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Design, synthesis and antibacterial activity of 3-methylenepyrrolidine formyl hydroxyamino derivatives as novel peptide deformylase inhibitors

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ABSTRACT

The synthesis and antibacterial activity of 3-methylenepyrrolidine formyl hydroxyamino derivatives are reported. The antibacterial activities of these derivatives were evaluated to discover SAR at $P^{1'}$ and $P^{3'}$ positions, and most of these derivatives exhibit better in vitro antibacterial activity than existing drugs against drug-resistant clinical isolates including MRSA, PRSP, and *Haemophilus influenzae*.

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The emergence of life threatening, multidrug-resistant (MDR) bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA) have been a serious concern both in the hospital and community settings.[1] MRSA infections are the leading cause of nosocomial infections and cause increased number of deaths every year.[2] The latest antibiotic Linezolid (Zyvox™) was approved by US-FDA in 2000, unfortunately, already a few cases of Linezolid resistant pathogens in hospital isolates have been reported.[3] While vancomycin is an efficient therapeutic agent for most antibiotic-resistant Gram-positive bacteria, vancomycin-resistant *S. aureus* (VRSA) was reported in the U.S. in 2002.[4] This crisis has resulted in an intensive research effort to develop a new class of compounds that exhibit novel mechanisms of antibacterial activity. Antibacterial drugs with a new mode of action are expected to have no pre-existing resistance.

Peptide deformylase (PDF) catalyzes the removal of the *N*-formyl group from the N-terminal methionine during bacterial protein maturation, and mammalian cytosolic protein synthesis does not produce N-formylated polypeptides,[5] making PDF an attractive target for developing antibiotics with novel mechanisms of action. Actinonin (Fig. 1), a naturally occurring antibiotic isolated in 1962 from an actinomycete,[6] was the first PDF inhibitor reported by the researchers from Vicuron. It showed moderate antibacterial activity against several Gram-positive and Gram-negative bacteria,[7] however, actinonin did not show good in vivo antibacterial activity due to poor pharmacokinetic properties, either

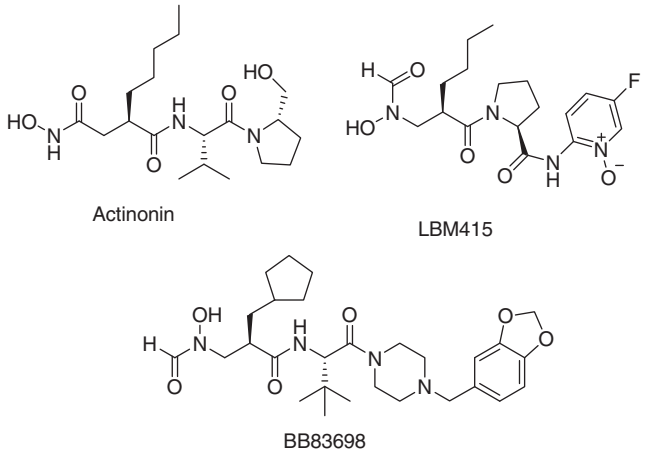

Figure 1. Structures of actinonin, LBM415 and BB83698.

poor absorption or quick clearance.[8] Many pharmaceutical companies and academic institutions have focused on the development of novel PDF inhibitors.[9] Thus far, two PDF inhibitors underwent human clinical trials, BB83698 (Fig. 1, discovered by British Biotech, in collaboration with Genesoft) and LBM415 (Fig. 1, discovered by Vicuron pharmaceuticals, in collaboration with Novartis), but there are no currently marketed PDF inhibitors. We have reported the synthesis of a series of PDF inhibitors with a 2,5-dihydropyrrole motif and evaluated their antibacterial activity.[10] The motif of

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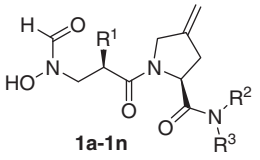


Figure 2. Generic structure of **1a**–**1n**.

