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Identification of Novel Potent Hydroxamic Acid Inhibitors of Peptidyl Deformylase and the Importance of the Hydroxamic Acid Functionality on Inhibition

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Abstract—Peptidyl deformylase (PDF) is a metallo protease that catalyzes the removal of a formyl group from the N-termini of prokaryotic prepared polypeptides, an essential step in bacterial protein synthesis. Screening of our compound collection using *Staphylococcus aureus* PDF afforded a very potent inhibitor with an IC_{50} in the low nanomolar range. Unfortunately, the compound that contains a hydroxamic acid did not exhibit antibacterial activity (MIC). In order to address the lack of activity in the MIC assay and to determine what portion of the molecule was responsible for binding to PDF, we prepared several analogues. This paper describes our findings that the hydroxamic acid functionality found in **1** is mainly responsible for the high affinity to PDF. In addition, we identified an alternative class of PDF inhibitors, the *N*-hydroxy urea **18**, which has both PDF and antibacterial activity.

Antibacterial resistance to a wide range of available antibiotics is a growing problem in the treatment of bacterial infections.[1–3] Currently available antibiotics inhibit several essential biological pathways in bacteria. Historically, antibacterial discovery programs have focused on protein targets, which have little homology or resemblance to mammalian counterparts. Recent advances in biology and automation have resulted in the discovery of new targets at an impressive rate. Antibacterial discovery programs can rely on target or cell based assays utilizing high throughput screening for lead generation.[4–6] Peptidyl deformylase (PDF) is a metallo protease, which catalyzes the removal of a formyl group from the N-termini of polypeptides biosynthesized in prokaryotes. This step in bacterial protein synthesis is essential for bacterial proliferation. The metal in the native enzyme is thought to be iron, but due to the extreme sensitivity of this protein during purification it has been isolated with a variety of cations in its active site including cobalt, nickel, and zinc.[7] Several solution[8] and solid-state[9,10] structural studies have recently been reported. Recent reports describing PDF inhibitors with metal chelating functional groups such as hydroxamic acids,[11,12] *N*-formylhydroxyl-amine,[13] thiol,[14] and biaryl acids[15] demonstrate a current interest in PDF as an antibacterial target.

Screening of our compound collection against *Staphylococcus aureus* PDF identified a very potent inhibitor **1** with an IC_{50} in the low nanomolar range (Fig. 1).[16] To our surprise, compound **1** did not exhibit antibacterial activity (MIC). The inhibitor contains a hydroxamic acid, a feature common to recently reported inhibitors

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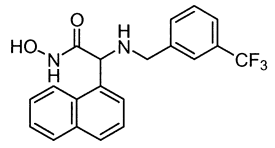
of PDF, which demonstrate antibacterial activity.[11,12] The lack of antibacterial activity for this compound was noteworthy, considering its potency against the PDF enzyme. We set out to address the lack of antibacterial activity in this series of compounds and to find a potent inhibitor of PDF with activity in our MIC assay. We have tested and prepared analogues addressing two issues: (a) the importance of the hydroxamic acid moiety to binding, and (b) the possibility of an alternative functional group existing for the hydroxamic acid with affinity for PDF.

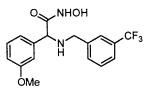
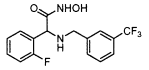
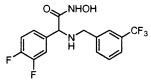
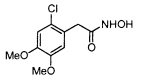
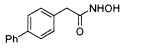
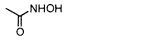
Similarity searches of our compound collection identified several related compounds shown in Table 1. These compounds (which also contain a hydroxamic acid) exhibit a wide range of inhibition against PDF, but were all devoid of any antibacterial activity. The shape of the molecule appears to contribute significantly to the tight binding of **1** to PDF. It is not sufficient to only have the hydroxamic acid functionality, since both **6** and **7** were inactive as inhibitors for PDF.



1
MIC >128 μg/mL
PDF IC_{50} 5 nM

Figure 1. Inhibitor of PDF identified by screening.

Table 1. Antibacterial and PDF activity of selected hydroxamic acids

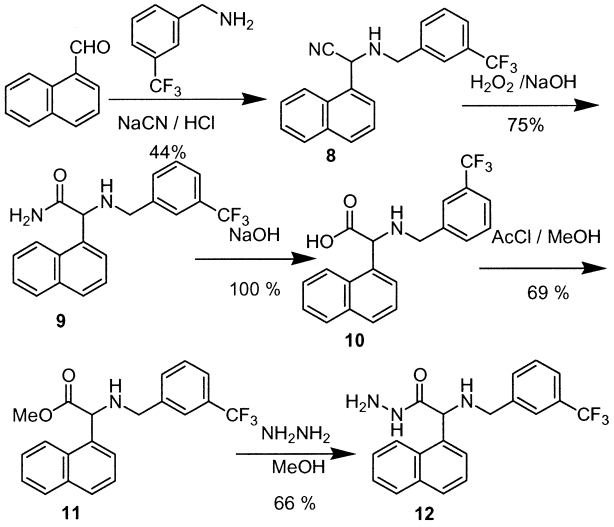
		SAUR 9218	SEPI 12084	SPNE 9912	ECOL 6674	PDF[a] IC_{50} (μM)
	**2**	> 128	> 128	> 128	> 128	0.059
	**3**	> 128	> 128	> 128	> 128	0.46
	**4**	> 128	> 128	> 128	> 128	0.69
	**5**	> 32	> 32	> 32	> 32	0.2
	**6**	> 128	> 128	> 128	> 128	> 50
	**7**	> 128	> 128	> 128	> 128	> 100

