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Peptide deformylase inhibitors with retro-amide scaffold: Synthesis and structure–activity relationships

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ABSTRACT

Peptide deformylase (PDF) is a metalloprotease catalyzing the removal of a formyl group from newly synthesized proteins. Thus inhibition of PDF activity is considered to be one of the most effective antibiotic strategies. Reported herein are the synthesis and structure–activity relationship studies of retro-amide inhibitors based on actinonin, a naturally occurring PDF inhibitor. Analysis of the structure–activity relationships led to the discovery of **7a**, which exhibits potent enzyme inhibition and antibacterial activity against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.

The increased prevalence of antibiotic resistance resulting from evolution of bacterial genes is becoming one of major health concerns. After an extensive search for new antibiotics, many effective antibacterial drugs have been developed since the 1940s. However, many of these agents share the same target and the actual number of antibacterial targets is very limited. Industrial and academic antibacterial research groups have been trying to find inhibitors that interfere with new targets and thus can shed a light in solving the antibiotic resistance problems.

All proteins produced in prokaryotic translation process begin with *N*-formyl methionine, which is commonly removed in a multi-step process beginning with deformylation. Peptide deformylase (PDF), which is an essential enzyme in protein synthesis in bacteria, has been recognized as a potent and useful target for antibacterial therapy.[1] It is metalloprotease, which has a function of removing the formyl group at the *N*-terminal methionine residue of nascent proteins.[2] The naturally occurring antibiotic actinonin, which was first isolated in 1962 from an actinomycete,[3] is a potent inhibitor of PDF.[4] There have been many reports on the PDF inhibitors containing peptide[5] or non-peptide[6] scaffolds, and peptide scaffold inhibitors[5] based on actinonin have shown potent effects on both PDF enzyme inhibition and cell-based antibacterial activity. Among various peptidomimetic scaffolds, retro-amide is one of possible bioisosteres of amide function.[7] To the best of our knowledge, there have been no reports on PDF inhibitors having *N*-alkyl retro-amide at P'_1 site. We undertook a comprehensive structure–activity relationship (SAR) study on PDF inhibitors equipped with the retro-amide scaffold, particularly focusing on the modification of P'_1, P'_2, and P'_3 residues. Our SAR study led to the discovery of **7a** that possesses a promising antibacterial activity (Fig. 1). Here, we describe the synthesis of retro-amide inhibitors and corresponding SAR around P'_1, P'_2, and P'_3 positions.

The synthesis of PDF inhibitors used in this study is outlined in Schemes 1 and 2.[8] Compound **2** was prepared from alkylation of glycine ethyl ester hydrochloride using corresponding alkyl tosylate in the presence of $NaHCO_3$. In this step, employment of a stron-

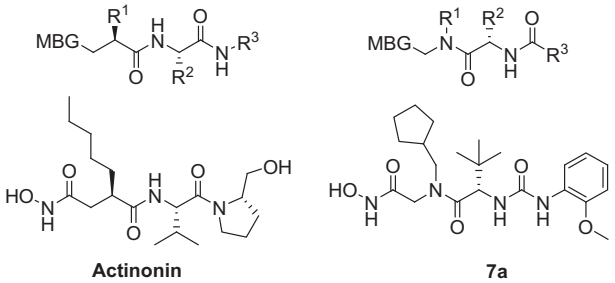

Figure 1. Pharmacophore and structure of PDF inhibitor actinonin and retro-amide **7a**.

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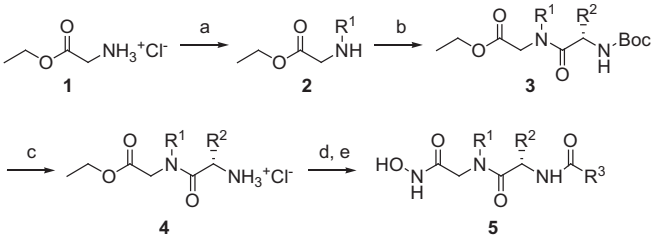
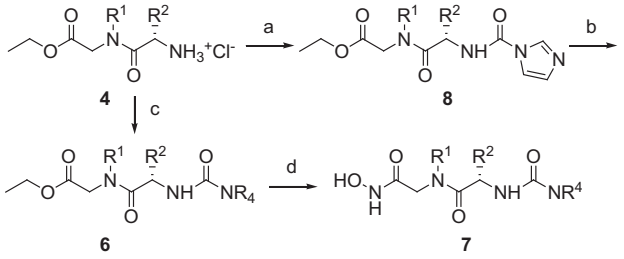


Scheme 1. Reagents and conditions: (a) R^1OTs, $NaHCO_3$, acetonitrile, 60 °C, 18 h; (b) *N*-Boc-L-amino acid (introduction of R^2), HOBt, EDC, diethylamine, dichloromethane, rt, 1 h; (c) 4 M HCl in 1,4-dioxane, rt, 18 h; (d) R^3COCl, triethylamine, dichloromethane; (e) hydroxylamine, MeOH, rt, 30 min.



