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2D-QSAR in Hydroxamic Acid Derivatives as Peptide Deformylase Inhibitors and Antibacterial Agents†

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Abstract—Peptide deformylase catalyzes the removal of *N*-formyl group from the *N*-formylmethionine of ribosome synthesized polypeptide in eubacteria. Quantitative structure–activity relationship (QSAR) studies have been carried out in a series of β-sulfonyl and β-sulfinyl hydroxamic acid derivatives for their PDF enzyme inhibitory and antibacterial activities against *Escherichia coli* DC2 and *Moraxella catarrhalis* RA21 which demonstrate that the PDF inhibitory activity in cell free and whole cell system increases with increase in molar refractivity and hydrophobicity. The comparison of the QSARs between the cell free and whole cell system indicate that the active binding sites in PDF isolated from *E. coli* and in *M. catarrhalis* RA21 are similar and the whole cell antibacterial activity is mainly due to the inhibition of PDF. Apart from this the QSARs on some matrixmetelloproteins (COL-1, COL-3, MAT and HME) and natural endopeptidase (NEP) indicate the possibilities of introducing selectivity in these hydroxamic acid derivatives for their PDF inhibitory activity.
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Peptide deformylase (PDF) is an essential iron containing metallo-enzyme that catalyzes the removal of *N*-formyl group from the *N*-formylmethionine of ribosome synthesized nascent polypeptide, which is an essential step in the maturation of proteins in eubacterias.[1–5] In view of the absence of deformylase activity in mammal cells, it is a novel target for antibacterial chemotherapy.

The PDF enzyme uses Fe^{++} (ferrous ion) as catalytic metal ion, which is very labile to number of assay conditions because of its conversion to Fe^{+++} (ferric ion) by molecular oxygen resulting in inactivation of PDF.[6,7] In view of better stability of PDF-Ni than PDF-Fe, PDF-Zn and PDF-Co, the PDF-Ni provide a useful tool for the assessment of enzymatic properties of PDF and in turn screening of PDF inhibitors.[8–10]

The crystal structure complexes of PDF-Ni with actinonin and with *N*-formylhydroxylamine derivative BB-3497 (potent inhibitors of PDF, Fig. 1) have shown that metal ion Ni^{++} is pentacoordinated by the two oxygen atoms of the hydroxamate group of actinonin or by that of the *N*-formylhydroxylamine group of BB-3497 and by Cys90, His132 and His136 side chains of PDF (Fig. 2). The both complexes show extensive hydrogen bonding between the side chains of Glu-133, Gln-50 and the main chain NH– of Leu-91 of PDF with hydroxamate group of actinonin and *N*-for-

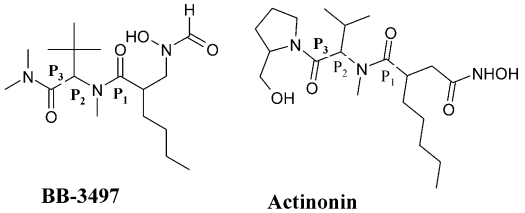

Figure 1. Structures of potent inhibitors of PDF.

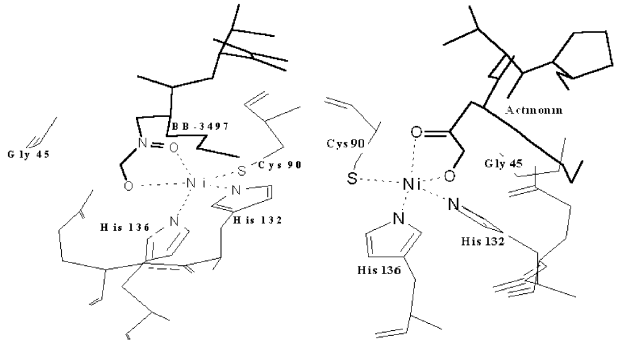

Figure 2. BB-3497 (left) and actinonin (right) bound to active site of *E. coli* PDF.

