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A Quantitative Structure–Activity Relationship Study on Some Matrix Metalloproteinase and Collagenase Inhibitors

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Abstract—A quantitative structure–activity relationship (QSAR) study is made on some hydroxamic acid-based inhibitors of matrix metalloproteinases (MMPs) and a bacterial collagenase, namely *Clostridium histolyticum* collagenase (ChC), that also belongs to an MMP family, M-31, using Kier's valence molecular connectivity index $^1\chi^v$ of the substituents and electrotopological state (E-state) indices of some atoms. The results indicate that out of the four MMPs (MMP-1, MMP-2, MMP-8, and MMP-9) studied, MMP-2 and MMP-9 can be structurally quite similar, but widely differing from MMP-1 and MMP-8 and ChC. For MMP-2 and MMP-9, the inhibition activity of compounds is shown to depend on both $^1\chi^v$ and E-state indices, while for MMP-1 and MMP-8 it is shown to depend only on E-state indices and for ChC only on $^1\chi^v$. However, in all the cases, an aromatic group like C_6F_5 or 3-CF_3–C_6H_4 attached to SO_2 moiety in the compounds is indicated to be equally beneficial, due to probably the involvement of fluorine atom(s) in charge–charge interactions with the Zn^{2+} ion of the enzymes or in the formation of the hydrogen bonds with some sites of the receptors.

Introduction

Recently, in the search of novel types of anticancer, antiarthritis, and other pharmalogical agents useful in the management of inflammatory processes, the matrix metalloproteinases (MMPs) have been found to be interesting targets.[1–5] The MMPs are a family of structurally related zinc metalloproteinases that degrade and remodel structural proteins in the extracellular matrix (ECM), such as membrane collagens, aggrecan, fibronectin, and liminin.[3,6] They have been implicated in tissue remodeling at various stages of human development, wound healing, and disease. Their enhanced activity results in tissue degradation, leading to a wide array of disease processes including cancer[7–9] and osteo-[10,11] and rheumatoid arthritis.[12–14] Multiple sclerosis[15–17] and congestive heart failure[18–20] are also associated with the overexpression and activation of these enzymes. It is thus possible to envisage many clinical applications by inhibiting the activity of these enzymes and, of course, several hydroxamic acid-based inhibitors of these enzymes, such as batimastat (**1**), marimastat (**2**), trocade (**3**), and AG3340 (**4**), are currently in advanced clinical trials as antimetastasis, anticancer, antiarthritis drugs, or as agents for the control of recurrant corneal growth (batimastat).[1–4,21,22] Like MMPs, there are other enzymes also, such as bacterial collagenases, that degrade ECM. A bacterial collagenase, isolated from *Clostridium histolyticum*, belongs to M-31 metalloproteinase family,[23,24] which is able to hydrolyze triple helical regions of collagen under physiological conditions, as well as an entire range of synthetic-peptide substrate. In fact, the crude homogenate of *C. histolyticum*, which contains several distinct collagenase isozymes,[23,24] is the most efficient system known for the degradation of connective tissue, being also involved in the pathogenicity of this and related clostridia, such as *C. perfringens*, which causes human gas gangrene and food poisoning among others.[25]

Like MMPs, the *C. histolyticum* collagenase (abbreviated as ChC) is also a multiunit protein. Both MMPs and ChC are considered to have similar mechanism of action for the hydrolysis of proteins and synthetic substrates,[23–27] though they are relatively different. It is thus of great interest to find the inhibitors that may inhibit both types of enzymes and may have a wide range of medicinal applications. Scozzafava and

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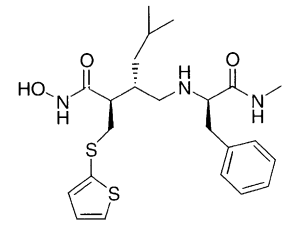
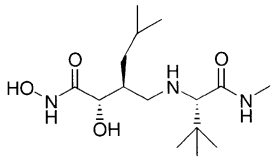


1

Batimastat



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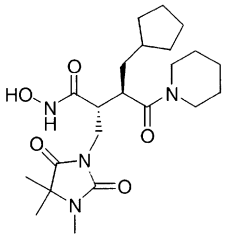
Marimastat



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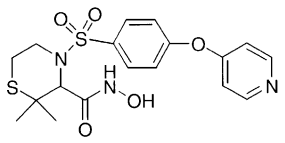
Trocade



4

AG3340

Supuran[28] recently reported some hydroxamic acid-based inhibitors that inhibited both MMPs and ChC. For progression of design and development of such inhibitors, a quantitative structure–activity relationship (QSAR) study has been made by us on some of these inhibitors. QSAR studies provide deeper insight into the mechanism of action of compounds, that ultimately becomes of great importance in the modification of the structure of the compounds, and predict the activity of the compounds even before their synthesis.

