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# A Comparative QSAR Study on Carbonic Anhydrase and Matrix Metalloproteinase Inhibition by Sulfonylated Amino Acid Hydroxamates

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**A quantitative structure–activity relationship (QSAR) study is made on the inhibition of a few isozymes of carbonic anhydrase (CA) and some matrix metalloproteinases (MMPs), both zinc containing families of enzymes, by sulfonylated amino acid hydroxamates. For both enzymes, the inhibition potency of the hydroxamates is found to be well correlated with Kier's first-order valence molecular connectivity index $^1\chi^v$ of the molecule and electrotopological state indices of some atoms. From the results, it is suggested that while hydroxamate-CA binding may involve mostly polar interactions, hydroxamate-MMP and hydroxamate-ChC (ChC: *Clostridium histolyticum* collagenase, another zinc enzyme related to MMPs) bindings may involve some hydrophobic interactions. Both MMPs and ChC also possess some electronic sites of exactly opposite nature to the corresponding sites in CAs. A group such as $C_6F_5$ present in the sulfonyl moiety is shown to be advantageous in both CA and MMP (also ChC) inhibitions, which is supposed to be due to the interaction of this group with $Zn^{2+}$ ion present in the catalytic site of both families of enzymes.**

*Keywords*: Quantitative structure–activity relationship (QSAR); Carbonic anhydrase inhibition; Matrix metalloproteinase inhibition; *Clostridium histolyticum* collagenase inhibition; Sulfonylated amino acid hydroxamates

## INTRODUCTION

A variety of carbonic anhydrase (CA) isozymes and different matrix metalloproteinases (MMPs) isolated up to now in higher vertebrates play important physiological functions in these organisms. Both have excellent structural similarities possessing very similar metal coordination spheres within their catalytic sites that consists of a $Zn^{2+}$ ion coordinated by three histidines, with the fourth ligand being a water molecule or a hydroxide ion, the latter being the nucleophile intervening in the catalytic cycle of both enzymes (Fig. 1).[1–5] They differ only in the residues with which this fourth ligand interacts. In CAs, it interacts with a threonine residue (Thr 199 in human CA II isozyme) and forms a hydrogen bond with its hydroxyl moiety, which in turn is hydrogen-bonded to the carboxylate moiety of a glutamate residue,[1–3,6] while in MMPs it interacts with the carboxylate moiety of a conserved glutamate residue (Glu 198 in MMP-8), probably forming two hydrogen bonds with it.[3–5] This interaction in both the cases, however, enhances the nucleophilicity of the water molecule/hydroxide ion thus leading to the formation of a very effective nucleophile which attacks the scissile amide bond of the peptide substrate. The principal difference between the enzymatic mechanisms of CAs and MMPs lies in the fact that the nucleophilic adduct formed after the attack of the zinc-bound nucleophile to the substrate is the reaction product in the case of the CAs ($HCO_3^-$ ion), whereas the nucleophilic adduct is only a reaction intermediate in the case of MMPs.[2,3] This difference is of crucial importance for the interaction of these enzymes with their inhibitors. Inhibition of CAs have been well exploited to develop the drugs against a variety of diseases such as glaucoma,[1,7] epilepsy,[8] congestive heart failure,[9] mountain sickness,[10] and gastric and duodenal ulcers,[11] or as diuretic agents.[12] Inhibitions of MMPs on the other

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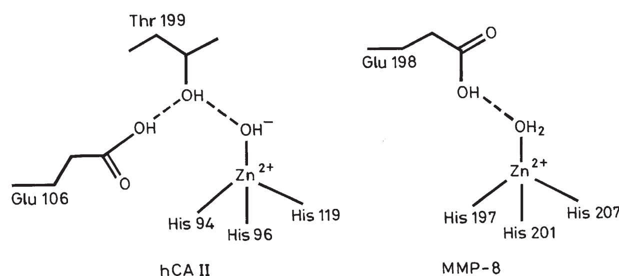
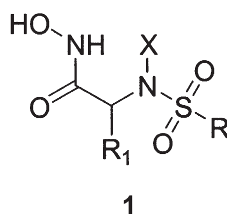



FIGURE 1 Schematic comparative models showing active site coordination of $Zn^{2+}$ ion in a CA (hCA II) molecule and in a MMP (MMP-8) molecule. The non-protein ligand of $Zn^{2+}$ in hCA II molecule may be a hydroxide ion (as shown above) or a water molecule, depending on the pH.[18]

hand have attracted attention for drug design only recently, but several drugs based on MMP inhibition might reach the clinics soon as anticancer or antiarthritis agents among others.[13–15] MMPs are involved in the degradation and remodeling of connective tissues, and, as a family, exhibit proteolytic activity towards virtually all of the constituents of the extracellular matrix.[16,17] Misregulation of these enzymes is believed to be a major factor in a number of disease states characterized by unwanted degradation of connective tissues, including arthritis and tumor invasion and metastasis.[15] Tumor invasion processes and carcinogenesis are related to CAs also. A series of sulfonylated amino acid hydroxamates (**1**) have been recently reported, acting against both CAs and MMPs as well as *Clostridium histolyticum* collagenase (ChC, another zinc enzyme related to MMPs).[18] A quantitative structure–activity relationship (QSAR) study on them may be of interest to explore the structural specificity for the inhibition of the two different but structurally very similar families of enzymes by a common group of inhibitors.




