Bioorganic & Medicinal Chemistry Letters 21 (2011) 1541–1544

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Synthesis and in vitro antibacterial activity of oxazolidine LBM-415 analogs as peptide deformylase inhibitors

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ARTICLE INFO

*Article history:*
Received 30 November 2010
Accepted 20 December 2010
Available online 24 December 2010

*Keywords:*
Bacterial peptide deformylase (PDF)
Enzyme inhibitors
LBM-415

ABSTRACT

The drug resistant bacteria pose a severe threat to human health. The increasing resistance of those pathogens to traditional antibacterial therapy renders the identification of new antibacterial agents with novel antibacterial mechanisms an urgent need. In this study, a series of (2*S*)-*N*-substituted-1-[(formyhydroxyamino)methyl]-1-oxohexyl]-2-oxazolidinecarboxamides were designed, synthesized and evaluated for in vitro antibacterial activity. Most of these compounds displayed good activities against Gram-positive organisms comparable to reference agent LBM-415.



Antibiotic resistance is a serious global concern. The emergence of superbugs, bacterial pathogens that are resistant to many known antibiotics, is an imminent threat to public health.[1] The discovery of novel antibiotics with completely new mechanisms of action are urgently desirable to circumvent the rapid rise of resistance.[2] In that essence, bacterial peptide deformylase (PDF) is an attractive target for the exploration of a new antibiotic agent.[3] In bacteria protein synthesis, PDF removes the formyl group of the *N*-formyl methionine of the nascent protein in the press to afford the mature protein, and it is essential for the bacterial growth.[4,5] However, in mammalian cells, PDF was much less active compared with its bacterial counterpart, which provided a sound basis for selective inhibition.[6] Additionally, given that PDF, encoded by *def* gene, are essential to bacteria and it seems all bacterial PDF adopt a similar overall tertiary structure,[7,8] the inhibitors targeted at PDF could lead to broad spectrum activity.

Based on the crystal structure of PDF in the cofactor and substrate bound conformations,[9–11] many bacterial PDF inhibitors were designed and evaluated.[12–15] The general structure was derived and structure–activity relationship discussed. (Fig. 1)[16,17] LBM-415 (Fig. 1), with a pyrrolidine moiety at the $P_2$ position, is a potential PDF inhibitor progressed to clinical trial.[18] In vitro drug susceptibilities tests and in vivo characterization in infected animal mode showed that LBM-415 had a good antimicrobial activity equivalent to the marketed antibiotic agents.[19–22] In our previous effort in searching for new antimicrobial agents,[23–27] we investigated the effects of substituent of $P_3$ position of LBM-415, and obtained several compounds with improved antimicrobial activity in vitro.[28] In this study, we focused on the modification of the $P_2$ position of LBM-415. Oxazolidine moiety is an important building block for many biological active agents.[29,30] It is called pseudoproline to mimic the proline skeleton for investigation of peptide biological activity.[31] With this in mind, we substituted the pyrrolidine moiety of LBM-415 with oxazolidine at the $P_2$ position. A series of oxazolidinyl LBM-415 analogs (Fig. 2) were synthesized and their in vitro antibacterial activities were evaluated.

