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Peptide deformylase inhibitors with non-peptide scaffold: Synthesis and structure–activity relationships

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ABSTRACT

Peptide deformylase (PDF), which removes the formyl group at the N-terminal methionine residue of nascent protein, has been recognized as a potent target for antibacterial therapy. We report herein the synthesis and structure–activity relationship studies of non-peptide PDF inhibitors.

Increasing antibacterial resistance poses a severe threat to human health.[1] As consequences, there is a growing need to identify new antibiotics that do not share the targets of existing antibacterial drugs. Many novel and potentially useful targets are discovered by analysis of microbial genomes, but only a few targets succeed to yield active antibiotics.[2,3] One of novel and potentially useful targets that have recently received a particular attention is peptide deformylase (PDF).[4] The difference in protein synthesis between bacteria and mammalian cells stems from transformylation and deformylation of initiating methionine. Protein synthesis in bacteria is initiated with *N*-formylmethionine which is generated by transformylation of methionine. PDF removes the formyl group at the N-terminal methionine residue of nascent protein.[5] The fact that the peptide deformylase is essentially required for producing mature protein in bacteria provides a rational basis to choose it as a potential antibacterial target.

In a previous study, we have reported that PDF inhibitor **1**[6] and its analogs have potent antibacterial activity against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* which are known to cause respiratory tract-associated infection (Fig. 1). But PDF inhibitor **1** and analogs have lower antibacterial activity against *Staphylococcus aureus*. During the course of our PDF inhibitor program, we found that replacement of the *N*-alkyl side chain and the hydroxamic acid moiety of compound **1** with chiral alkyl chain and reverse hydroxamic acid, respectively, significantly increases antibacterial activity against *S. aureus*. Here, we describe the synthesis of non-peptide PDF inhibitors **2** and corresponding SAR around the P_3' positions.

The synthesis of PDF inhibitors used in this study is outlined in Schemes 1–3.[7] Coupling of *N*-Boc amino acid **3** with Meldrum's acid in the presence of DCC and DMAP gave acyl Meldrum's acid and subsequent treatment with benzyl alcohol provided β-keto ester **4**.[8] In this step tetramic acid, which was produced from undesired cyclization reaction, was easily removed by silica-gel column chromatography. Compound **5** was prepared from alkylation with butyl group at the α-position of the β-keto ester **4**. Removal of benzyl group by hydrogenolysis in the presence of 10% Pd/C afforded a β-keto acid, which was treated with formaldehyde and piperidine to give compound **6**. Since the β-keto acid was easily decarboxylated at room temperature, storing the β-keto acid is not recommended. Conjugate addition of benzylhydroxylamine formed diastereomeric adducts (72:28, *RS*:*SS* diastereomeric ratio). The desired (*R*)-diastereomer **7** was isolated from purification through silica-gel column chromatography. Treatment of compound **7** with formic acid and acetic anhydride provided compound **8**. Final compound **9** was obtained from removal of the benzyl group through hydrogenolysis.

For the synthesis of PDF inhibitors with amide moiety at P_3' position, compound **8b** was treated with trifluoroacetic acid to afford compound **10**. Amine **10** was coupled with corresponding carboxylic acid to provide compound **11**. Removal of *O*-benzyl group with hydrogenolysis gave final compound **12**.