## MATERIALS AND METHOD

The series of sulfonylated amino acid hydroxamates as listed in Table I were reported by Scozzafava and Supuran[18] with their inhibition activities against four MMPs (MMP-1, MMP-2, MMP-8, and MMP-9), three CA isozymes (two human isozymes hCA I and hCA II and one bovine isozyme bCA IV), and type II ChC. The inhibition constant $K_i$ was reported in each case in terms of nanomolar (nM) concentration. For correlation purposes, we have taken log $(1/K_i)$ values as listed in Tables II and III and attempted to correlate them with Kier's first-order valence molecular connectivity index $^1\chi^v$.[19] Since no experimental data for any physiochemical properties of these compounds are available, some theoretical parameters have to be depended on. Though octanol–water partition coeffcient (log P) can be theoretically obtained,[20] it is not always fully reliable. The molecular connectivity index $^1\chi^v$ signifies the degree of branching, connectivity of atoms, and the unsaturation in the molecule. It is calculated according to the equation:

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

where $\delta_i^v$ and $\delta_j^v$ are the vertex connectivity indices of atoms i and j, respectively, and the summation extends to all bonded pairs of nonhydrogenic atoms in the group or molecule. For second and third rows of atoms, a unified definition of $\delta^v$, as expressed by Equation (2), was given.[21] In this equation, $Z_i^v$ is the number of valence electrons of atom i, $h_i$ is the number of hydrogen atoms attached to it, and $Z_i$ is its atomic number.

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

To account for any electronic effects of atoms/substituents, we have also calculated the electrotopological state (E-state) index (S) of atoms.[22] To calculate $S_i$ of an atom i, we first define the intrinsic state of that atom, $I_i$, as

$$I_i = (\delta_i^v + 1)/\delta_i \tag{3}$$

where $\delta_i$ is the σ electron count on atom i. Then a factor $\Delta I_i$ is defined as

$$\Delta I_i = \Sigma_{j=1}(I_i - I_j)/n^2 \tag{4}$$

where n refers to the number of atoms in the path i to j including both i and j.[22] $I_i$ and $\Delta I_i$ are then used to find the value of $S_i$ according to the equation:

$$S_i = I_i + \Delta I_i \tag{5}$$

Using these procedures, the $^1\chi^v$ values for the molecules and the S values for some relevant atoms were calculated and are listed in Table I.

## RESULTS AND DISCUSSION

When a multiple regression analysis was performed on the activity data of Tables II and III, excellent correlations were obtained between the activity and $^1\chi^v$ of the molecules and the E-state indices of some atoms for both MMPs and CAs as well as ChC as shown below.

## Matrix Metalloproteinases

### *MMP-1:*

$$\log(1/K_i) = 0.194(\pm 0.082)^1\chi^v + 0.423(\pm 0.144)S_S - 0.862(\pm 0.242)S_N + 0.75(\pm 0.142)I + 8.859(\pm 1.614)$$

$$n = 31, \quad r = 0.945, \quad R^2 = 0.876, \quad s = 0.17, \quad F_{4,26} = 53.78(4.14) \qquad (6)$$

### *MMP-2:*

$$\log(1/K_i) = 0.204(\pm 0.080)^1\chi^v + 0.198(\pm 0.127)S_S - 0.682(\pm 0.211)S_N + 0.616(\pm 0.144)I + 7.938(\pm 1.563)$$

$$n = 31, \; r = 0.964, \; R^2 = 0.921, \; s = 0.21, \; F_{4,34} = 111(3.93) \qquad (7)$$

### *MMP-8:*

$$\log(1/K_i) = 0.244(\pm 0.118)^1\chi^v + 0.264(\pm 0.194)S_S - 0.757(\pm 0.320)S_N + 0.667(\pm 0.219)I + 7.933(\pm 2.366)$$

$$n = 37, \quad r = 0.940, \quad R^2 = 0.869, \quad s = 0.31, \quad F_{4,32} = 60.20(3.97) \qquad (8)$$