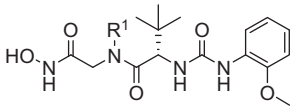
Scheme 2. Reagents and conditions: (a) carbonyl diimidazole, *N*,*N*-diisopropylethylamine, dichloromethane, rt, 4 h; (b) R^4NH, toluene, reflux, 18 h; (c) R^4NCO, triethylamine, dichloromethane, rt, 18 h; (d) hydroxylamine, MeOH, rt, 30 min.

ger base such as K_2CO_3 led to lower yield due to hydrolysis of the product. The corresponding alkyl tosylate was obtained conventionally from alkyl alcohol through reaction with tosyl chloride in the presence of DMAP and triethylamine. Compound **2** was coupled to *N*-Boc-L-amino acid through standard peptide coupling methodology using EDC and HOBt. Removal of the *N*-Boc group with 4 M HCl solution in 1,4-dioxane gave ammonium chloride salt **4**. For the synthesis of bis(retro-amide) **5**, compound **4** was treated with corresponding acid chloride in the presence of triethylamine and subsequent substitution with hydroxylamine produced the final amide **5**. For the synthesis of retro-amide–urea derivatives, compound **4** was directly coupled with corresponding isocyanate to provide compound **6**. When the corresponding isocyanate was not commercially available, compound **4** was treated with carbonyl diimidazole[9] and the resulting imidazolide **8** was coupled with the corresponding amine in toluene to give compound **6**. The final urea **7** was obtained through treatment of compound **6** with a solution of hydroxylamine in ethyl alcohol.[10]

Compounds were tested using a *Pseudomonas aeruginosa* Ni-PDF enzyme assay and primary in vitro antibacterial activity was tested against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. These strains are known to cause respiratory tract-associated infection. Data from these assays on PDF inhibitors are summarized in Tables 1–3.

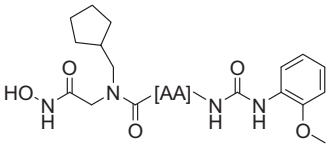
The size of the ring substituted at P'_1 side chain appears to have little effect on the enzyme inhibition activity, but showed difference in antibacterial activity against tested strains (Table 1). Introduction of the cyclopropyl at P'_1 site (**7d**) caused decrease in antibacterial activity compared to other analogs (**7a** and **7e**). As the ring size was increased from a three-membered ring to a five-membered ring, antibacterial activity was improved, and cyclopentylmethyl analog (**7a**) showed optimal activity. Incorporation of cyclopentylethyl (**7b**) resulted in slightly reduced activity relative to that of cyclopentylmethyl derivative (**7a**), which shows that the cyclopentylmethyl group is optimal for the P'_1 binding pocket. Branched alkyl chain (**7c**) is also tolerated in hydrophobic

Table 1
PDF inhibition and in vitro antibacterial activities for P'_1 side chain



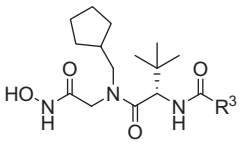
Compound	R^1	IC_{50} (nM)	MIC (μg/ml)		
		P. aer PDF	S. pne	H. inf	M. cat
7a	Cyclopentylmethyl	29	0.2	0.2	0.1
7b	Cyclopenylethyl	31	0.4	0.8	0.1
7c	*i*-Pentyl	21	0.8	0.8	0.1
7d	Cyclopropylmethyl	15	12.5	6.3	1.6
7e	Cyclobutylmethyl	13	0.4	0.4	0.1

Table 2
PDF inhibition and in vitro antibacterial activities for P'_2 side chain



Compound	AA	IC_{50} (nM)	MIC (μg/ml)		
		P. aer PDF	S. pne	H. inf	M. cat
7a	*t*-Leucine	29	0.2	0.2	0.1
7f	Valine	13	0.8	0.8	0.1
7g	Alanine	42	6.3	12.5	12.5
7h	Phenylalanine	61	25.0	25.0	12.5
7i	*i*-Leucine	32	3.2	1.6	0.2
7j	Leucine	36	3.2	3.2	0.8
7k	Proline	116	200.0	200.0	50.0

Table 3
PDF inhibition and in vitro antibacterial activities for P'_3 retro-amides

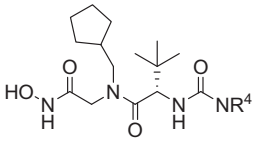


Compound	R^3	IC_{50} (nM)	MIC (μg/ml)		
		P. aer PDF	S. pne	H. inf	M. cat
5a	*i*-Propyl	52	100.0	50	0.8
5b	Phenyl	41	12.5	3.2	0.1
5c	Thiophene-2-yl	19	12.5	1.6	0.2
5d	Ph(2-MeO)	11	50.0	0.4	0.1

P'_1 binding pocket, but compound **7c** displayed reduced antibacterial activity compared to **7a**.

