A	9.795	−4.127	4.097	0	1	0	6.09	5.91	5.94	7.31	7.58	7.62	7.11	7.19	7.20
10B	11.456	−2.738	4.893	0	1	0	6.21	6.24	6.28	7.68	7.74	7.68	7.23	7.31	7.31
10C	12.577	−2.080	5.260	0	1	0	6.21	6.40	6.32	7.60	7.80	7.78	7.19	7.34	7.35
10D	13.343	−0.882	5.626	0	1	0	6.20	6.47	6.55	7.43	7.82	7.81	7.12	7.27	7.28
11A	11.821	−5.649	4.461	0	1	0	6.26	6.30	6.34	7.74	7.76	7.70	7.17	7.29	7.32
11B	13.482	−4.260	5.257	0	1	0	6.42	6.48	6.53	7.92	7.82	7.89	7.51	7.50	7.50
11C	14.603	−3.602	5.624	0	1	0	6.40	6.53	6.53	7.89	7.82	7.74	7.46	7.57	7.58
11D	15.369	−2.404	5.989	0	1	0	6.33	6.53	6.39	7.82	7.79	7.70	7.31	7.57	7.59
12A	11.331	−5.415	4.269	0	1	0	6.44	6.22	6.20	7.72	7.73	7.67	7.19	7.24	7.23
12B	12.992	−4.026	5.065	0	1	0	6.46	6.44	6.47	7.92	7.81	7.87	7.52	7.44	7.44
12C	14.113	−3.368	5.433	0	1	0	6.46	6.51	6.56	7.82	7.82	7.92	7.44	7.50	7.51
12D	14.879	−2.170	5.798	0	1	0	6.46	6.53	6.55	7.74	7.81	7.88	7.34	7.50	7.50
13A	11.581	−5.294	4.276	0	1	0	6.49	6.26	6.23	7.72	7.75	7.69	7.24	7.25	7.25
13B	13.242	−3.905	5.072	0	1	0	6.52	6.46	6.49	8.00	7.82	7.86	7.48	7.44	7.44
13C	14.363	−3.247	5.439	0	1	0	6.51	6.52	6.58	7.85	7.82	7.72	7.48	7.50	7.50
13D	15.129	−2.049	5.804	0	1	0	6.51	6.53	6.57	7.77	7.80	7.89	7.38	7.48	7.48
14A	8.662	−4.981	3.337	0	0	1	6.52	6.60	6.65	8.00	7.83	7.83	7.30	7.41	7.41
14B	10.323	−3.592	4.133	0	0	1	6.69	7.01	6.43	8.30	8.04	7.97	7.77	7.58	7.57
14C	11.444	−2.934	4.500	0	0	1	6.52	7.22	7.27	8.15	8.14	8.20	7.60	7.64	7.64
14D	12.210	−1.736	4.865	0	0	1	6.52	7.33	7.43	8.05	8.18	8.14	7.44	7.61	7.62
15A	9.058	−4.126	3.606	0	0	1	6.48	6.71	6.77	8.10	7.89	7.88	7.22	7.47	7.50
15B	10.719	−2.737	4.426	0	0	1	6.70	7.09	7.17	8.22	8.08	8.12	7.80	7.60	7.59
15C	11.840	−2.079	4.791	0	0	1	6.52	7.28	7.36	8.15	8.16	8.10	7.60	7.63	7.63
15D	12.606	−0.881	5.142	0	0	1	6.52	7.38	7.47	8.10	8.20	8.15	7.46	7.56	7.56
16A	10.324	−5.380	4.037	0	0	1	7.37	7.01	7.03	8.15	8.04	7.98	7.74	7.59	7.57
16B	11.985	−3.991	4.833	0	0	1	7.48	7.30	7.29	8.40	8.17	8.19	7.82	7.79	7.79
16C	13.106	−3.333	5.200	0	0	1	7.39	7.43	7.48	8.40	8.21	8.26	7.72	7.85	7.86
16D	13.872	−2.135	5.565	0	0	1	7.39	7.48	7.53	8.22	8.22	8.31	7.80	7.84	7.84
17A	12.708	−4.529	4.811	1	0	1	7.82	8.26	8.33	8.22	8.64	8.71	7.92	7.80	7.78
17B	14.369	−3.140	5.607	1	0	1	8.00	8.38	8.46	8.70	8.65	8.72	8.05	7.95	7.94
17C	15.490	−3.333	5.974	1	0	1	7.92	8.38	8.36	8.30	8.62	8.68	8.05	8.06	8.06
17D	16.256	−1.284	6.339	1	0	1	7.85	8.36	8.39	8.30	8.58	8.66	8.00	7.94	7.93