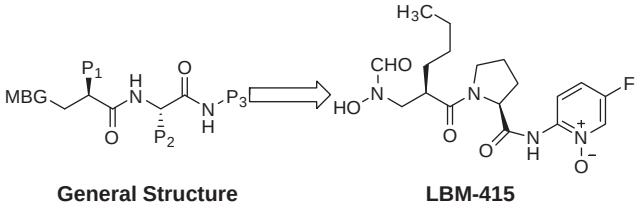


**Figure 1.** General structure of PDF inhibitor; structure of LBM-415.

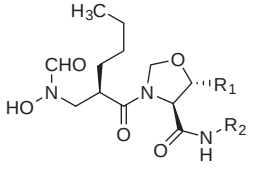


**Figure 2.** General structure of **1a**–**1r**.

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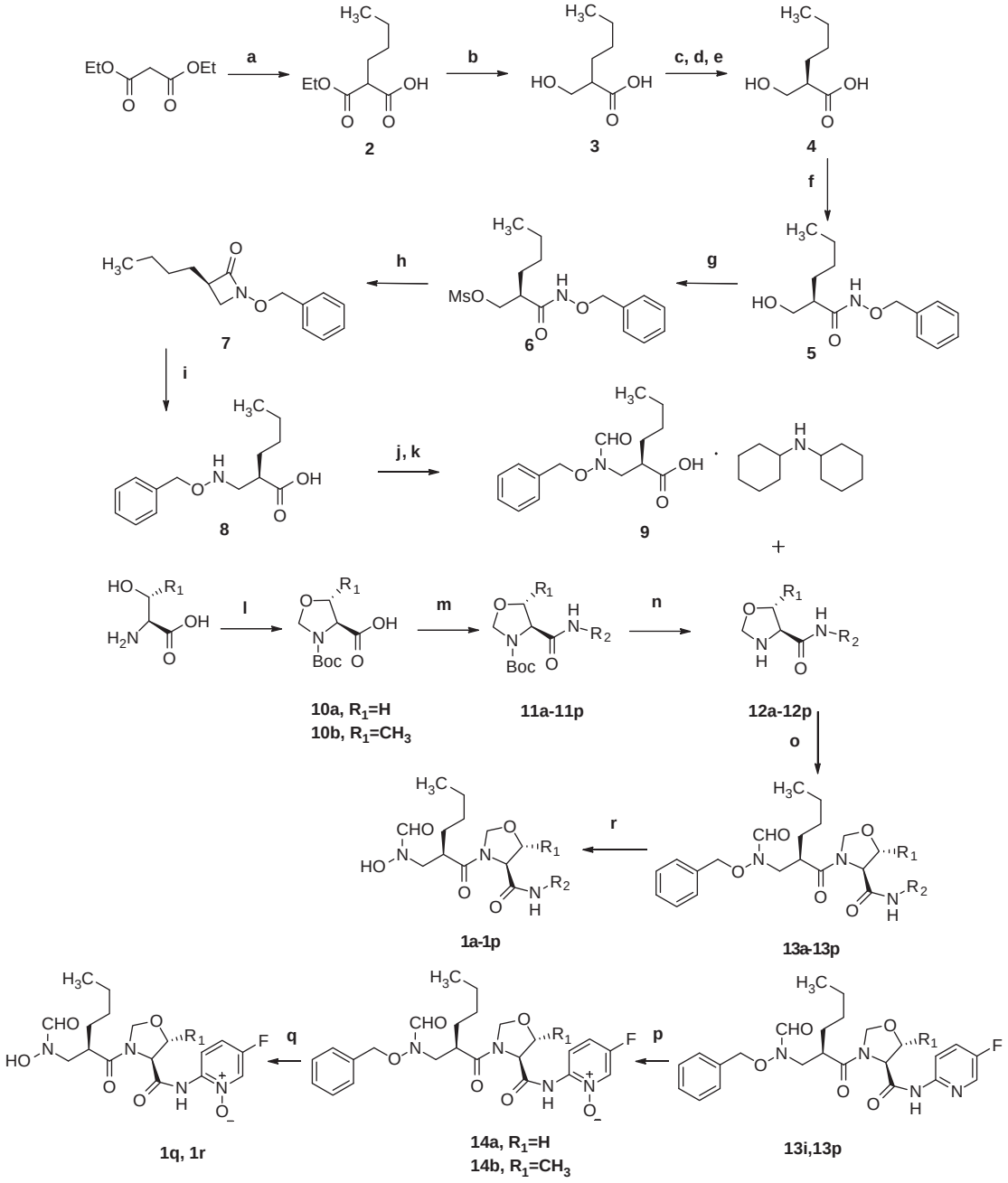