Materials and Methods

The QSAR study has been made on the compounds reported by Scozzafava and Supuran.[28] These compounds are listed in Table 1. The enzyme inhibition constants K_i of these compounds for different enzymes are given in Table 2. These K_i values were obtained by Scozzafava and Supuran from Easson–Stedman plots,[29] using a linear regression programme and using at least three different assays.

All the K_i values of the compounds were, in fact, reported in terms of nanomolar (nM) concentration. For correlation purposes, we have taken $\log(1/K_i)$ values and attempted to correlate them with Kier's first-order valence molecular connectivity index $^1\chi^v$.[30] Since no experimental data for any physiochemical properties of these compounds are available, one has to depend on some theoretical parameters. Though octanol–water partition coefficient (log P) can be theoretically obtained,[31] it is not always fully reliable. On the other hand, $^1\chi^v$ has been found in a number of cases to be significantly correlated with the hydrophobicity (log P) of the molecules.[30] It signifies the degree of branching, connectivity of atoms, and the unsaturation in the molecule. It is calculated according to the equation:

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

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 nonhydrogenic atoms in the group or molecule. For second and third rows of atoms, a unified definition of δ^v, as expressed by eq 2, was given.[32] 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.

$$\delta_i^v = \left(Z_i^v - h_i\right)/\left(Z_i - Z_i^v - 1\right) \quad (2)$$

To account for any electronic effects of atoms/substituents, we have also calculated the electrotopogical state (E-state) indices (S) of some atoms.[33,34] To calculate S_i of an atom i, we first define the intrinsic state of that atom, I_i, as

$$I_i = \left(\delta_i^v + 1\right)/\delta_i \quad (3)$$

where δ_i is the σ electron count on atom i. Then a factor ΔI_i is defined as

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

where n refers to the number of atoms in the path i to j including both i and j.[33,34] I_i and ΔI_i are then used to find the value of S_i according to the equation

$$S_i = I_i + \Delta I_i \quad (5)$$

Now using these procedures, the $^1\chi^v$ of R-substituents and S of some relevant atoms have been calculated and listed in Table 1. The δ^v and δ values for some important atoms that have been used in the calculations are given in Table 3.

Results and Discussion

A multiple regression analysis was performed on all observed inhibition activity data using the variables as given in Table 1 and the results obtained were as follows:

MMP-1

$$\log(1/K_i) = 7.441(\pm 0.114) + 0.198(\pm 0.122)S_N + 0.893(\pm 0.259)D$$
$$n = 28,\ \ r = 0.830,\ \ R_A^2 = 0.66,\ \ s = 0.17,$$
$$F_{2,\ 25} = 27.67\ (5.57) \quad (6)$$

MMP-2

$$\log(1/K_i) = 9.447(\pm1.052) - 1.172(\pm0.489)S_N + 0.432(\pm0.312)S_S + 0.149(\pm0.082)^1\chi^v + 0.898(\pm0.207)D$$
$$n = 33,\ r = 0.928,\ R^2_A = 0.84,\ s = 0.19,\ F_{4,\ 28} = 43.47(4.07) \quad (7)$$

MMP-8

$$\log(1/K_i) = 8.205(\pm0.163) - 0.466(\pm0.172)S_N + 0.909(\pm0.300)D$$
$$n = 31,\ r = 0.844,\ R^2_A = 0.69,\ s = 0.24,\ F_{2,\ 28} = 34.64(5.45) \quad (8)$$

MMP-9

$$\log(1/K_i) = 9.581(\pm1.332) - 1.249(\pm0.618)S_N + 0.512(\pm0.394)S_S + 0.166(\pm0.103)^1\chi^v + 1.057(\pm0.262)D$$
$$n = 33,\ r = 0.902,\ R^2_A = 0.79,\ s = 0.23,\ F_{4,\ 28} = 30.50(4.07) \quad (9)$$

ChC

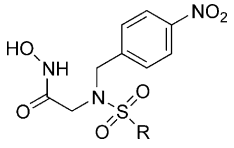
$$\log(1/K_i) = 0.253(\pm0.069)^1\chi^v + 0.550(\pm0.220)D + 6.816(\pm0.246)$$
$$n = 32,\ r = 0.856,\ R^2_A = 0.71,\ s = 0.20,\ F_{2,\ 29} = 39.87(5.42) \quad (10)$$