3-methylenepyrrolidine is found in many biologically active products.[11] In continuation of our efforts to find new PDF inhibitors through structural modification of LBM415, we continued research on the exploring the structure–activity relationship (SAR) by replacing the pyrrolidine functionality at the $P^{2'}$ position with 3-methylenepyrrolidine. A number of 3-methylenepyrrolidine formyl hydroxyamino derivatives **1a–1n** (Fig. 2) were synthesized as novel PDF inhibitors, and their in vitro antibacterial activities were evaluated.

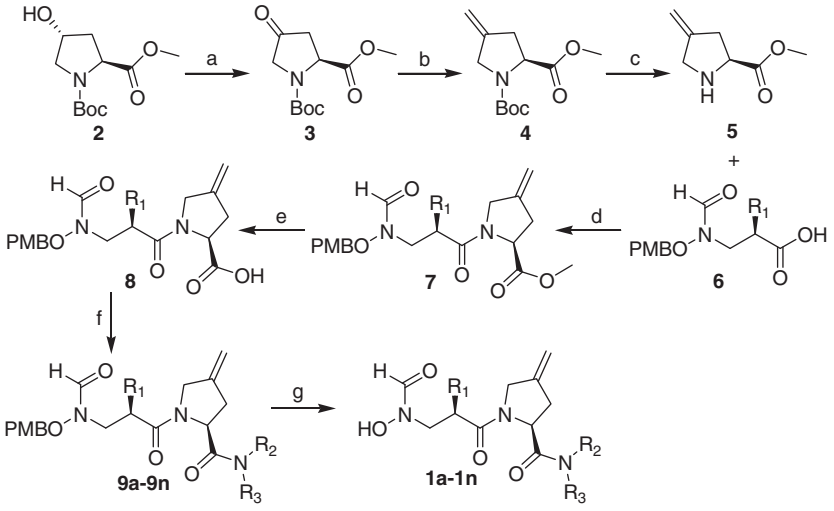
A general synthesis of 3-methylenepyrrolidine formyl hydroxyamino derivatives **1a–1n** is illustrated in Scheme 1, and the synthetic route is similar to that we reported previously.[10] In the synthesis shown in Scheme 1, we started from the methyl ester of *N*-Boc-*trans*-4-hydroxy-L-proline (**2**). A Swern oxidation of **2** afforded corresponding ketone **3** in good yield according to a literature procedure.[12] Treatment of **3** with a Wittig reagent (prepared by methyl triphenyl phosphonium bromide and potassium *tert*-butoxide) gave olefin **4**,[13] followed by removal of the Boc group to afford building block **5** in a quantitative yield. The coupling reaction between **5** and **6** (R_1 is *n*-butyl or cyclopentyl methyl) was carried out using 1-hydroxy-benzotriazole monohydrate (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as coupling reagents in the presence of *N*-methylmorpholine (NMM) to produce coupling product **7** smoothly, which gave corresponding acid **8** after hydrolysis. Penultimate compounds **9a–9n** were obtained by coupling of **8** with various amines R_2R_3NH. Finally, deprotection of the PMB group with trifluoroacetic acid successfully provided the desired 3-methylenepyrrolidine derivatives **1a–1n**. All the compounds were characterized by ^1H NMR, ^{13}C NMR and HR-MS (ESI), and HPLC purity of the compounds ranges from 90% to 97% AP.