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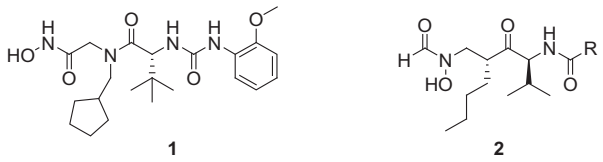
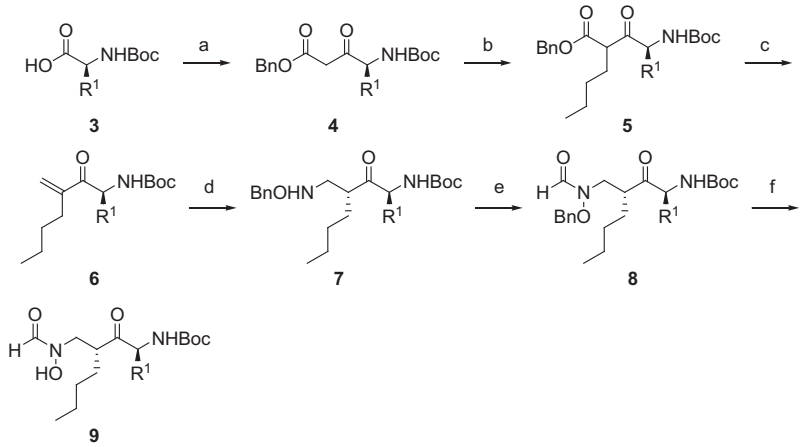


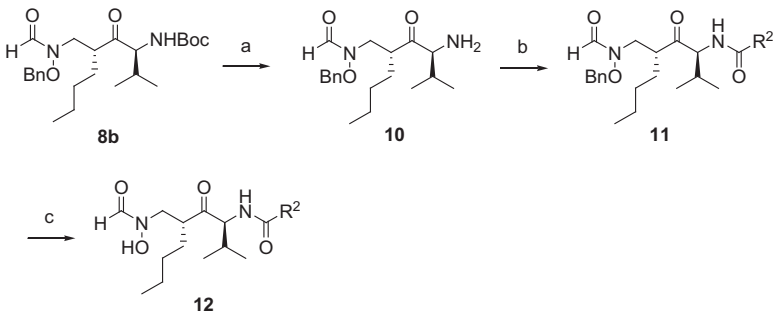
Figure 1. Structures of PDF inhibitor **1** and non-peptide PDF inhibitor **2**.

Compound **10** was treated with carbonyl diimidazole[9] and the resulting imidazolide **13** was coupled with the corresponding amine in toluene to give compound **14**. The final urea **15** was obtained by deprotection of benzyl group using hydrogenolysis.

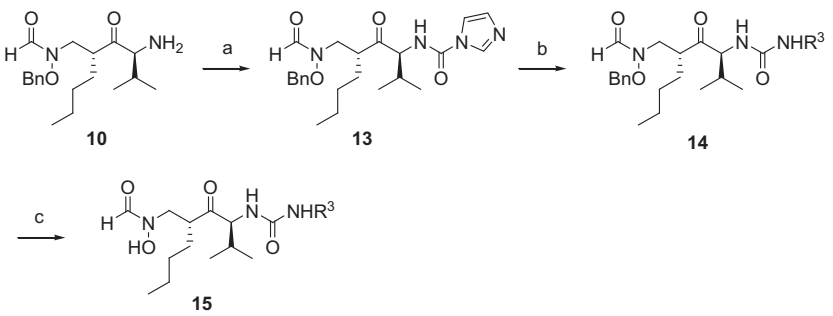
Compounds were tested using a *P. aeruginosa* Ni-PDF enzyme assay[10] and primary in vitro antibacterial activity was tested against *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. aureus*. The size of the amino acid side chain at P_2' position appears to have significant effect on the enzyme inhibition activity, and antibacterial activity against *S. aureus*. From the assay data summarized in Table 1, preferred side chain for P_2' site appears to be an isopropyl group because valine derivative (**9b**) revealed potent inhibition against enzyme and bacterial strains. The loss in activity of *t*-leucine derivative (**9c**) against *S. aureus* shows that P_2' binding pocket is not suitable for binding with side chain bigger than isopropyl group.