### *MMP-9:*

$$\log(1/K_i) = 0.283(\pm 0.106)^1\chi^v + 0.433(\pm 0.143)S_N + 0.683(\pm 0.240)I + 5.510(\pm 0.951)$$

$$n = 37, \quad r = 0.920, \quad R^2 = 0.832, \quad s = 0.34, \quad F_{3,33} = 60.70(4.44) \qquad (9)$$

TABLE I A series of sulfonylated amino acid hydroxamates (**1**) with molecular connectivity and E-state indices. I is an indicator parameter used with a value of unity for compounds having R = $C_6F_5$

| No | $R_1$ | R | X | $^1\chi^v$ | I | $S_S$ | $S_N$ |
|---|---|---|---|---|---|---|---|
| 1 | H | $n$-$C_4F_9$ | H | 5.76 | 0.0 | −8.354 | 0.474 |
| 2 | H | $C_6F_5$ | H | 5.99 | 1.0 | −6.660 | 1.284 |
| 3 | H | 4-MeO−$C_6H_4$ | H | 5.97 | 0.0 | −5.127 | 2.147 |
| 4 | H | $n$-$C_4F_9$ | $C_6H_5CH_2$ | 8.44 | 0.0 | −8.675 | −0.646 |
| 5 | H | $C_6F_5$ | $C_6H_5CH_2$ | 8.47 | 1.0 | −6.980 | 0.281 |
| 6 | H | 4-MeO−$C_6H_4$ | $C_6H_5CH_2$ | 8.45 | 0.0 | −5.448 | 1.118 |
| 7 | Me | $n$-$C_4F_9$ | H | 6.39 | 0.0 | −8.434 | 0.572 |
| 8 | Me | $C_6F_5$ | H | 6.42 | 1.0 | −6.740 | 1.382 |
| 9 | Me | 4-MeO−$C_6H_4$ | H | 6.40 | 0.0 | −5.207 | 2.245 |
| 10 | Me | $n$-$C_4F_9$ | $C_6H_5CH_2$ | 8.94 | 0.0 | −8.755 | −0.604 |
| 11 | Me | $C_6F_5$ | $C_6H_5CH_2$ | 8.97 | 1.0 | −7.060 | 0.323 |
| 12 | Me | 4-MeO−$C_6H_4$ | $C_6H_5CH_2$ | 8.95 | 0.0 | −5.528 | 1.161 |
| 13 | $i$-Pr | $n$-$C_4F_9$ | H | 7.29 | 0.0 | −8.519 | 0.709 |
| 14 | $i$-Pr | $C_6F_5$ | H | 7.32 | 1.0 | −6.824 | 1.519 |
| 15 | $i$-Pr | 4-MeO−$C_6H_4$ | H | 7.30 | 0.0 | −5.292 | 2.382 |
| 16 | $i$-Pr | $n$-$C_4F_9$ | $C_6H_5CH_2$ | 9.84 | 0.0 | −8.960 | −0.530 |
| 17 | $i$-Pr | $C_6F_5$ | $C_6H_5CH_2$ | 9.87 | 1.0 | −7.145 | 0.398 |
| 18 | $i$-Pr | 4-MeO−$C_6H_4$ | $C_6H_5CH_2$ | 9.75 | 0.0 | −5.613 | 1.235 |
| 19 | $i$-Bu | $n$-$C_4F_9$ | H | 7.76 | 0.0 | −8.527 | 0.740 |
| 20 | $i$-Bu | $C_6F_5$ | H | 7.79 | 1.0 | −6.833 | 1.551 |
| 21 | $i$-Bu | 4-MeO−$C_6H_4$ | H | 7.77 | 0.0 | −5.300 | 2.414 |
| 22 | $i$-Bu | $n$-$C_4F_9$ | $C_6H_5CH_2$ | 10.21 | 0.0 | −8.848 | −0.506 |
| 23 | $i$-Bu | $C_6F_5$ | $C_6H_5CH_2$ | 10.24 | 1.0 | −7.154 | 0.421 |
| 24 | $i$-Bu | 4-MeO−$C_6H_4$ | $C_6H_5CH_2$ | 10.32 | 0.0 | −5.621 | 1.259 |
| 25 | H | $n$-$C_4F_9$ | 2-$O_2NC_6H_4CH_2$ | 8.94 | 0.0 | −9.002 | −0.944 |
| 26 | H | $C_6F_5$ | 2-$O_2NC_6H_4CH_2$ | 8.97 | 1.0 | −7.308 | −0.016 |
| 27 | H | 4-MeO−$C_6H_4$ | 2-$O_2NC_6H_4CH_2$ | 8.95 | 0.0 | −5.775 | 0.821 |
| 28 | H | $n$-$C_4F_9$ | 4-$O_2NC_6H_4CH_2$ | 8.92 | 0.0 | −8.855 | −0.794 |
| 29 | H | $C_6F_5$ | 4-$O_2NC_6H_4CH_2$ | 8.95 | 1.0 | −7.161 | 0.134 |
| 30 | H | 4-MeO−$C_6H_4$ | 4-$O_2NC_6H_4CH_2$ | 8.93 | 0.0 | −5.628 | 0.972 |
| 31 | Me | $n$-$C_4F_9$ | 2-$O_2NC_6H_4CH_2$ | 9.51 | 0.0 | −9.082 | −0.901 |
| 32 | Me | $C_6F_5$ | 2-$O_2NC_6H_4CH_2$ | 9.54 | 1.0 | −7.387 | 0.026 |
| 33 | Me | 4-MeO−$C_6H_4$ | 2-$O_2NC_6H_4CH_2$ | 9.52 | 0.0 | −5.855 | 0.864 |
| 34 | Me | $n$-$C_4F_9$ | 4-$O_2NC_6H_4CH_2$ | 9.49 | 0.0 | −8.935 | −0.751 |
| 35 | Me | $C_6F_5$ | 4-$O_2NC_6H_4CH_2$ | 9.52 | 1.0 | −7.241 | 0.176 |
| 36 | Me | 4-MeO−$C_6H_4$ | 4-$O_2NC_6H_4CH_2$ | 9.50 | 0.0 | −5.709 | 1.014 |
| 37 | Me | $n$-$C_4F_9$ | 2-$ClC_6H_4CH_2$ | 9.49 | 0.0 | −8.779 | −0.574 |
| 38 | Me | $C_6F_5$ | 2-$ClC_6H_4CH_2$ | 10.08 | 1.0 | −7.085 | 0.353 |
| 39 | Me | 4-MeO−$C_6H_4$ | 2-$ClC_6H_4CH_2$ | 9.50 | 0.0 | −5.552 | 1.191 |

