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Design, synthesis, and antibacterial activity of 2,5-dihydropyrrole formyl hydroxyamino derivatives as novel peptide deformylase inhibitors

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ABSTRACT

The synthesis and antibacterial activity of 2,5-dihydropyrrole formyl hydroxyamino derivatives are reported. The antibacterial activities of these derivatives were evaluated, and some of these derivatives showed better in vitro antibacterial activity than existing drugs, including penicillin, ciprofloxacin, vancomycin, and linezolid.

The rising morbidity caused by antibiotic-resistant Gram-positive pathogenic bacteria has become a major concern for clinicians and the public health system. For example, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in U.S. hospitals is presently in the range of 33–55% in 2006,[1] and treatment for infections caused by antibiotic-resistant bacteria has become complex and costly. One worrisome trend is the high prevalence of antibiotic-resistant bacteria strains in community-acquired infections.[2] 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.[3] Therefore, it is imperative to search for novel drug candidates with new modes of action against drug-resistant bacteria, because antibacterial drugs with a new mode of action are expected to have no pre-existing resistance.

Bacterial peptide deformylase (PDF), an iron-containing metalloproteinase, is an attractive target for studies focusing on developing new antibiotics for the following reason: while PDF does not share close homology with any mammalian equivalent, it catalyzes the removal of the *N*-formyl group from the N-terminal methionine during bacterial protein synthesis.[4] The proposed transition state structure corresponds to a formylmethionyl peptide bound to a ferrous ion at the active site of the deformylase enzyme. The transition state indicates that the generic inhibitor structure should combine the characteristics of both the chelator and the peptidomimetic backbone (Fig. 1).[5] Actinonin (Fig. 2), a naturally occurring antibiotic isolated in 1962 from an actinomycete,[6] was the first reported PDF inhibitor. It showed moderate antibacterial activity against several Gram-positive and Gram-negative bacteria,[7] but did not show good in vivo antibacterial activity due to poor pharmacokinetic properties, which could be attributed to either poor absorption or quick clearance.[8]

Thus far, many pharmaceutical companies and academic institutions have focused on the development of novel PDF inhibitors.[9] The first two PDF inhibitors that underwent human clinical trials are BB83698 (Fig. 2, discovered by British Biotech, in collaboration with Genesoft) and LBM415 (Fig. 2, discovered by Vicuron pharmaceuticals, in collaboration with Novartis).[8] LBM415 was reported to have better in vitro antibacterial activity than BB83698.[8,10] Investigations of the structure–activity relationships based on LBM415 modification have been reported.[11] α,β-Dehydroamino acids were found in many biologically active natural products, including antrimycins, tentoxin, and the phosphatase inhibitors microcystin and nodularin.[12] In this study, we report our approach to the modification of LBM415, in which the pyrrolidine functionality at the $P^{2'}$ position is replaced by 2,5-dihydropyrrole. A number of 2,5-dihydropyrrole formyl hydroxyamino derivatives **1a**–**1r** (Fig. 3) were synthesized as novel PDF inhibitors, and their in vitro antibacterial activities were evaluated.[13]

A general synthesis of 2,5-dihydropyrrole formyl hydroxyamino derivatives (**1a**–**1r**) is illustrated in Scheme 1 and is similar to a previously reported route.[14] In the synthesis, dimethyl 2-butylmalonate was hydrolyzed with 30% aqueous sodium hydroxide to afford 2-butylmalonic acid **2**, which was converted to 2-methylenehexanoic acid **3** by treatment with formaldehyde. Reaction of **3** with pivaloyl chloride gave an anhydride intermediate, which was treated with the anion of (*S*)-4-benzyl-2-oxazolidinone to

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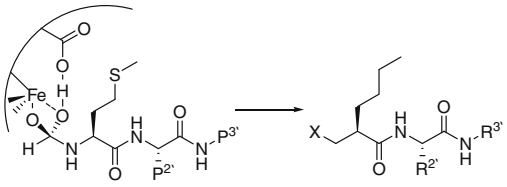


Figure 1. Generic PDF inhibitor structure.

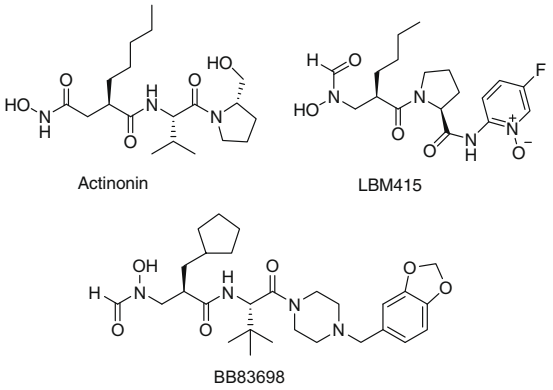


Figure 2. Structures of Actinonin, LBM415 and BB83698.