Scheme 1. Reagents and conditions: (a) Meldrum's acid, DCC, DMAP, rt, then toluene, BnOH, 80 °C, 30–50%; (b) K_2CO_3, NaI, *n*-BuBr, acetone, reflux, 18 h, 50–60%; (c) 10% Pd/C, H_2, EtOH, then CH_2O, piperidine, 80 °C to rt, 55–65%; (d) $BnONH_2$, 40 °C, 12 h, 55–60%; (e) HCO_2H, Ac_2O, EtOAc, rt, 1 h, 90–95%; (f) 10% Pd/C, H_2, MeOH, rt, 2 h, 40–50%.

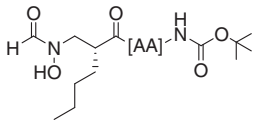


Scheme 2. Reagents and conditions: (a) TFA, dichloromethane, rt, 6 h, 98%; (b) R^2CO_2H, HOBt, EDC, *N*,*N*-diisopropylamine, dichloromethane, rt, 12 h, 35–50%; (c) 10% Pd/C, H_2, MeOH, rt, 2 h, 40–50%.



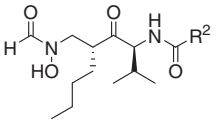
Scheme 3. Reagents and conditions: (a) carbonyl diimidazole, *N*,*N*-diisopropylethylamine, dichloromethane, rt, 4 h, 88%; (b) R^3NH_2, toluene, reflux, 18 h, 35–50%; (c) 10% Pd/C, H_2, MeOH, rt, 2 h, 40–50%.

Table 1
PDF inhibition and in vitro antibacterial activities for P_2' side chain



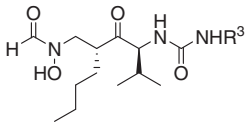
Compound	AA	IC_{50} (nM) *P. aeruginosa* PDF	MIC (μg/ml)			
			S. pneumoniae	*H. influenzae*	*M. catarrhalis*	*S. aureus*
1		29	0.2	0.2	0.1	50.0
9a	Alanine	330	3.2	0.8	1.6	12.5
9b	Valine	183	1.6	1.6	0.1	12.5
9c	*t*-Leucine	603	6.3	3.2	0.1	100
9d	Proline	169	6.3	0.4	0.4	12.5

Table 2
PDF inhibition and in vitro antibacterial activities for P_3' amides



Compound	R^2	IC_{50} (nM) *P. aeruginosa* PDF	MIC (μg/ml)			
			S. pneumoniae	*H. influenzae*	*M. catarrhalis*	*S. aureus*
1		29	0.2	0.2	0.1	50.0
9b	*O*-*t*-Butyl	183	1.6	1.6	0.1	12.5
12a	*i*-Propyl	60	0.8	0.2	1.6	3.2
12b	Ph	51	3.2	0.2	0.4	3.2
12c	Pyridin-2-yl	117	0.2	0.1	0.1	1.6
12d	Ph(3-MeO)	22	0.2	0.2	0.8	0.8
12e	Ph(3-F)	43	0.4	0.2	1.6	1.6
12f	Pyridin-3-yl	73	0.2	0.1	0.1	0.8
12g	Pyridin-4-yl	52	0.4	0.1	0.1	1.6
12h	Quinolin-2-yl	8	0.1	0.2	0.1	0.4
12i	*i*-Quinolin-1-yl	5	0.4	0.4	0.1	6.4

Table 3
PDF inhibition and in vitro antibacterial activities for P_3' ureas



Compound	NHR^3	IC_{50} (nM) *P. aeruginosa* PDF	MIC (μg/ml)			
			S. pneumoniae	*H. influenzae*	*M. catarrhalis*	*S. aureus*
1		29	0.2	0.2	0.1	50.0
9b	*O*-*t*-Butyl	183	1.6	1.6	0.1	12.5
15a	NH-*i*-Propyl	16	0.4	0.1	0.8	1.6
15b	NH-*c*-Pentyl	10	1.6	0.2	0.1	1.6
15c	NH-Ph	49	0.4	0.4	0.1	1.6
15d	NH-Ph(2-MeO)	41	0.1	0.2	0.1	0.8
15e	NH-Ph(2-F)	41	0.2	0.1	0.1	0.4
15f	NH-Pyridin-2-yl	43	1.6	0.1	0.1	0.8