[a]PDF assays were performed with the *S. aureus* enzyme utilizing the assay relying on coupling the PDF peptide product to an aminopeptidase as described in ref 17a (data shown). In order to validate PDF inhibition, the assay described in ref 17b, in which formate dehydrogenase utilizes the formic acid released to reduce NAD, was performed (data complementary). In addition, in selected cases where the aminopeptidase[17a] was inhibited by a compound, the assay[17a] was modified to an HPLC end point assay measuring PDF product formation, verifying PDF inhibition.[17]

In order to evaluate the importance of the hydroxamic acid, the corresponding carboxyl, amide, and hydrazide surrogates were prepared (Scheme 1). These analogues contain a constant attachment of the backbone of **1** to the metal coordination group and are able to coordinate the metal in a mono- or bidentate fashion.

The hydrazide **12** that achieves a bidentate chelate with the metal inhibited PDF with an IC_{50} of 4.1 μM (Table 2). This is a similar metal coordination to the hydroxamate **1** but is 100-fold less potent. The remainder of the compounds **9**–**10**, which are monodentate ligands, were inactive. These results clearly demonstrate that the hydroxamic acid functionality is essential for the high affinity of **1** against PDF.

Crystal structures of PDF[10] and our computer modeling efforts suggested that **1** binds to PDF in a C-shape (Fig. 2a). Based on this data, it was our desire to design a new set of inhibitors that would have the potential of achieving a five-membered bidentate chelate based on the importance of the hydroxamic acid functionality found in **1**. In order to increase the basicity of the carbonyl and thereby enhance its coordination, we elected to pursue amides. The data in Table 1 suggest that the basic nitrogen found in **1** was not essential for PDF activity. Our approach was to prepare α-hetero-substituted amides that could attain a C-shaped conformation similar to **1** based on molecular modeling (Fig. 2b).



Scheme 1.

Table 2. Antibacterial and PDF activity of alternative chelators

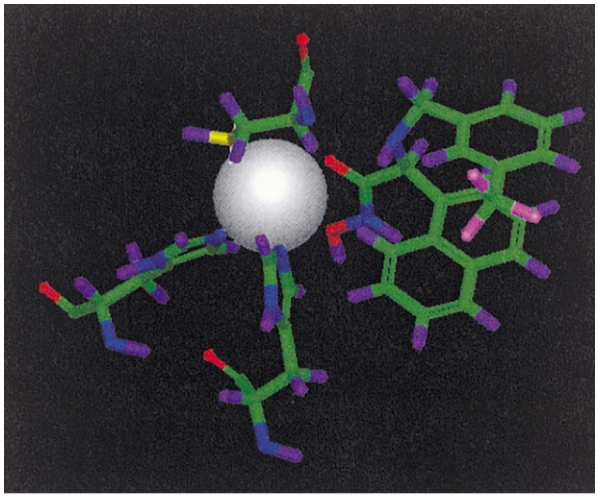
		SAUR 9218	SEPI 12084	SPNE 9912	ECOL 6674	PDF IC_{50} (μM)
1	NHOH	> 64	> 64	64	> 64	0.046
10	OH	> 128	> 128	128	> 128	> 100
9	NH_2	64	64	16	> 128	> 100
12	$NHNH_2$	64	128	32	> 128	4.1

The synthesis of the series of analogues is outlined in Scheme 2. 1-Naphthylamine was acylated with α,α,α-trifluoro-*m*-tolylacetic acid affording amide **13**. The amide was subsequently reduced to afford **14** in excellent overall yield. Amine **14** was then acylated with several α-hetero substituted acid chlorides, which upon deprotection afforded thiol **15** and hydroxy compound **16**. The corresponding hydroxamate and the hydrazine were prepared by acylation of **14** with a phosgene equivalent, followed by the addition of excess hydrazine or hydroxylamine to afford the desired compounds **17** and **18**.