18A	13.314	−4.405	1	0	7.82	8.32	8.37	8.30	8.65	8.76	8.00	7.88	7.86
18B	14.975	−3.016	1	0	7.96	8.39	8.33	8.70	8.64	8.73	8.10	8.02	8.02
18C	16.096	−2.358	1	0	7.89	8.36	8.53	8.52	8.59	8.65	8.00	8.06	8.06
18D	16.862	−1.160	1	0	7.82	8.32	8.40	8.40	8.53	8.67	8.00	8.00	8.00
19A	12.907	−3.671	1	0	7.26	6.80	6.95	7.80	7.76	7.79	7.11	7.17	7.18
19B	14.568	−2.282	1	0	7.39	6.90	7.21	8.10	7.77	7.66	7.27	7.27	7.27
19C	15.689	−1.624	1	0	7.34	6.89	7.44	7.96	7.72	7.67	7.20	7.29	7.30
19D	16.455	−0.426	1	0	7.30	6.86	7.52	7.82	7.68	7.73	7.14	7.20	7.20
20A	12.907	−3.671	1	0	7.21	6.80	6.89	7.82	7.76	7.76	7.07	7.17	7.18
20B	14.568	−2.282	1	0	7.42	6.90	7.18	8.05	7.77	7.69	7.26	7.27	7.27
20C	15.689	−1.624	1	0	7.37	6.89	7.46	7.92	7.72	7.65	7.22	7.29	7.29
20D	16.455	−0.426	1	0	7.28	6.86	7.49	7.82	7.68	7.73	7.14	7.20	7.20
21A	10.663	−3.776	0	0	7.28^e	5.63	–	7.72^f	7.24	–	7.16	7.04	7.04
21B	12.324	−2.387	0	0	7.46^e	5.90	–	7.96^f	7.37	–	7.30	7.14	7.13
21C	13.445	−1.729	0	0	7.35^e	6.02	–	7.82^f	7.42	–	7.24	7.16	7.16
21D	14.211	−0.531	0	0	7.33^e	6.06	–	7.80^f	7.43	–	7.21	7.07	7.06
22A	10.200	−3.068	1	0	7.82	6.99	7.48	8.05	8.03	8.08	7.30	7.55	7.57
22B	11.861	−1.679	1	0	8.00	7.29	7.62	8.30	8.16	8.19	7.72	7.62	7.61
22C	12.982	−1.021	1	0	7.96	7.42	7.74	8.22	8.21	8.29	7.52	7.62	7.62
22D	13.748	0.177	1	0	7.89	7.48	7.99	8.10	8.22	8.32	7.42	7.49	7.50
23A	10.109	−3.128	1	0	7.89	6.97	7.51	8.10	8.02	7.97	7.54	7.53	7.53
23B	11.770	−1.739	1	0	7.96	7.27	7.63	8.30	8.16	8.19	7.77	7.60	7.59
23C	12.891	−1.081	1	0	7.96	7.41	7.71	8.15	8.21	8.14	7.57	7.61	7.60
23D	13.657	0.117	1	0	7.92	7.47	7.95	8.10	8.22	8.32	7.41	7.49	7.50
24A	11.186	−2.472	1	0	7.92	7.18	7.60	8.15	8.12	8.17	7.48	7.59	7.60
24B	12.847	−1.083	1	0	8.05	7.41	7.67	8.40	8.21	8.08	7.80	7.62	7.62
24C	13.968	−0.425	1	0	8.00	7.49	7.86	8.30	8.22	8.33	7.60	7.61	7.61
24D	14.734	0.773	1	0	7.96	7.51	8.18	8.15	8.21	8.07	7.52	7.46	7.44
25A	8.958	−3.939	0	0	5.74	5.20	6.02	7.10	6.99	6.96	6.85	6.82	6.79
25B	10.619	−2.550	0	0	5.87	5.59	6.09	7.42	7.18	7.21	7.12	6.94	6.93
25C	11.740	−1.892	0	0	5.77	5.78	6.34	7.29	7.27	7.34	7.01	6.96	6.96
25D	12.506	−0.694	0	0	5.75	5.88	6.52	7.15	7.31	7.28	6.92	6.88	6.88
26A	9.458	−3.572	0	0	5.77	5.33	5.98	7.11	7.05	7.01	6.89	6.85	6.85
26B	11.119	−2.183	0	0	5.96	5.68	6.12	7.47	7.23	7.23	7.16	6.95	6.94
26C	12.240	−1.525	0	0	5.82	5.85	6.43	7.34	7.30	7.36	7.08	6.96	6.96
26D	13.006	−0.327	0	0	5.78	5.94	6.58	7.15	7.33	7.30	6.94	6.87	6.86