## Carbonic Anhydrases

### *hCA I:*

$$\log(1/K_i) = -0.177(\pm0.060)^1\chi^v - 0.335(\pm0.103)S_S + 0.513(\pm0.161)S_N + 0.427(\pm0.121)I + 5.755(\pm1.181)$$
$$n = 23,\ r = 0.969,\ R^2 = 0.925,\ s = 0.13,\ F_{4,19} = 68.89(4.50) \quad (10)$$

### *hCA II:*

$$\log(1/K_i) = -0.089(\pm0.087)^1\chi^v - 0.353(\pm0.128)S_S + 0.597(\pm0.209)S_N + 0.305(\pm0.159)I + 5.065(\pm1.561)$$
$$n = 28,\ r = 0.920,\ R^2 = 0.820,\ s = 0.19,\ F_{4,23} = 31.55(4.26) \quad (11)$$

### *bCA IV:*

$$\log(1/K_i) = -0.158(\pm0.038)^1\chi^v - 0.231(\pm0.062)S_S + 0.338(\pm0.101)S_N + 0.364(0.076)I + 6.580(\pm0.742)$$
$$n = 28,\ r = 0.978,\ R^2 = 0.949,\ s = 0.09,\ F_{4,23} = 124.24(4.26) \quad (12)$$

### *C. Histolyticum* collagenase (ChC)

$$\log(1/K_i) = 0.156(\pm0.025)^1\chi^v + 0.141(\pm0.040)S_S - 0.396(\pm0.066)S_N + 0.396(\pm0.045)I + 7.478(\pm0.491)$$
$$n = 39,\ r = 0.991,\ R^2 = 0.980,\ s = 0.07,\ F_{4,34} = 447.28(3.93) \quad (13)$$

TABLE II Hydroxamates and their observed and calculated inhibition potencies against matrix metalloproteinases (MMPs)