Variation in P'_2 amino acids displayed significant effect on enzyme inhibition and antibacterial activity. From the assay data summarized in Table 2, preferred side chain for P'_2 site appears to be a branched ethyl group because valine and *t*-leucine derivatives (**7a** and **7f**, respectively) revealed potent inhibition against enzyme and bacterial strains. Amino acid derivatives (**7g–7j**) with a shorter or longer side chain than valine displayed reduced activity. Interestingly proline derivative **7k** showed significantly reduced antibacterial activity. The loss in activity can be explained by the

Table 4
PDF inhibition and in vitro antibacterial activities for P'_3 ureas



Compound	NR^4	IC_{50} (nM)	MIC (μg/ml)		
		P. aer PDF	S. pne	H. inf	M. cat
7a	NH-Ph(2-MeO)	29	0.2	0.2	0.1
7l	NH-Ph	17	3.2	0.4	0.1
7m	Piperidine-1-yl	47	12.5	12.5	0.8
7n	N(Me)-Ph	50	50.0	25.0	0.4
7o	Morpholin-4-yl	28	25.0	12.5	0.2
7p	NH-*c*Hex	55	6.3	6.3	0.1
7q	NH-ethyl(2-MeO)	42	6.3	6.3	0.1
7r	NH-thiazol-2-yl	43	12.5	0.4	0.1
7s	NH-pyridin-2-yl	54	12.5	0.8	0.1
7t	NH-pyridin-4-yl	65	25.0	6.3	0.2
7u	NH-pyridin-3-yl	49	6.3	3.2	0.1

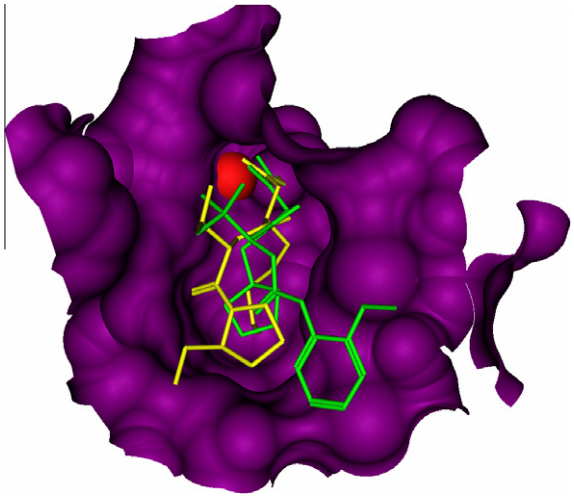


Figure 2. Compound **7a** (green) is superimposed with an X-ray crystal structure of actinonin (yellow) that is bound to *Pseudomonas aeruginosa* Zn–PDF (RCSB protein data bank ID: 1LRY). The Zn^{2+} atom is colored in red. The accessible surface of the binding site is shown in purple.

absence of hydrogen bonding of NH hydrogen with a nearby water molecule or a carbonyl oxygen in the enzyme backbone. One of major differences between actinonin and our retro-amide inhibitors is the number of backbone atoms between P'_1 and P'_2 side chains. In spite of this difference in backbone chain length, it appears that side chains of retro-amide inhibitors may be properly located in the binding pocket.

The introduction of urea derivative (**7l**) at P'_3 site (Table 4) led to compounds having more potent antibacterial activity than the corresponding amide compound **5b** (Table 3). Phenyl urea (**7l**) was found to be a preferred substituent for the P'_3 position. Introduction of a heterocyclic ring (**7r–7u**) instead of the phenyl group resulted in the reduction of antibacterial activity. For urea derivatives, methoxy group substitution at the 2-position of the phenyl ring (**7a**) significantly increased the antibacterial activity against the tested strains.

We conducted a modeling study based upon the X-ray crystal structure of actinonin/*P. aeruginosa* Zn–PDF complex[11] to gain an insight on the structural differences between **7a** and actinonin in their binding modes. The compound **7a** fits into the active site of *P. aeruginosa* Zn–PDF and the resulting 3D structure of **7a** is superimposed with X-ray crystal structure of actinonin/*P. aeruginosa* Zn–PDF complex (Fig. 2). Superimposition of these structures yields a close overlap between the P'_1 and P'_2 side chains of **7a** and that of actinonin.

An extensive SAR study of retro-amide PDF inhibitors led us to discover **7a**, which displays a good in vitro antibacterial activity against pathogens associated with respiratory tract infection. Modeling study showed that the P'_1 and P'_2 side chains of **7a** are very nicely overlapped with those of actinonin. Our results show that the retro-amide scaffold can be used as an excellent bioisostere of amide group for peptide inhibitors in the PDF inhibitor design.

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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.2010.06.088.

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