[a] Taken from ref 5.
[e] Not used in the derivation of Equation 6.
[f] Not used in the derivation of Equation 7.
[g] Not used in the derivation of Equation 8.

For the charged derivatives (**1A–D to 26A–D**) of these sulfonamides, using the data of variables and the activity as given in Table II the best correlations that could be found were:

hCA I (compounds **1C, D; 2C, D; 4C, D; 21A–D** deleted)

$$\log(1/K_i) = 0.671(\pm 0.530)^1\chi^v - 0.022(\pm 0.022)$$
$$\times (^1\chi^v)^2 + 0.870(+0.389)I$$
$$+ 0.503(\pm 0.265)I_1 + 1.486(\pm 0.242)I_A$$
$$+ 0.972(\pm 3.165)$$

$$n = 94, \quad r = 0.881, \quad r_{cv}^2 = 0.80, \quad s = 0.48,$$
$$F_{5,88} = 61.11(3.22), \quad (^1\chi^v)_{opt} = 15.25 \quad (6)$$

hCA II (same compounds, as in hCA I, deleted)

$$\log(1/K_i) = 0.402(\pm 0.247)^1\chi^v - 0.014(\pm 0.010)$$
$$\times (^1\chi^v)^2 + 0.435(\pm 0.181)I$$
$$+ 0.489(\pm 0.124)I_1 + 0.885(\pm 0.112)I_A$$
$$+ 4.546(\pm 1.473)$$

$$n = 94, \quad r = 0.903, \quad r_{cv}^2 = 0.78, \quad s = 0.22,$$
$$F_{5,88} = 77.87(3.22), \quad (^1\chi^v)_{opt} = 14.36 \quad (7)$$

bCA IV (compounds **1C, D; 2C, D; 4A–D** deleted)

$$\log(1/K_i) = 0.265(\pm 0.058)Pol$$
$$- 0.200(\pm 0.078)\log P - 0.020(\pm 0.014)$$
$$\times (\log P)^2 + 0.252(\pm 0.082)I_1$$
$$+ 0.664(\pm 0.078)I_A + 5.369(\pm 0.352)$$

$$n = 96, \quad r = 0.903, \quad r_{cv}^2 = 0.79, \quad s = 0.16,$$
$$F_{5,.90} = 79.21(3.22) \quad (8)$$

Now it is observed that for the charged compounds, the correlation obtained for the membrane-bound enzyme bCA IV (Equation 8) is quite different from those obtained for the cytosolic enzymes hCA I and hCA II (Equations 6 and 7). On the other hand, the correlations obtained for the cytosolic enzymes are exactly parallel to each other, exhibiting the dependence of inhibitory activity of the molecules in both the cases on the molecular connectivity and some indicator variables that describe the role of some specific characteristics present in the molecule. In both Equations 6 and 7, I, I_1 and I_A stand, with a value of unity each, for the presence in any molecule of an $NHSO_2$ bridge group between two aromatic rings, halogens, and a 5-membered ring, respectively. The positive coefficients of all the three parameters in both the equations indicate that all these three structural features will be favorable to the inhibitory activity of the compounds against both cytosolic isozymes.