Introduction of amide derivatives at P_3' site (Table 2) led to compounds having more potent activity than the carbamate compound (**9b**) (Table 1). Variation in the P_3' amide derivatives did not show much difference in PDF inhibition and antibacterial activity. Substitution at the phenyl ring (**12d, 12e**) slightly improved the enzyme inhibition and antibacterial activity. Nitrogen atom's position in the pyridine ring appeared to have little effect on antibacterial activity. Introduction of quinoline (**12h**) and isoquinoline (**12i**) showed increased enzyme inhibition, but antibacterial activity was similar to that of pyridine derivatives.

Urea derivatives (Table 3) also displayed potent PDF inhibition and antibacterial activity against tested strains. Both alkyl and aromatic ureas were well tolerated at the P_3' position. Compound **15d** having the same urea P_3' moiety in compound **1** displayed significantly increased antibacterial activity against *S. aureus* than compound **1**, which showed that chiral alkyl chain and reverse hydroxamic acid might be important factor to improve antibacterial activity against *S. aureus*.

In order to obtain an insight on the structural differences between **1** and **15d** in their binding modes, we conducted a modeling

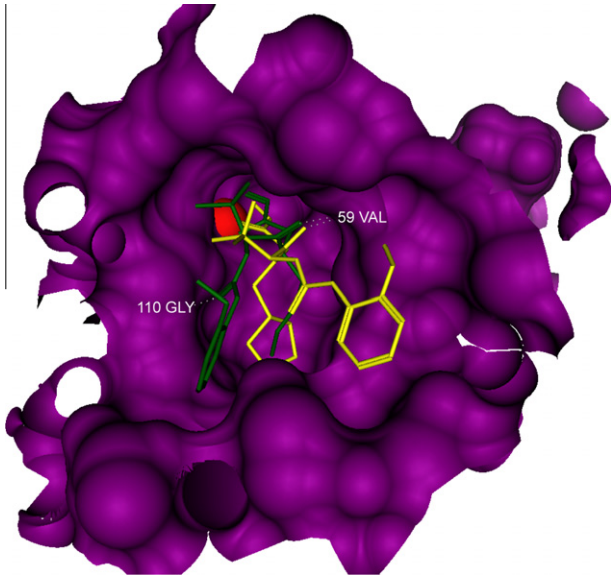


Figure 2. Docking model of compound **1** (yellow) and compound **15d** (green) were superimposed with active site of X-ray crystal structure of *S. aureus* PDF (RCSB protein data bank ID: 1Q1Y). The Zn^{2+} atom is colored in red. The accessible surface of the binding site is shown in purple.

study based upon the X-ray crystal structure of *S. aureus* PDF.[11] The compound **1** and **15d** fit into the active site of *S. aureus* PDF and the resulting 3D structures of **1** and **15d** are superimposed on the active site (Fig. 2). Superimposition of these structures yields a close overlap between the P_1' side chains of **1** and **15d**, but P_2' and P_3' side chains of **1** and **15d** show the different binding modes. P_2' side chain of compound **15d** is located more deeply in the binding pocket than that of compound **1**. Additionally P_3' site of compound **15d** is not overlapped with compound **1** and oxygen atom of urea group in compound **15d** is hydrogen bonding with backbone atom (Gly110). These factors may increase antibacterial activity of compound **15** against *S. aureus*.

A series of non-peptide, reverse hydroxamide inhibitors was designed based on the known peptide inhibitor **1.** An SAR study of these non-peptide inhibitors led to identification of potent PDF inhibitors. Modeling study shows that compound **1** and **15d** have different binding modes on active site of *S. aureus* PDF.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.056.

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