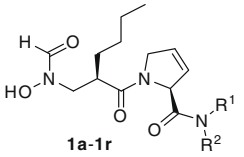
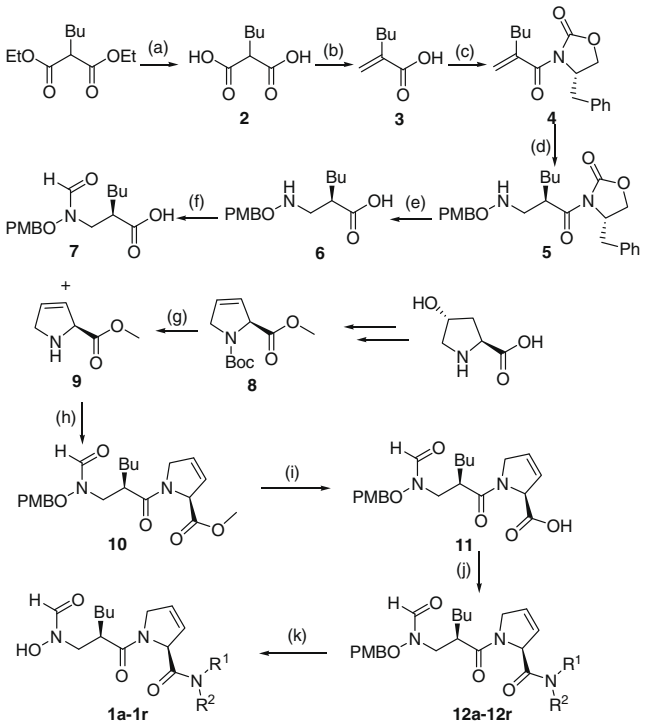


Figure 3. Generic structure of 1a–1r.



Scheme 1. Reagents and conditions: (a) NaOH, H_2O, reflux, 3–4 h, 89%; (b) 37% HCHO, Et_2NH, EtOH, reflux, 16 h, 84%; (c) i—**3**, pivaloyl chloride, Et_3N, THF, −60 °C; ii—LDA; (*S*)-4-benzyl-2-oxazolidinone, −60 °C; iii—−60 to 25 °C, 3 h, 63%; (d) i—$PMBONH_2$, 50 °C, 24 h; ii—PTSA, Na_2CO_3, EA/H_2O, 58%; (e) LiOH, 30% H_2O_2, Na_2SO_3, 0 °C, 1 h, 58%; (f) HCO_2Et, 60 °C, 16 h, 80%; (g) TFA/CH_2Cl_2, Et_3N, 25 °C, 30 min, quantity; (h) HOBt, EDCI, NMM, CH_2Cl_2, 25 °C, 16 h, 84%; (i) LiOH, dioxane/H_2O, 25 °C, 5 h, 99%; (j) Et_3N, $ClCO_2Et$, R_1R_2NH, THF, 0–25 °C, 16 h, 60–81%; (k) TFA/CH_2Cl_2, purified by chromatograph column, elute with CH_2Cl_2/MeOH 50:1, 35–45%.

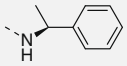
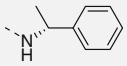
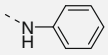
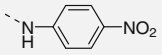
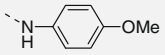
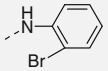
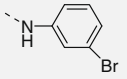
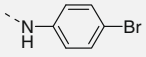
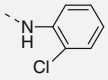
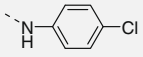
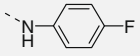
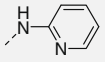
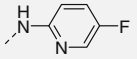
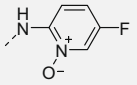
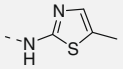
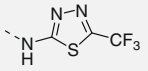
yield conjugated enone **4**. The diastereoselective Michael addition reaction of **4** with *O*-(4-methoxybenzyl)hydroxylamine[15] afforded **5** as a single diastereomer with a yield of 58% after recrystallization in *t*-butyl methyl ether. The chiral auxiliary was removed by LiOH and H_2O_2 to give the corresponding carboxylic acid derivative **6**. Formylation of **6** using ethyl formate yielded **7**.