| | $\log(1/K_i)$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MMP-1 | | MMP-2 | | MMP-8 | | MMP-9 | |
| No | Obsd. | Calcd. Eq (6) | Obsd. | Calcd. Eq (7) | Obsd. | Calcd. Eq (8) | Obsd. | Calcd. Eq (9) |
| 1 | – | – | 7.12 | 7.14 | 6.88 | 6.78 | 6.90 | 6.93 |
| 2 | 6.83 | 6.85 | 7.35 | 7.58 | 6.90 | 7.33 | 7.00 | 7.33 |
| 3 | – | – | 6.95 | 6.68 | 6.80 | 6.41 | 6.84 | 6.27 |
| 4 | 7.52 | 7.39 | 8.40 | 8.39 | 8.27 | 8.19 | 8.30 | 8.18 |
| 5 | 8.15 | 8.06 | 8.82 | 8.71 | 8.95 | 8.61 | 8.92 | 8.47 |
| 6 | 7.22 | 7.23 | 7.74 | 7.82 | 7.50 | 7.71 | 7.37 | 7.41 |
| 7 | – | – | 7.16 | 7.18 | 6.92 | 6.84 | 6.91 | 7.07 |
| 8 | 6.82 | 6.82 | 7.39 | 7.59 | 6.93 | 7.34 | 7.01 | 7.41 |
| 9 | – | – | 7.06 | 6.68 | 6.90 | 6.42 | 6.86 | 6.35 |
| 10 | 7.58 | 7.41 | 8.49 | 8.44 | 8.30 | 8.26 | 8.36 | 8.30 |
| 11 | 8.15 | 8.09 | 9.04 | 8.77 | 8.95 | 8.68 | 8.85 | 8.59 |
| 12 | 7.23 | 7.26 | 7.82 | 7.87 | 7.72 | 7.78 | 7.45 | 7.54 |
| 13 | – | – | 7.16 | 7.26 | 6.95 | 6.93 | 6.92 | 7.26 |
| 14 | 6.85 | 6.84 | 7.38 | 7.66 | 6.98 | 7.44 | 7.05 | 7.60 |
| 15 | – | – | 7.08 | 7.76 | 6.89 | 6.52 | 6.89 | 6.54 |
| 16 | 7.67 | 7.44 | 8.62 | 8.53 | 8.37 | 8.37 | 8.36 | 8.52 |
| 17 | 8.15 | 8.16 | 9.09 | 8.88 | 9.00 | 8.82 | 8.92 | 8.81 |
| 18 | 7.36 | 7.31 | 7.96 | 7.98 | 7.88 | 7.90 | 7.56 | 7.73 |
| 19 | – | – | 7.20 | 7.33 | 6.96 | 7.02 | 7.44 | 7.38 |
| 20 | 6.80 | 6.90 | 7.40 | 7.73 | 6.99 | 7.52 | 6.91 | 7.72 |
| 21 | – | – | 7.07 | 6.83 | 6.91 | 6.60 | 7.10 | 6.66 |
| 22 | 7.79 | 7.54 | 8.72 | 8.62 | 8.48 | 8.47 | 8.39 | 8.62 |
| 23 | 7.22 | 7.21 | 9.09 | 8.94 | 9.22 | 8.89 | 8.95 | 8.91 |
| 24 | 7.35 | 7.40 | 8.00 | 8.07 | 8.04 | 8.02 | – | – |
| 25 | 7.60 | 7.60 | 8.43 | 8.63 | 8.25 | 8.46 | 8.33 | 8.45 |
| 26 | 8.22 | 8.28 | 8.85 | 8.95 | 9.00 | 8.87 | 8.88 | 8.74 |
| 27 | 7.26 | 7.45 | 7.82 | 8.06 | 7.56 | 7.97 | 7.40 | 7.69 |
| 28 | 7.20 | 7.53 | 8.82 | 8.55 | 8.62 | 8.37 | 8.69 | 7.38 |
| 29 | 8.52 | 8.21 | 9.15 | 8.87 | 10.00 | – | 9.22 | 8.67 |
| 30 | 7.55 | 7.38 | 7.74 | 7.99 | 9.68 | – | 7.50 | 7.61 |
| 31 | 7.61 | 7.64 | 8.54 | 8.70 | 8.29 | 8.54 | 8.35 | 8.59 |
| 32 | 8.15 | 8.32 | 9.09 | 9.02 | 8.95 | 8.96 | 9.00 | 8.88 |
| 33 | 7.40 | 7.48 | 7.88 | 8.13 | 7.69 | 8.06 | 7.61 | 7.83 |
| 34 | 7.22 | 7.57 | 8.85 | 8.62 | 8.63 | 8.46 | 8.82 | 8.52 |
| 35 | 8.39 | 8.25 | 9.15 | 8.94 | 9.52 | 8.88 | 9.22 | 8.81 |
| 36 | 7.60 | 7.42 | 7.82 | 8.06 | 7.74 | 7.98 | 7.55 | 7.76 |
| 37 | 7.43 | 7.48 | 8.43 | 8.53 | 8.19 | 8.37 | 8.30 | 8.44 |
| 38 | 8.00 | 8.27 | 8.82 | 8.97 | 8.89 | 8.92 | – | – |
| 39 | 7.27 | 7.33 | 7.92 | 7.97 | 7.56 | 7.89 | 7.50 | 7.68 |

TABLE III Hydroxamates and their observed and calculated inhibition potencies against isozymes of carbonic anhydrase (hCA I, hCA II, and bCA IV) and *Clostridium histolyticum* collagenase (ChC).