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Table 1. Observed and calculated inhibitory activity data of β-sulfonyl and β-sulfinyl hydroxamic acid derivatives against PDF-Fe, *E. coli* DC2 and *M. catarrhalis* RA21

Compd.	PDF-Fe			*E. coli* DC2			*M. catarrhalis* RA21		
	Observed		Calcd	Observed		Calcd	Observed		Calcd
	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$
1	22.00	−1.34	−0.92	>32	—	−3.0	>32	—	−0.72
2	3.00	−0.48	−0.27	>64	—	−2.32	>64	—	−0.72
3	0.16	0.80	0.26	64	−1.80	−1.76	4	−0.60	−0.72
4	0.04	1.46	0.80	8	−0.90	−1.20	<0.25	—	−0.72
5	0.14	0.85	1.38	2	−0.30	−0.60	<0.06	—	−0.72
6	0.16	0.80	0.20	32	−1.50	−1.88	8	−0.90	−0.72
7	0.53	0.28	0.71	64	−1.81	−1.20	4	−0.60	−0.84
8	0.23	0.64	0.59	8	−0.90	−1.20	<0.25	—	−1.0
9	0.19	0.72	0.77	128	−2.11	−1.20	16	−1.20	−0.76
10	0.02	1.64	1.32	8	−0.90	−1.20	0.5	0.30	−0.02
11	0.03	1.50	1.18	16	−1.20	−1.20	0.5	0.30	−0.22
12	0.09	1.07	1.18	16	−1.20	−1.20	2	−0.30	−0.22
13	0.01	1.96	1.58	16	−1.20	−1.20	1	0.0	0.32
14	0.12	0.92	1.24	16	−1.20	−1.20	2	−0.30	−0.13
15	0.10	1.00	0.80	1	0.0	0.24	<0.25	—	0.09
16	0.10	1.00	1.18	2	−0.30	0.24	<0.25	—	0.60
17	0.09	1.03	1.18	4	−0.60	0.24	0.25	0.60	0.60
18	0.06	1.19	1.32	2	−0.30	0.24	<0.25	—	0.79

Table 2. Observed and calculated inhibitory activity data of some selected hydroxamic acid derivatives against several MMPs

Compd	NEP			COL-1			COL-3			MAT			HME		
	Observed		Calcd	Observed		Calcd	Observed		Calcd	Observed		Calcd	Observed		Calcd
	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$	IC_{50}	$-\log IC_{50}$	$-\log IC_{50}$
4	18.00	−1.26	−0.83	0.11	0.96	0.99	0.02	1.66	1.57	1.10	−0.04	0.11	0.01	1.92	1.92
8	1.65	−0.22	0.07	0.05	1.27	1.22	0.10	1.00	1.04	0.29	0.54	0.46	0.01	1.85	1.87
10	0.50	0.30	−0.02	0.07	1.16	1.20	0.01	2.05	2.09	1.50	−0.18	−0.25	0.01	1.89	1.87
15	2.60	−0.42	−0.83	0.09	1.03	0.99	—	—	—	—	—	—	—	—	

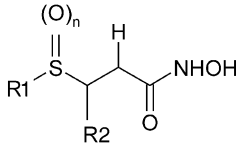
—Denotes no activity data.

mylhydroxylamine group of BB-3497. The other H-bonds are formed between NH– of Ile-44 with the **P1** carbonyl group and with main chain carbonyl oxygen and NH– group of Gly-89 with the NH- at **P2** and carbonyl group at **P3** of the BB-3497. This H-bonding network helps to make intimate association of PDF to inhibitors and results in better enzyme inactivation. The chemical groups at **P2** and **P3** position are generally accessible with solvent molecules and hence proposed as attractive sites for modification to improve inhibitory properties of PDF inhibitors.[11] In view of this, the low molecular weight β-sulfonyl and β-sulfinyl hydroxamic acid derivatives having structural variation in this region have been investigated as inhibitors of *E. coli* PDF.[12] The reported SAR of these compounds and others PDF inhibitor indicate the importance of hydrophobicity and hydrogen bonding in the binding of inhibitor to enzyme but do not precisely indicate the role of substitution by chemical groups in the phenyl ring for modulation of inhibitory activity in terms of physicochemical factors at **R1** and **R2** positions. In addition no where quantitative structure activity analysis have been reported to study the role of variation of PDF inhibitory activity in cell free enzyme or in whole cell system with different physicochemical (hydrophobicity, electronic and steric) and structural properties. Such studies if carried out for different matrix-metalloproteases (MMPs) may help in identification of structural differences between the substrate recognition sites of PDF and MMPs and thus may lead to the design and synthesis of more active and selective PDF inhibitors.