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

Now it is to be noted that for MMP-2 and MMP-9, eqs 7 and 9, respectively, represent almost parallel correlations, exhibiting the dependence of inhibition activities in both the cases on the valence molecular connectivity ($^1\chi^v$) of the R-substituent and the electrotopological state indices S_S and S_N of the sulfur and adjacent nitrogen. An additional parameter D is an indicator variable that has been used with a value of 1 for the compounds having $R = C_6F_5$ or 3-CF_3–C_6H_4. Both the correlations are highly significant and account equally well for the variance in the activity of the compounds for the inhibitions of both MMP-2 and MMP-9 (for MMP-2, $R^2_A = 0.84$, and for MMP-9, $R^2_A = 0.79$).

As suggested by eq 1, $^1\chi^v$ depends on δ^v. It will increase as δ^v decreases and eq 2 suggests that δ^v will decrease when there is a decrease in the valence electrons of the atom and/or an increase in the number of hydrogen atoms attached to it. Thus $^1\chi^v$ will be higher for a group or molecule which will have less electronegative and more saturated atoms. Such a group or molecule will be less polar, or that is to say, more hydrophobic in nature. Thus the dependence of activity on $^1\chi^v$ suggests that it may be the hydrophobicity of the R-group that plays a role in the inhibition of MMP-2 and MMP-9. This group may have the hydrophobic interaction with the

Table 1. A series of hydroxamates and molecular connectivity ($^1\chi^v$) of R-substituents and electrotopological state indices (S) of some atoms



Compd	R	$^1\chi^v$	S_S	S_N	D
1	CH_3	1.224	−1.701	0.957	0.0
2	CF_3	1.179	−4.041	−0.085	0.0
3	CCl_3	2.313	−1.967	1.082	0.0
4	n-C_4F_9	3.063	−4.258	−0.792	0.0
5	n-C_8F_{17}	5.575	−6.377	−1.231	0.0
6	Me_2N	1.442	−2.053	0.957	0.0
7	C_6H_5	2.523	−2.231	0.994	0.0
8	$PhCH_2$	3.130	−2.084	1.033	0.0
9	4-F–C_6H_4	2.623	−2.428	0.881	0.0
10	4-Cl–C_6H_4	3.000	−2.255	1.008	0.0
11	4-Br–C_6H_4	3.416	−2.241	1.019	0.0
12	4-I–C_6H_4	3.700	−2.238	1.021	0.0
13	4-CH_3–C_6H_4	2.934	−2.261	1.003	0.0
14	4-NO_2–C_6H_4	3.022	−2.530	0.847	0.0
15	3-NO_2–C_6H_4	3.022	−2.639	0.803	0.0
16	2-NO_2–C_6H_4	3.028	−2.820	0.737	0.0
17	3-Cl-4–NO_2–C_6H_3	3.921	−2.564	0.867	0.0
18	4-AcNH–C_6H_4	3.638	−2.429	0.937	0.0
19	4-BocNH–C_6H_4	5.046	−2.510	0.925	0.0
20	3-BocNH–C_6H_4	5.046	−2.597	0.884	0.0
21	C_6F_5	3.057	−3.869	0.135	1.0
22	3-CF_3–C_6H_4	3.251	−2.875	0.661	1.0
23	2,5-$Cl_2C_6H_3$	3.484	−2.313	1.043	0.0
24	4-MeO–C_6H_4	3.046	−2.335	0.973	0.0
25	2, 4, 6-$Me_3C_6H_2$	3.767	−2.700	1.045	0.0
26	4-MeO-3-BocNH–C_6H_3	5.575	−2.702	0.863	0.0
27	2-HO-3, 5-Cl_2–C_6H_2	3.624	−2.610	0.804	0.0
28	3-HONHCO-C_6H_4	3.361	−2.774	0.794	0.0
29	4-HONHCO-C_6H_4	3.361	−2.516	0.875	0.0
30	1-Naphthyl	3.934	−2.481	1.028	0.0
31	2-Naphthyl	3.928	−2.770	1.016	0.0
32	5-Me_2N-1-naphthyl	4.968	−2.576	1.037	0.0
33	2-Thienyl	2.887	−2.039	1.067	0.0

enzymes. The enzymes may have the deep hydrophobic pocket to accommodate sufficiently a large group.