The new compounds **1a–1n** were screened against Gram-positive bacterial strains such as *S. aureus* and *S. epidermidis*, and the results are summarized in Table 1. Moderate antibacterial activity (MIC 8–16 μg/ml) was observed for compound **1a** that bears an aliphatic amino morpholine group at $P^{3'}$ position. This is interesting because no antibacterial activity was observed from corresponding 2,5-dihydropyrrole derivatives in our previous study.[10] Compounds **1b–1d** bearing aromatic amines at $P^{3'}$ position typically gave moderate to good antibacterial activity (MIC 0.5–8 μg/ml). Good to excellent antibacterial activities (MIC 0.125–4 μg/ml) were observed when heterocyclic aromatic amines were introduced at the $P^{3'}$ position in compounds **1e–1g**. Preliminary SAR study at $P^{1'}$ position was also investigated. Because cyclopentylmethyl group at $P^{1'}$ position in BB83698 showed excellent antibacterial activity, we decided to screen antibacterial activity of compounds **1h–1n** that bear a cyclopentyl methyl group at the $P^{1'}$ position. We were gratified to find that compounds **1h–1n** exhibited better antibacterial activity than corresponding analogs **1a–1g** that bear an *n*-butyl group (**1a** vs **1h**, **1b** vs **1j**, **1c** vs **1k**, **1f** vs **1m** and **1g** vs **1n**). Similar SAR at $P^{3'}$ position was observed for **1h–1n**. Among the compounds screened, **1n** gave the best antibacterial activity (0.0625–0.5 μg/ml) against *S. aureus*, MSSA, MRSA and *S. epidermidis*.

As PDF inhibitors were originally pursued with the belief to generate a respiratory drug, we tested the antibacterial activity of **1g**, **1i**, **1k–1n** against MRSA, MRSE, *Streptococcus pneumoniae*, PRSP, *Haemophilus influenzae* clinical isolates. The screening results are summarized in Table 2. While moderate antibacterial activity (1–32 μg/ml) against Gram-negative strain *H. influenzae* was observed, excellent antibacterial activity (0.0625–4 μg/ml) against the other Gram-positive tested strains was observed. Here, compound **1m** showed better antibacterial activity than LBM415 and other existing drugs such as Penicillin, Ciprofloxacin, Linezolid and Vancomycin.

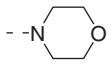
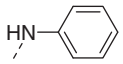
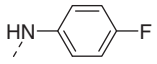
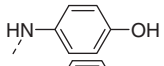
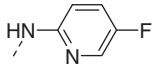
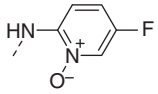
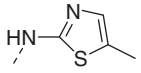
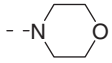
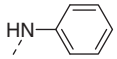
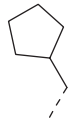
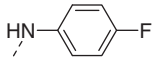
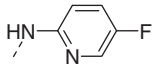
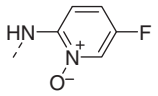
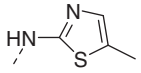
At last, two representative compounds **1e** and **1g**, along with the control compound amoxicillin, were screened against a panel of 14 isolates representing a broad-spectrum of activity. The results are summarized in Table 3. Compound **1g** shows good activity against all isolates tested except for *E. coli*.

In summary, we have synthesized a number of 3-methylenepyrrolidine formyl hydroxyamino derivatives **1a–1n**, and screened their antibacterial activity against a wide range of drug-resistant bacteria including some clinical isolates. Preliminary SAR on $P^{1'}$ and $P^{3'}$ positions of the compounds was studied. Compounds **1g**,



Scheme 1. Reagents and conditions: (a) (i) DMSO, oxalyl chloride, CH_2Cl_2, −78 °C, 10 min; (ii) Et_3N, −78 °C, 80%; (b) (i) $Ph_3P^+CH_3{\cdot}Br^-$, *t*-BuOK, THF, 0 °C–25 °C, 1 h; (ii) **3**, 1 h, 60%; (c) TFA/CH_2Cl_2, Et_3N, 30 min, quantitative yield; (d) HOBT, EDCI, NMM, CH_2Cl_2, 25 °C, 16 h, 84%; (e) LiOH, dioxane/H_2O, 25 °C, 5 h, 90%; (f) Et_3N, $ClCO_2Et$, R_1R_2NH, THF, 0 °C–25 °C, 16 h, 60–81%; (g) TFA/CH_2Cl_2, column chromatography purification, 35–45%.