Thus, the quantitative structural–activity relationship (QSAR) studies in β-sulfonyl and β-sulfinyl hydroxamic acid derivatives for the variation in their PDF-Fe and MMPs inhibitory activities, in cell free and whole cell system with the physicochemical and structural properties have been carried out and are described in this paper.

The 2D-QSAR studies in β-sulfonyl and β-sulfinyl hydroxamic acid derivatives have been carried out for their antibacterial activities as dependent and different physicochemical and structural parameters as independent variables on Compaq PC using SYSTAT software version 7.0.1. The biological activities data for these hydroxamic acid derivatives were taken from literature (Tables 1 and 2).[12] The values for physicochemical constants viz. hydrophobic fragment constant, molar refractivity and electronic field effect were calculated and/or computed from literature values (Table 3).[13] An

Table 3. Physicochemical parameters corresponding to different functional groups at position **R1** and **R2** of β-sulfonyl ($n=2$) and β-sulfinyl ($n=1$) hydroxamic acid derivatives



Compd	Groups		[a]f^{R1}	[b]Fr^{R1}	[c]MR^{R1}	[b]Fr^{R2}	[c]MR^{R2}	[d]In
	R1	R2						
1	Ph-	Me	0.08	1.90	30.51	0.77	5.65	1
2	Ph-	Et	0.08	1.90	30.51	1.43	10.30	1
3	Ph-	Pr	0.08	1.90	30.51	1.97	14.96	1
4	Ph-	Bu	0.08	1.90	30.51	2.51	19.61	1
5	Ph-	Pentyl	0.08	1.90	30.51	3.10	24.26	1
6	Ph-	Ph	0.08	1.90	30.51	1.90	30.51	1
7	Hexyl-	Bu	−0.06	3.69	28.91	2.51	19.61	1
8	Cyclohexyl-	Bu	−0.06	3.33	26.82	2.51	19.61	1
9	Benzyl-	Bu	−0.08	2.44	30.01	2.51	19.61	1
10	2-Naphthyl-	Bu	0.22	3.20	39.82	2.51	19.61	1
11	4-MeOPh-	Bu	0.34	1.88	37.30	2.51	19.61	1
12	3-MeOPh-	Bu	0.34	1.88	37.30	2.51	19.61	1
13	4-AcNHPh-	Bu	0.36	0.93	44.41	2.51	19.61	1
14	4-Br Ph-	Bu	0.52	2.76	38.36	2.51	19.61	1
15	Ph-	Bu	0.08	1.90	30.51	2.51	19.61	0
16	4-MeOPh-	Bu	0.34	1.88	37.30	2.51	19.61	0
17	3- MeOPh-	Bu	0.34	1.88	37.30	2.51	19.61	0
18	2-Naphthyl-	Bu	0.22	3.20	39.82	2.51	19.61	0
19	4-BrPh-	Bu	0.52	2.76	38.36	2.51	19.61	0

1–**14** β-sulfonyl and **15**–**19** β-sulfinyl hydroxamic acid derivatives.
[a]Electronic field effect at R1.
[b]Hydrophobic fragment constants values.
[c]Molar refractivity parameters at R1 and R2.
[d]Indicator variable In = 1 for two and 0 for β-sulfonyl and β-sulfinyl groups, respectively.

indicator variable In = 1 was taken for the presence of sulfonyl group. The antibacterial activity values [C] of dependant variables were transformed to −log[C].

The correlation between different physicochemical parameters like hydrophobic (Fr), steric (MR) and electronic (f) as independent and inhibitory activity viz. PDF-Fe, *E. coli*. DC2, *M. catarrhalis* RA21, NEP, COL-1, COL-3, MAT and HME as dependent variable are described in Tables 4 and 5. Different permutations and combinations showing some correlation with activity with good orthogonality between them (inter-correlation value <0.2) were carried and those with statistical significant correlation are discussed in Table 6 (eqs 1–9).