It has been pointed out in many studies[4] that SO_2 moiety of the inhibitor is involved in strong hydrogen bonding with amino acid residues from the active site cleft of the enzyme. This is indicated in a way by the occurrence of E-state index S_S of the sulfur atom in the equations. In SO_2, sulfur itself would not participate in the hydrogen bonding but it can enhance the participation of the two oxygen atoms by donating them a major share of the lone pairs of electrons, now forming the coordinate bonds. This leads to the development of partial negative charge on the oxygen atoms to the extent they attract the lone pairs of the sulfur and this makes them capable of forming the hydrogen bonds. The strength of the hydrogen bonds will depend on the partial negative charge developed on them.

The occurrence of E-state index S_N of nitrogen, adjacent to sulfur, indicates the role of this nitrogen also. The nitrogen has a free lone pair of electrons. S_N then can be taken synonymous to electron density at nitrogen and then nitrogen can be assumed to participate in some charge-transfer phenomenon with the receptor, where it can act as a donor or acceptor of the charge, depending upon the positive or negative coefficient of S_N in the correlation. A negative coefficient, as in eqs 7 and 9, will mean that the nitrogen is acting as an acceptor so that as the value of S_N increases, its ability to accept the electron decreases, decreasing the strength of charge-transfer phenomenon. A positive coefficient of S_N, on the other hand, as in eq 6, will mean that the nitrogen would act as a donor so that as the value of S_N increases, it becomes more capable of donating the electron, resulting in stronger charge-transfer phenomenon.

In both eqs 7 and 9, a positive coefficient of D indicates that an R-substituent like C_6F_5 or 3-CF_3–C_6H_4 will be more beneficial to the activity than any other type of R-substituent. The similarity in these two groups is that they are aromatic groups containing fluorine or fluorine-substituted group at the 3-position. These groups can be assumed to bind, through fluorine atom, with Zn^{2+} ion of the enzyme, having charge–charge interaction, or with any hydrogen bonding site of the enzyme, forming the hydrogen bond. The effect of such groups seems to be equally important in the inhibition of all

Table 2. Observed and calculated enzyme inhibition activities of hydroxamates against matrix metalloproteinases (MMPs) and *C. histolyticum* collagenase (ChC)

Compd	log ($1/K_i$)									
	MMP-1		MMP-2		MMP-8		MMP-9		ChC	
	Obsd[a]	Calcd eq 6	Obsd[a]	Calcd eq 7	Obsd[a]	Calcd eq 8	Obsd[a]	Calcd eq 9	Obsd[a]	Calcd eq 10
1	7.13[b]	—	7.72	7.77	7.62	7.76	7.52	7.72	7.07	7.12
2	7.68	7.42	7.83	7.98	7.70	8.25	7.54	7.81	7.14	7.11
3	7.62	7.66	7.77	7.68	7.72	7.70	7.60	7.61	7.16	7.40
4	7.21	7.25	8.82	8.99	8.62	8.57	8.70	8.90	7.92	7.59
5	7.10	7.20	9.05	8.97	8.89	8.78	8.89	8.78	8.10	8.23
6	7.59	7.63	7.44	7.65	7.46	7.76	7.37	7.57	7.10	7.18
7	7.68	7.64	7.75	7.70	7.62	7.74	7.82	7.62	7.29	7.45
8	7.62	7.65	7.75	7.80	7.59	7.72	7.80	7.74	7.30	7.61
9	7.72	7.62	7.82	7.76	7.82	7.80	7.85	7.67	7.38	7.48
10	7.66	7.64	7.82	7.74	7.75	7.74	7.77	7.67	7.39	7.57
11	7.60	7.64	7.89	7.80	7.68	7.73	7.68	7.73	7.44	7.68
12	7.54	7.64	7.72	7.84	7.77	7.73	7.80	7.77	7.50	7.75
13	7.52	7.64	7.70	7.73	7.62	7.74	7.57	7.66	7.35	7.56
14	7.82	7.61	8.05	7.81	8.16	7.81	8.10	7.73	7.89	7.58
15	7.75	7.60	7.92	7.82	8.00	7.83	8.10	7.73	7.92	7.58
16	7.23	7.59	7.82	7.82	7.89	7.86	7.85	7.72	7.75	7.58
17	7.51	7.61	8.16	7.91	8.22	7.80	8.16	7.84	8.00	7.81
18	7.85	7.63	8.10	7.84	8.05	7.77	7.92	7.77	8.00	7.74
19	7.77	7.62	8.00	8.03	7.96	7.77	8.00	7.98	8.05	8.09
20	7.59	7.62	7.96	8.04	7.82	7.79	7.85	7.99	8.05	8.09
21	8.52	8.36	9.16	8.97	10.00[c]	—	9.22	9.00	8.30	8.14
22	8.30	8.46	8.96	8.81	9.16	8.81	9.10	8.88	8.30	8.19
23	8.05[b]	—	8.00	7.75	7.89	7.72	7.77	7.67	7.80	7.70
24	7.55	7.63	7.75	7.75	7.68	7.75	7.51	7.68	7.70	7.59
25	7.44	7.65	7.60	7.62	7.54	7.72	7.41	7.52	7.62	7.77
26	7.60	7.61	8.30	8.10	8.16	7.80	8.16	8.05	8.22	8.23
27	7.92	7.60	7.92	7.92	7.82	7.83	7.80	7.84	8.05	7.73
28	7.39	7.60	8.52	8.72	8.48	8.74	8.60	8.78	8.00	8.22
29	7.47	7.61	8.60	8.74	8.62	8.71	8.55	8.82	8.16	8.22
30	7.06[b]	—	7.41	7.76	7.35	7.73	7.36	7.68	7.82	7.81
31	7.21[b]	—	7.48	7.65	7.37	7.73	7.30	7.55	8.00	7.81
32	7.08[b]	—	7.39	7.86	7.24[c]	—	7.28	7.79	7.92	8.07
33	7.85	7.65	7.89	7.75	7.92	7.71	8.00	7.68	8.05[d]	—