For both cytosolic isozymes, the dependence of the inhibitory activity of the compounds on $^1\chi^v$ is shown to be parabolic with an optimum value of $^1\chi^v$ equal to 15.25 for hCA I and 14.36 for hCA II, both values being almost identical. These optimum values are quite high, suggesting that the limit of bulk tolerance of receptors is appreciably good.

For the membrane-bound enzyme, no correlation was found to exist between the activity and $^1\chi^v$, rather the polarizability of the molecule was found to play a good role in the inhibition of this enzyme (Equation 8). And as can be expected from the favorable role of polarizability, the hydrophobicity of molecules is shown to play a negative role. Since no mutual correlation is found to exist between $\log P$ and polarizability (r = -0.204), the molecules can be made highly selective by attaching the sulfonamide molecules to a highly polarizable or highly polarizing entity, and as in the case of cytosolic isozymes, the presence of I_1 and I_A variables in Equation 8, too, with positive coefficients indicate that the presence of halogens and 5-membered rings in the molecule will be favorable to the inhibition of the membrane-bound enzyme.

In deriving Equations 6–8, however, certain compounds as mentioned in the equations themselves were not included. The deletion of these compounds are primarily based on their aberrant behavior in the correlations, but the most notable aspect of this deletion is that the same compounds were found to be worth deleting for both cytosolic enzymes. It is worth noting in Table II that among all the outliers of Equations 6 and 7, compounds **1C, D, 2C, D**, and **4C, D** have much lower activity against both cytosolic enzymes than predicted from the respective equations. The one reason for this aberration can be attributed to the less bulky nature of the sulfonamides (**1, 2**, and **4**) and to their attachment through small amino chains to the trisubstituted-pyridinium-methylcarboxy (TPMC) moeity. TPMC's to which these compounds are attached (TPMC **C** and **D**) have bulky groups (**C**, R = Et; **D**, R = i − Pr) adjacent to nitrogen on both sides, and the sulfonamides with small amino chains, when attached to this nitrogen, may be very close to these groups. This situation may lead to a steric hindrance in the conformation flexibility of the sulfonamide molecules, reducing the scope of their interaction with the receptors.

The outliers **21A–D** are found to possess higher observed activities than predicted ones for both hCA I and hCA II inhibition. This anomaly in these compounds can be attributed to the presence in sulfonamide **21** of an NH bridge group between the two aromatic rings. This NH group might be participating unhindered in hydrogen bond interaction with the receptor, acting as a hydrogen-bond donor (unhindered in the sense that in the SO_2NH-like bridge it may form an intramolecular hydrogen bond with one of the oxygen atoms of the SO_2 moiety, losing the opportunity to interact with the receptor). This unhindered interaction of NH might appreciably increase the activity of the compounds. These compounds (**21A–D)** are not behaving as outliers in the case of membrane-bound enzyme (Equation 8). An obvious reason of this could be that in the membrane-bound enzyme there is no site available to form the hydrogen bond with the NH bridge group of these compounds.

All this analysis has been made using *in vitro* data which shows that a good selectivity can be achieved for the charged compounds to selectively interact with the membrane-bound CA. For this selectivity, the polarizability of the molecules seems to be an important factor. Highly polarized molecules can have not only the strong interaction with this isozyme, but also drastically reduce the lipophilic character of the molecules, which is otherwise detrimental to the inhibition potency of the molecules. We believe that if a remarkable selectivity is achieved with *in vitro* data, it may also bring encouraging results *in vivo*.

From the QSAR studies on several series of CA inhibitors, including some positively charged derivatives, Supuran et al. [8–11] observed that the increase in the CA inhibitory activity of the compounds can be well correlated with the positive charges on the heterocyclic/aromatic rings present in the molecules. In a recent review, it has been discussed that electronic properties of the sulfonamides, particularly of the sulfonamide group in them, is more important than the hydrophobic property [12].

Acknowledgements

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