The PDF inhibitory activity ($-\log IC_{50\ PDF\text{-}Fe}$) shows positive correlation with molar refractivity at position **R1** and **R2** and hydrophobicity at **R2**. The equations eqs 1 and 2 derived by taking $-\log IC_{50PDF\text{-}Fe}$ as dependent and MR^{R1}, MR^{R2} and MR^{R1}, Fr^{R2} as independent variables respectively. Both equations, eqs 1 and 2, describe the good correlation coefficient value $r=0.813$ and 0.855, respectively, of high statistical significance (>99.90%) ($F_{2.16\ \alpha\ 0.001}=12.7$, $F_{2.16}=15.6$).

These equations indicate that PDF inhibitory activity of hydroxamate derivatives may be increased with the

Table 4. Correlation coefficient values of different physicochemical parameters with inhibitory activities against PDF-Fe, *E. coli* DC2 and *M. catarrhalis* RA21

	f^{R1}	Fr^{R1}	MR^{R1}	Fr^{R2}	MR^{R2}	In
$-\log IC_{50\ PDF\text{-}Fe}$	0.37	−0.10	0.56	0.78	0.65	−0.13
$-\log IC_{50\ E.\ coli\ DC2}$	0.39	−0.05	0.27	0.46	0.03	−0.74
$-\log IC_{50\ M.\ cata.\ RA21}$	0.68	−0.16	0.73	0.45	−0.27	−0.53

Table 5. Correlation coefficient values of different physicochemical parameters with inhibitory activities against NEP, COL-1, COL-3, MAT and HME

	f^{R1}	Fr^{R1}	MR^{R1}
$-\log IC_{50NEP}$	0.33	0.76	0.54
$-\log IC_{50COL\text{-}I}$	−0.33	0.94	−0.04
$-\log IC_{50COL\text{-}3}$	0.99	−0.23	0.92
$-\log IC_{50MAT}$	−0.94	0.41	−0.83
$-\log IC_{50HME}$	0.57	−0.87	0.35

NEP, natural endopeptidase; COL-1, collagenase-1 (MMP-1); COL-3, collegenase-3 (MMP13); MAT, matrilysin (MMP-7); HME, human macrophage elastage (MMP-12).

increase in molar refractivity at the **R1** and **R2** positions and the substituents, which increase steric interactions in polar space at **R1** and steric/hydrophobic interactions at **R2** positions positively contribute for the PDF inhibitory activity. Thus activities of these inhibitors should increase with increase in molar refractivity on the both **R1** and **R2** positions. The compound **13** is the most active in this series and has highest molar refractivity at **R1** position, the **–NHCOCH3** group at *para*-position of phenyl ring in addition may form additional H-bonds with respective amino acids of PDF and thus provide its more effective interactions with PDF. Thus substitution of bulky group at **R1**, with capability of H-bonding and hydrophobic groups at **R2** position may result in improving the activity.

The inhibitory activity against *E. coli* DC2($-\log MIC_{E.coli\ DC2}$) is best correlated with Fr^{R2} and In. eq 3 describes a good correlation value ($r=0.841$) of high (>99.90%) statistical significance. [$F_{2.14\ \alpha\ 0.001}=13.4$, $F_{2.14}=17.0$]. The comparison of eq 3 correlating whole cell activity, the minimum inhibitory concentration (MIC) of *E. coli* DC2 with eq 2 shows similar slope values 0.985 (±0.198) and 1.024 (±0.373) for eqs 2 and 3, respectively for the same hydrophobic Fr^{R2} parameter which unequivocally prove that the antibacterial activities against *E. coli*. DC2 is due to the inhibition of PDF and the negative contribution of the indicator variable may be interpreted in terms of putting steric hindrance in the H-binding of the one oxygen present in one sulphur atom with NH of Ile 44. The whole cell antibacterial activity of all hydroxamic acid derivatives having definite MICs values against *M. catarrhalis* RA21 ($-\log MIC_{M.\ cata RA21}$) is also best correlated with molar refractivity (MR) at **R1** and inclusion of indicator variable In for taking one sulfonyl compound with definite MIC value is to account and for the comparison the whole cell inhibitory activity in the PDF-Fe enzyme from the two different bacterial species *E. coli* DC2 and