| | Log (1/$K_i$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ChC | | hCA I | | hCA II | | bCA IV | |
| No | Obsd. | Calcd. Eq (13) | Obsd. | Calcd. Eq (10) | Obsd. | Calcd. Eq (11) | Obsd. | Calcd. Eq (12) |
| 1 | 7.09 | 7.01 | 7.74 | 7.76 | 7.82 | 7.78 | 7.79 | 7.76 |
| 2 | 7.26 | 7.36 | 8.15 | 8.01 | 8.09 | 7.95 | 8.00 | 7.97 |
| 3 | 6.92 | 6.84 | 7.52 | 7.52 | 7.49 | 7.62 | 7.53 | 7.55 |
| 4 | 7.88 | 7.83 | 6.97 | 6.84 | 7.07 | 6.99 | 7.00 | 7.03 |
| 5 | 8.22 | 8.10 | 7.04 | 7.16 | 7.44 | 7.24 | 7.37 | 7.31 |
| 6 | 7.56 | 7.59 | – | – | 6.92 | 6.90 | 6.83 | 6.88 |
| 7 | 7.10 | 7.06 | 7.67 | 7.74 | 7.79 | 7.81 | 7.76 | 7.71 |
| 8 | 7.34 | 7.38 | 8.15 | 8.01 | 8.09 | 8.00 | 8.00 | 7.96 |
| 9 | 6.88 | 6.86 | 7.49 | 7.52 | 7.45 | 7.67 | 7.52 | 7.53 |
| 10 | 7.92 | 7.88 | 6.91 | 6.79 | 7.03 | 7.00 | 6.97 | 6.99 |
| 11 | 8.22 | 8.15 | 7.07 | 7.13 | 7.42 | 7.25 | 7.36 | 7.27 |
| 12 | 7.69 | 7.64 | 6.70 | 6.62 | 6.92 | 6.91 | 6.86 | 6.84 |
| 13 | 7.10 | 7.14 | 7.53 | 7.68 | 7.82 | 7.84 | 7.69 | 7.64 |
| 14 | 7.39 | 7.45 | 8.09 | 7.95 | 7.95 | 8.03 | 7.88 | 7.88 |
| 15 | 6.98 | 6.93 | 7.48 | 7.46 | 7.40 | 7.70 | 7.42 | 7.46 |
| 16 | 8.00 | 7.96 | 6.85 | 6.74 | 6.96 | 7.03 | 6.90 | 6.92 |
| 17 | 8.30 | 8.25 | 7.05 | 7.03 | 7.34 | 7.25 | 7.31 | 7.17 |
| 18 | 7.76 | 7.72 | – | – | – | – | 6.73 | 6.76 |
| 19 | 7.16 | 7.20 | 7.44 | 7.62 | 7.74 | 7.82 | 7.52 | 7.58 |
| 20 | 7.42 | 7.51 | 8.00 | 7.89 | 7.95 | 8.01 | 7.72 | 7.82 |
| 21 | 7.02 | 6.99 | 7.30 | 7.39 | 8.30 | 7.62 | 7.40 | 7.39 |
| 22 | 8.09 | 8.03 | 6.74 | 6.65 | 6.93 | 6.97 | 6.85 | 6.84 |
| 23 | 8.30 | 8.30 | 7.00 | 6.98 | 7.25 | 7.23 | 7.12 | 7.12 |
| 24 | 7.88 | 7.80 | – | – | – | – | 6.72 | 6.68 |
| 25 | 7.88 | 7.98 | – | – | 7.00 | 6.88 | 6.92 | 6.93 |
| 26 | 8.22 | 8.25 | 6.89 | 7.03 | 7.13 | 7.14 | 7.30 | 7.21 |
| 27 | 7.61 | 7.74 | – | – | 6.76 | 6.79 | 6.85 | 6.78 |
| 28 | 7.92 | 7.94 | – | – | 6.92 | 6.92 | – | – |
| 29 | 8.30 | 8.21 | 6.82 | 7.07 | 6.97 | 7.18 | 6.88 | 7.23 |
| 30 | 7.69 | 7.70 | – | – | – | – | – | – |
| 31 | 8.00 | 8.04 | – | – | – | – | – | – |
| 32 | 8.30 | 8.31 | – | – | 6.79 | 7.14 | – | – |
| 33 | 7.72 | 7.80 | – | – | – | – | – | – |
| 34 | 7.95 | 8.00 | – | – | – | – | – | – |
| 35 | 8.30 | 8.27 | – | – | – | – | – | – |
| 36 | 7.67 | 7.76 | – | – | – | – | – | – |
| 37 | 7.95 | 7.95 | – | – | – | – | – | – |
| 38 | 8.30 | 8.31 | – | – | – | – | – | – |
| 39 | 7.65 | 7.71 | – | – | – | – | – | – |

In these equations, n is the number of data points, r is the correlation coefficient, $R^2$ is the adjusted squared value of r, also called explained variance (EV), s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities, and the data within parentheses with ± sign are 95% confidence intervals. The figure within parentheses following the F-value in each equation is the theoretical F-value of 99% level. The $R^2$ (or EV) value, that is calculated as $R^2 = r^2(1 - 1/F)$, is to account, in percentage when multiplied by 100, for the variance in the activity.