[a]Taken from ref 28
[b]Not included in the derivation of eq 6.
[c]Not included in the derivation of eq 8.
[d]Not included in the derivation of eq 10.

MMPs eqs 6–9. However, these groups appear to be little less effective in ChC inhibition, as the coefficient of *D* in this case eq 10 is slightly lower than in the case of MMPs. This may be due to improper orientation of these groups towards Zn^{2+} ion or hydrogen bonding sites in ChC.

Leaving aside the role of C_6F_5 or 3-CF_3–C_6H_4, the bindings of the inhibitors with other two MMPs, that is MMP-1 and MMP-8, appear to be quite different from those with MMP-2 and MMP-9. For MMP-1 and MMP-8, eqs 6 and 8 suggest that the only other parameter (other than *D*) important for the inhibition of these two MMPs is the E-state index S_N of the nitrogen, which also has the opposite signs in the two equations. Thus the nitrogen seems to play a crucial role also in the binding of the inhibitors with both MMP-1 and MMP-8 through the formation of charge-transfer complexes with the receptors, but performing the role of donor in the former and acceptor in the latter.

Table 3. δ^v and δ values of some important atoms and their calculated intrinsic state (*I*) values

Atom (group)	δ^v	δ	*I*
>C<	4	4	1.25
>CH–	3	3	1.33
>CH_2	2	2	1.50
–CH_3	1	1	2.00
>C=	4	3	1.67
=CH<	3	2	2.00
=CH_2	2	1	3.00
>N–	5	3	2.00
≡C–	4	2	2.50
–N(H)–	4	2	2.50
≡CH	3	1	4.00
–NH_2	3	1	4.00
=N–	5	2	3.00
=NH	4	1	5.00
–O–	6	2	3.50
≡N	5	1	6.00
–OH	5	1	6.00
=O	6	1	7.00
–F	7	1	8.00
-SO_2-	0.67	4	0.42
-Cl	0.78	1	1.78

The inhibition of ChC was found to depend only on the connectivity index of R-substituents and the indicator variable *D* eq 10, indicating that the inhibition activity of hydroxamates against this enzyme is solely controlled by the R-substituents and, as already indicated in the case of MMP-2 and MMP-9, they might affect the activity through their hydrophobic property, in addition to a charge-charge or hydrogen bonding interaction of a group like C_6F_5 or 3-CF_3–C_6H_4.

From this study we find that MMP-2 and MMP-9 may be structurally quite similar to each other, but both differing widely from MMP-1, MMP-8, and ChC. In the case of MMP-2 and MMP-9, highly significant correlations were obtained, but in the case of MMP-1, MMP-8, and ChC, the correlations obtained were little inferior. Moreover, in deriving the equations for these enzymes, some outliers, as indicated in Table 2, were excluded. However, all the correlations were found to have good predictive ability when judged from the square of cross-validated correlation coefficient (r^2_{cv}) obtained by leave-one-out jacknife procedure. The values of r^2_{cv} for eqs 6–10 were 0.614, 0.772, 0.628, 0.704, and 0.692, respectively, all of which are greater than the minimum one (0.60) required for any equation to have good predictive ability.

Acknowledgements

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