The *N*-Boc protected 2,5-dihydropyrrole derivative **8** was prepared in accordance with a previously described procedure.[16] Successful deprotection of **8** with trifluoroacetic acid afforded **9** in quantitative yield. The coupling reaction between **7** and **9** was successfully carried out using 1-hydroxy-benzotriazole monohydrate (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of *N*-methylmorpholine (NMM) to produce **10** with a yield of 84%. Hydrolysis of **10** followed by coupling with amines R_1R_2NH gave compounds **12a**–**12r**. Finally, removal of the PMB protecting group using trifluoroacetic acid successfully provided the desired 2,5-dihydropyrrole formyl hydroxyamino derivatives **1a**–**1r**. These compounds were characterized by ^1H NMR, ^{13}C NMR and HR-MS(ESI), and HPLC purity of the compounds ranges from 90% to 97%.

The new compounds **1a**–**1r** were screened against Gram-positive bacterial strains such as *S. aureus* and *Staphylococcus epidermidis* and against the Gram-negative strain *Escherichia coliform*. The screening results are summarized in Table 1. The compounds containing aliphatic amines such as morpholine and cyclopropyl amine (**1a** and **1b**) exhibited unsatisfactory antibacterial activity against all the tested strains. Moderate antibacterial activity (MIC 4–16 μg/ml) was observed when using aliphatic 1-phenylethylamines that contain an aromatic ring at the $P^{3'}$ position (compounds **1c** and **1d**). Taking these results into account, we decided to replace the aliphatic amines at the $P^{3'}$ position with aromatic amines. Compounds **1e**–**1m**, each bearing aromatic amide moieties, were synthesized, and they show moderate to good antibacterial activity against the Gram-positive bacterial strains. Finally, heterocyclic aromatic amines were introduced at the $P^{3'}$ position, resulting in compounds **1n**–**1q**. Good to excellent antibacterial activities were observed with these compounds. Notably, compound **1q**, which contains a thiazolyl functionality, exhibited MIC values ranging from 0.0625 to 0.25 μg/ml against Gram-positive bacterial strains including *S. aureus*, MSSA, MRSA, and *S. epidermidis*. It is worth mentioning that **1q** also showed moderate antibacterial activity (MIC 16–32 μg/ml) against Gram-negative bacteria *Escherichia coli*. Because compound **1f**, which contains an electron-withdrawing group, showed favorable antibacterial activity, we incorporated the electron-withdrawing trifluoromethyl group in a heterocyclic amine to synthesize compound **1r**. Unfortunately, a dramatic decrease in antibacterial activity was observed for compound **1r**.

In summary, replacement of the pyrrolidine ring of LBM415 with a 2,5-dihydropyrrole backbone resulted in the synthesis of novel compounds having in vitro antibacterial activity against drug-resistant bacteria. The aliphatic amine derivatives **1a** and **1b** are poor antibacterial agents, but the aromatic amine derivatives **1e**–**1m** and heterocyclic amine derivatives **1n**–**1q** are more active against bacteria. Compound **1q** showed excellent in vitro antibacterial activity (MIC 0.0625–0.25 μg/ml) against Gram-positive bacterial strains, including drug-resistant bacteria MRSA. Further in vivo studies of **1q** are currently in progress in our lab.

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

Compound	$-NR^1R^2$	*S. aureus* ATCC25923	MSSA ATCC29213	MRSA ATCC43300	*S. epidermidis* ATCC12228	*E. coli* 1 ATCC25922	*E. coli* 2 ATCC35218
1a		>64	>64	32	>64	>64	>64
1b		64	32	2	4	64	32
1c		4	16	8	8	>64	>64
1d		4	16	2	4	>64	>64
1e		4	8	1	2	>64	64
1f		0.5	2	0.5	0.5	>64	32
1g		0.5	2	1	2	>64	>64
1h		32	4	1	8	>64	>64
1i		8	1	0.125	1	64	16
1j		4	1	0.5	2	>64	>64
1k		0.5	2	0.25	1	>64	64
1l		8	2	1	4	>64	>64
1m		2	2	1	4	>64	64
1n		0.125	1	0.125	0.5	>64	32
1o		0.5	1	0.25	0.5	>64	64
1p		0.5	1	0.5	1	>64	64
1q		0.0625	0.25	0.125	0.25	32	16
1r		32	16	8	16	>64	>64
LBM415		0.25	1	0.5	0.5	64	64
Linezolid		2	2	4	0.5	>64	>64
Penicillin		0.0625	1	64	2	>64	>64
Ciprofloxacin		0.5	0.5	1	0.25	<0.03125	<0.03125
Vancomycin		1	1	1	2	>64	>64

[a] MIC was determined by the microbroth dilution technique. MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

Acknowledgments

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