All correlations expressed by Equations (6)–(13) exhibit the excellent correlations between $^1\chi^v$ of the molecule and $S_S$ and $S_N$, the E-state indices of sulfur and adjacent nitrogen in the molecule (**1**), respectively. The additional parameter I in each equation is a dummy parameter that has been used for the R-substituent in the molecule. It is equal to 1 for $R = C_6F_5$ and zero for others.

Now the correlations obtained for MMPs [Equations (6)–(9)] and ChC [Equation (13)], which also belongs to the MMP family, exhibit the positive dependence of activity on $^1\chi^v$. The value of $^1\chi^v$ depends, as suggested by Equation (1), on $\delta^v$. The $^1\chi^v$ will increase if $\delta^v$ decreases and Equation (2) suggests that $\delta^v$ will decrease when there is a decrease in the valence electrons of the atom and/or increase in the number of hydrogen atoms attached to it. Thus, $^1\chi^v$ value will be higher for a group or molecule which has less electronegative and more saturated atoms. Such a group or molecule will be less polar in nature. Thus, the positive dependence of MMP and ChC inhibitions on $^1\chi^v$ suggests that less polar molecules will have better activity. Less polar molecules probably might have some hydrophobic

interactions with the enzymes. The negative dependence on $^1\chi^v$ of CA inhibitions suggests that polar compounds would be beneficial to the inhibition of CAs.

In each correlation [Equations (6)–(13)], the coefficient of I is positive, indicating that in each case the $C_6F_5$ group attached to the sulfur atom will be conducive to the inhibition potency of the compounds. This group may have a polar interaction with the $Zn^{2+}$ ion of the enzymes. This interaction, however, seems to be slightly more effective in the inhibition of MMPs than in the inhibition of CAs and ChC, as the coefficients of I in the case of the former [Equations (6)–(9)] are slightly higher than in the case of the latter two [Equations (10)–(13)].

For the inhibition of both CAs and MMPs, the inhibitors are first ionized (as anions) and then coordinated to $Zn^{2+}$ ion with or without replacing the metal bound water molecule or hydroxyl ion.[1–3,13]

Our QSAR study suggests that hydroxamate-like CA and MMP inhibitors may also have polar or hydrophophic interactions with certain polar and hydrophobic sites available within the enzyme molecules. The polar interactions may dominate in CA inhibitions and the hydrophobic ones in MMP and ChC. In the inhibition of both CAs and MMPs as well as ChC by hydroxamates (**1**), it is however pointed out that a group like $C_6F_5$ attached to the sulfur atom ($R = C_6F_5$) will have an added effect. This group can be assumed to strongly bind with the $Zn^{2+}$ ion through charge–charge interactions, involving at least two of its fluorine atoms (Fig. 2). The better effect of this group in the inhibition of MMPs than in the inhibitions of CAs and ChC may be assumed due to its better orientation towards the $Zn^{2+}$ ion in MMPs than in CAs and ChC.

E-state index of atoms is a measure of the availability of π and lone pair electrons at the atoms. The more electronegative atoms or groups have a richer content of π and lone pair electrons, giving rise to a higher calculated value of S. The occurrence of $S_S$ and $S_N$ in the correlations indicate that sulfur and the adjacent nitrogen atom play some electronic roles in the binding of the hydroxamates with both MMPs and CAs and as well as ChC. However, in the case of all MMPs, except MMP-9 where $S_S$ does not appear, and ChC, the coefficient of $S_S$ is positive and that of $S_N$ negative (Equations (6)–(8) and (13)). The $S_N$ with negative sign is present even in the case of MMP-9. The reverse is the case for all CAs (Equations (10)–(12)). This shows a major difference in the electronic interactions of hydroxamates with MMPs (or ChC) and CAs, where sulfur and nitrogen play exactly opposite roles in the two systems.