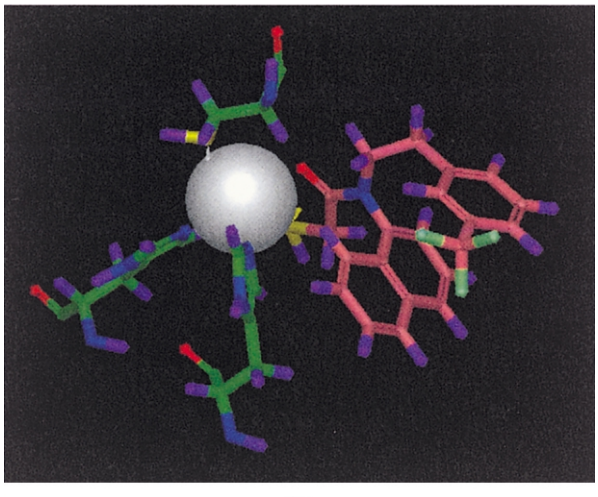
The compounds exist as atropisomers and as a mixture of *E*/*Z* amide isomers, which is solvent dependent. The energy barrier for a few derivatives[18] was measured to be approximately $\Delta G^{\ddagger}$ of 24.0 kcal/mol. This data indicates that the compounds rapidly equilibrate at room temperature and the separation of each enantiomer is not justifiable.

Amine **14** was found to be inactive in both the MIC and the PDF screen. Compounds **15**–**16** containing a methylene group between the coordination groups were inactive against PDF, but the hydroxy compound **16** was active as an antibacterial agents (Table 3).[19] Replacement of the carbon in **16** with a nitrogen, such as in the hydrazine urea **17**, also results in complete loss of antibacterial activity. A more significant observation is that the *N*-hydroxyurea **18** is active against PDF with an IC_{50} around 2.2 μM and has antibacterial activity. The ability of a hydroxamic urea to coordinate PDF has not been previously reported. In compound **18**, we have overcome the lack of MIC found in **1** despite a decrease in PDF activity. This functionality appears not to have the same disadvantage as the hydroxamic acid. One can speculate that the inactivity in the MIC assay of **1** is due to efflux and that **18** is not a high affinity substrate for the efflux pumps.[12]

This report describes an initial effort to design inhibitors of PDF with in vitro antibacterial activity. It was demonstrated that the high affinity of compound **1** is mainly due to the hydroxamic acid functionality, but the backbone is also important. The initial lead **1** and the understanding of its inhibition of PDF was subsequently utilized to design several α-hetero substituted amides. The urea derivative **18** was found to have PDF activity with an IC_{50} of 2.2 μM in addition to antibacterial activity. One can speculate that the lack of MIC activity of **1** is due to efflux or poor membrane permeability. The discovery of **18** circumvents that

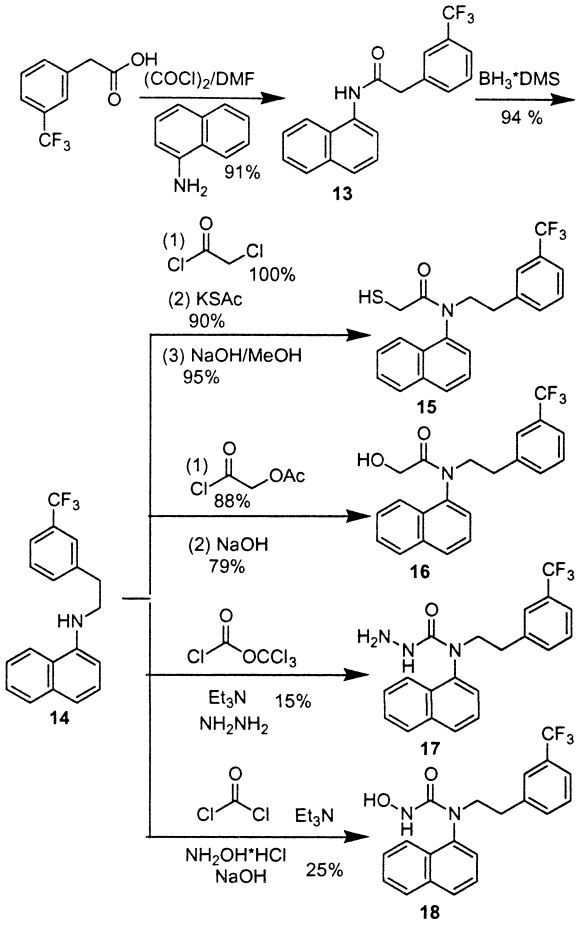


(a)



(b)

Figure 2. Modeling of analogues in the active site of PDF: (a) **1**, and (b) α-thioacetamide **15**.



Scheme 2.

Table 3. Antibacterial and PDF activity of α-hetero substituted acetoamides

	R	SAUR 9218 MIC	SEPI 12084 MIC	SPNE 9912 MIC	ECOL 6674 MIC	PDF IC_{50} (μM)
15	CH_2SH	>128	>128	>128	>128	>100
16	CH_2OH	32	>128	32	>128	>100
17	$NHNH_2$	>128	>128	>128	>128	>100
18	NHOH	32	32	32	>128	2.2

problem and fulfills our initial goal of a PDF inhibitor with antibacterial activity. The SAR of this general template and the optimization of its antibacterial activity along with the search of additional polar functional groups will be the topic of future reports.

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18. Compounds not shown but are related to **16**.
19. The antibacterial activity of **16** is due to an unknown mechanism and appears to be unrelated to PDF.