Table 6. Statistically significant QSAR models

Eq no.		Regression equation	Regression parameters			
			n	*r*	*s*	*F*
1	$-\log IC_{50\ PDF\text{-}Fe}$	$=0.075(\pm0.022)\ MR^{R1}+0.089(\pm0.022)\ MR^{R2}-3.424(\pm0.828)$	19	0.81	0.46	15.6
2	$-\log IC_{50\ PDF\text{-}Fe}$	$=0.056\ (\pm0.020)\ MR^{R1}+0.985(\pm0.198)\ Fr^{R2}-3.386(\pm0.727)$	19	0.86	0.41	21.7
3	$-\log MIC_{E.\ coli\ DC2}$	$=1.024(\pm0.373)Fr^{R2}-0.964(\pm0.198)In-2.810(\pm0.950)$	17	0.84	0.37	17.0
4	$-\log MIC_{M.\ cata\ .RA21}$	$=0.075(\pm0.022)\ MR^{R1}-0.812\ (\pm0.360)\ In-2.188(\pm0.891)$	10	0.85	0.34	9.4
5	$-\log IC_{50NEP}$	$=0.624(\pm0.378)\ Fr^{R1}-2.011(\pm1.009)$	4	0.76	0.52	2.7
6	$-\log IC_{50COL-1}$	$=0.164(\pm0.041)\ Fr^{R1}+0.681(\pm0.110)$	4	0.94	0.05	15.8
7	$-\log IC_{50HME}$	$=-0.038(\pm0.022)\ Fr^{R1}+1.995\ (\pm0.064)$	3	0.87	0.02	3.0
8	$-\log Ic_{50COL-3}$	$=3.750(\pm0.557)\ f^{R1}+1.270(\pm0.78)$	3	0.99	0.11	45.0
9	$-\log IC_{50MAT}$	$=-2.571\ (\pm0.907)\ f^{R1}+0.312(\pm0.127)$	3	0.94	0.18	8.03

M. catarrhalis RA21 led to eq 4. This equation has good correlation coefficient ($r=0.853$) of more than 95% statistical significance. ($F_{2.7\ \alpha\ 0.05}=6.6$, $F_{2.7}=9.4$). The observed similar regression coefficient with MR^{R1}, in eq 4 for whole cell and in eq 1 describing the correlation of PDF-Fe inhibitory activity in the cell-free system, suggest that this whole cell antibacterial activity is mainly governed by the PDF enzyme inhibitory activity and that the active site of the enzyme binding or interacting with the **R1** substituent is same between the PDF isolated from *E.coli* and that of *M. catarrhalis* RA21. Further the negative contribution of the same magnitude by Indicator parameter in both eqs 3 and 4 suggest that sulfonyl group competes for the similar binding sites in the enzyme in both from *E. coli* DC2 and *M. catarrhalis* RA21.

In order to understand the influence of the physicochemical factors on the selectivity towards PDF-Fe from other MMPs (COL-1, COL-3, HME and MAT) and endopeptidase (NEP), the limited number of available compounds with definite MICs values were considered. These were divided into two sets A and B with four and three compound data respectively. Set A of four compounds (**4**, **8**, **10**, and **15**) shows inhibitory activity against NEP and COL-1 and set B of three compounds (**4**, **8** and **10**) shows inhibitory activity for COL-3, MAT and HME.

Knowing that the activity data of the three or four compounds are too less to correlate with single independent variable, the equations were developed to see the trend of variation in the inhibitory activity of different compounds against the MMPs with different physicochemical parameters. The inhibitory activity for NEP, COL-1, HME enzymes correlated best with Fr^{R1}, (eqs 5, 6 and 7), while for enzyme COL-3 and MAT correlated best with f^{R1} (eqs 8 and 9). Thus it appears that the variation in activities are mainly influenced by hydrophobicity (Fr^{R1}) but with different rate of variation; being positive in compounds of set A (eqs 5 and 6) and negative in compounds of set B (eq 7). A comparison of the rate of variation of enzyme inhibitory activities for PDF-Fe and NEP, COL-1 and HME with respect to Fr^{R1} clearly indicate the possibility of increasing the activity and selectivity towards PDF-Fe versus different MMPs and endopeptidase through structural modulation. Unlike the MMPs, namely NEP, COL-1 and HME, where hydrophobicity of the inhibitor has major influence on activity, the inhibition of other MMPs; COL-3 and MAT shows correlation with electronic parameter f^{R1} (eqs 8 and 9). Since these parameters do not show good correlation (Table 4) with PDF-Fe in the hydroxamic acid derivatives, their selectivity towards PDF-Fe can be optimized by judicious substitution at **R1**.

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