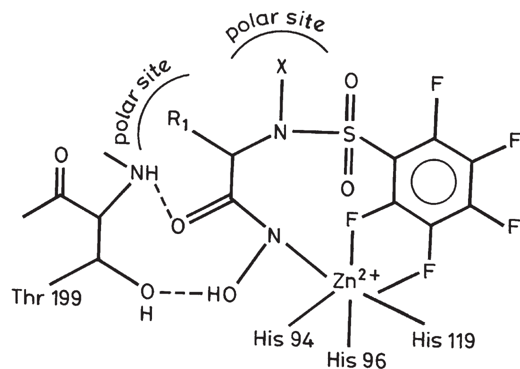



FIGURE 2 A hypothetical model for the binding of a sulfonylated amino acid hydroxamate with a CA molecule (hCA II). The polar sites shown here may be hydrophobic sites in an MMP molecule.

Thus the above QSAR analysis indicates that MMPs and ChC appreciably differ from CAs with regard to the nature of their active sites. Both families of enzymes appear to have electronic sites but of opposite nature. Further, CAs seem to provide more opportunity for polar interactions than MMPs and ChC *vis a vis* the dominance of hydrophobic interactions in MMPs and ChC.

All of our correlations are statistically highly significant. For all the enzymes, the calculated values of the inhibition activities of the compounds are found to match highly with the observed ones. Further, except in the case of MMP-8, in no other case were any outliers found. For MMP-8, Equation (8) was derived excluding only two compounds 29 and 30 (Table II). The aberrant behaviour of these two compounds, however, is not apparent.

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### References

[1] Smith, K.S., Jakubzick, C., Whittam, T.S. and Ferry, J.G. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96**, 15184–15189.
[2] Coleman, J.E. (1998) *Curr. Opin. Chem. Biol.* **2**, 222–234.
[3] Supuran, C.T. and Scozzafava, A. (2002) In: Smith, H.J. and Simons, C., eds, *Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development* (Taylor and Francis, London & New York), pp 35–61.
[4] Grams, F., Crimmin, M., Hinnes, L., Huxley, P., Piper, M., Tschesche, H. and Bode, W. (1995) *Biochemistry* **34**, 14012–14020.
[5] Lovejoy, B., Hassel, A.M., Luther, M.A., Weigl, D. and Jordon, S.R. (1994) *Biochemistry* **33**, 8207–8217.
[6] Briganti, F., Mangani, S., Scozzafava, A., Vernaglione, G. and Supuran, C.T. (1999) *J. Biol. Inorg. Chem.* **4**, 528–536.
[7] Scozzafava, A., Menabuoni, L., Mincione, F., Briganti, F., Mincione, G. and Supuran, C.T. (1999) *J. Med. Chem.* **42**, 2641–2650, references therein.
[8] Chufan, E.E., Pedregosa, J.C., Baldini, O.N. and Bruno-Blanch, L. (1999) *Farmaco* **54**, 838–886.
[9] Maren, T.H. (1991) In: Botré, F., Gross, G. and Storey, B.T., eds, *Carbonic Anhydrase—From Biochemistry and Genetics to Physiology and Clinical Medicine* (VCH, Veinheim), pp 186–207.

[10] Bernhard, W.N., Schalik, L.M., Delaney, P.A., Bernhard, T.M. and Barnas, G.M. (1998) *Aviat. Space Environ. Med.* **69**, 883–886.
[11] Puscas, I. and Supuran, C.T. (1996) In: Coelho, J., ed, *Aparelho Digestivo* (MEDSI, Rio de Janeiro), pp 1704–1734.
[12] Jackson, E.K. (1996) In: Hardman, J.G., Lumbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G., eds, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, $9^{th}$ed. (McGraw-Hill, New York), pp 685–713.
[13] Whittaker, M., Floyd, C.D., Brown, P. and Gearing, A.J. (1999) *Chem. Rev.* **99**, 2735–2776.
[14] Leung, D., Abbenante, G. and Fairlie, D. (2000) *J. Med. Chem.* **43**, 305–341.
[15] Babine, R.E. and Bender, S.L. (1997) *Chem. Rev.* **97**, 1359–1472.
[16] Woessner, Jr., J.F. (1991) *FASEB J.* **5**, 2145–2154.
[17] Hagmann, W.K., Lark, M.W. and Becker, J.W. (1996) *Annu. Rep. Med.* **31**, 231–240.
[18] Scozzafava, A. and Supuran, C.T. (2000) *J. Med. Chem.* **43**, 3677–3687.
[19] Kier, L.B. and Hall, L.H. (1976) *Molecular Connectivity in Chemistry and Drug Research* (Academic press, New York).
[20] Hansch, C. and Leo, A. (1979) *Substituted Constants for Correlation Analysis in Chemistry and Biology* (John Wiley, New York).
[21] Kier, L.B. and Hall, L.H. (1983) *J. Pharm. Sci.* **72**, 1170–1173.
[22] Kier, L.B. and Hall, L.H. (1999) *Molecular Structure Descriptors: Electrotopological State Indices* (Academic Press, San Diego).

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