Table 1
In vitro minimum inhibitory concentration (MIC, μg/ml) values of **1a–1r** against bacterial strains[a]

Compound	R^1	$-NR^2R^3$	*S. aureus* ATCC25923	MSSA ATCC29213	MRSA ATCC43300	*S. epidermidis* ATCC12228
1a			8	>64	8	16
1b			2	8	1	2
1c			1	4	0.5	1
1d			8	32	4	8
1e			1	2	0.125	0.5
1f			2	4	1	2
1g			0.5	1	0.125	0.125
1h			4	8	2	4
1i			4	8	1	4
1j			0.5	1	0.25	0.5
1k			1	2	0.5	1
1l			0.5	1	0.0625	0.125
1m			0.125	2	0.125	0.5
1n			0.0625	0.5	0.0625	0.125
	LBM415		0.5	2	0.5	1
	Penicillin		0.0625	1	64	2
	Ciprofloxacin		0.5	0.5	1	0.25
	Linezolid		2	2	4	0.5
	Vancomycin		1	1	1	2

[a] MIC were determined by broth micro dilution technique. MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2
In vitro minimum inhibitory concentration (MIC, μg/ml) values of **1g**, **1i**, **1k–1n** against various clinical isolates[a]

Compound	MSSA 1 strain	MRSA 3 strains	MSSE 1 strain	MRSE 3 strains	PSSP 1 strain	PRSP 3 strains	H. F 4 strains
1g	1	0.5–1	1	2	0.25	0.5–32	4–32
1i	2	0.5–2	1	4	1	1–2	4–32
1k	1	0.25–4	0.5	0.25–2	0.25	0.25–4	4–8
1l	0.5	0.25–1	0.5	0.5–4	0.25	0.25–0.5	2–8
1m	0.5	0.125–2	0.125	0.25–0.5	0.25	0.0625–0.25	2–8
1n	1	0.0625–1	1	0.5–1	0.25	0.25	1–8
LBM415	0.5	0.125–1	0.5	0.125–1	0.125	0.5–1	2–8
Penicillin	0.5	64–>64	4	64–>64	2	64–>64	16–>64
Ciprofloxacin	1	1, 32–64	0.25	1, 64–>64	2	0.5–8	0.125–4
Linezolid	1	0.5–2	1	1	0.5	1, 8	ND
Vancomycin	1	1–2	1	1–4	1	1, 16	ND

[a] MIC were determined by agar dilution method. MSSE, methicillin-susceptible *Staphylococcus epidermidis*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; H. F, *Haemophilus influenzae*.

Table 3
In vitro minimum inhibitory concentration (MIC, μg/ml) values of **1e**, **1g** against 14 bacterial strains[a]

	1e	1g	Amoxicillin
Staphylococcus aureus OXFORD	4	0.5	0.125
Staphylococcus aureus WCUH29	0.5	0.125	64
Enterococcus faecalis I	32	4	0.5
Enterococcus faecium X7501	4	2	16
Haemophilus influenzae Q1	16	4	0.25
Haemophilus influenzae H128	16	4	32
Haemophilus influenzae H128 Acr A-	0.5	⩽0.06	64
Moraxella catarrhalis 1502	0.25	⩽0.06	⩽0.06
Streptococcus pneumoniae 1629	16	2	⩽0.06
Streptococcus pneumoniae N1387	4	1	2
Streptococcus pneumoniae ERY2	8	2	⩽0.06
Escherichia coli 3	>64	64	1
Streptococcus pyogenes 1307006P	4	2	⩽0.06
Streptococcus pyogenes 1308007P	4	2	⩽0.06

[a] MIC endpoints were determined by broth microdilution according to CLSI guidelines

1m and **1n** showed very promising antibacterial activity, and their in vivo studies are currently in progress.

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