**Scheme 1.** Reagents and conditions: (a) EtONa, *n*-$C_4H_9Br$, EtOH; (b) KOH, EtOH; (c) $LiBH_4$, THF/*i*-PrOH; (d) (*R*)-1-phenylethanamine, EtOAc/*i*-PrOH; (e) HCl, EtOAc; (f) *O*-benzylhydroxylamine hydrochloride, EDCI, NaOH, $H_2O$; (g) MsCl, pyridine; (h) $K_2CO_3$, tetrabutylammonium bromide, THF; (i) LiOH, THF/$CH_3OH$/$H_2O$; (j) HCOOH/$Ac_2O$; (k) dicyclohexylamine, *n*-heptane; (l) HCHO, NaOH, $(Boc)_2O$; (m) CDMT, $R_2NH_2$; (n) HCl/EtOAc; (o) 2-propane phosphonic acid anhydride, EtOAc; (p) urea hydrogen peroxide; (q) 10% Pd/C, $HCOONN_4$, EtOH.

The synthesis of oxzaolidine formyl hydroxyamino derivatives is outlined in Scheme 1. The general strategy relies on the coupling of **9**, bearing the protected formyl hydroxyl amine group in place, with oxazolidinyl carboxamide (**12**) to form the amide linkage. Compound **9** was prepared from diethylmalonate according to the literature.[32,33]

For the synthesis of **12**, commercially available serine or threonine was condensed with formaldehyde to construct the desired oxazolidine ring, which is then N-protected with Boc anhydride, and coupled with $R_2NH_2$ to give *N*-Boc protected oxazolidine derivatives **11a–11p**. Deprotection of **11a–11p** with HCl in EtOAc,[34,35] followed by coupling with **9** in the presence of 2-propane phosphonic acid anhydride gave the compounds **13a–13p**. Debenzylation of **13a–13p** by Pd/C hydrogenation provide the desired oxazolidine formyl hydroxyamino derivatives **1a–1p**. For **13i** and **13p**, oxidation with urea hydrogen peroxide and debenzylation afford the N-oxides **1q** and **1r**. These novel compounds were characterized by $^1H$ NMR and ESI-MS. The melting point and optical rotation of the desired compounds were also measured (Table 1).

The minimum inhibitory concentrations (MICs) of oxazolidine formyl hydroxyamino derivatives against several Gram-positive and Gram-negative bacterium were summarized in Table 2, along with data of LBM-415 for comparison. MICs were determined by the agar dilution method as outlined by the National Committed for Clinical Laboratory Standards. The MIC was defined as the low-

**Table 1**
Structure of **1a–1r**

| Compd | $R_1$ | $R_2$ | Compd | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| **1a** | H | Phenyl | **1j** | H | (2*S*)-Methyl-1-phenylmethyl |
| **1b** | H | 4-Methylphenyl | **1k** | H | 1-Phenylmethyl |
| **1c** | H | 4-Fluorophenyl | **1l** | $CH_3$ | 4-Methylphenyl |
| **1d** | H | 2,5-Difluorophenyl | **1m** | $CH_3$ | 4-Fluorophenyl |
| **1e** | H | 4-Methoxylphenyl | **1n** | $CH_3$ | 3-Fluoro-4-morpholinephenyl |
| **1f** | H | 3-Fluoro-4-morpholinylphenyl | **1o** | $CH_3$ | 3-Chloro-4-morpholinephenyl |
| **1g** | H | 3-Chloro-4-morpholinylphenyl | **1p** | $CH_3$ | 5-Fluoro-2-pyridinyl |
| **1h** | H | 4-Morpholinylphenyl | **1q** | H | 5-Fluoro-1-oxido-2-pyridinyl |
| **1i** | H | 5-Fluoro-2-pyridinyl | **1r** | $CH_3$ | 5-Fluoro-1-oxido-2-pyridinyl |

**Table 2**
In vitro antibacterial activity of **1a-1r**

| Compd | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|
| | Gram-positive organism | | | Gram-negative organism | |
| | *S. aureus* 26003 | *S. pneumoniae* 31002 | *S. albus* 260101 | *S. boydii* 51313 | *S. flexneri* 92475 |
| LBM-415 | 0.39 | 0.195 | 0.39 | 6.25 | >6.25 |
| **1a** | 0.39 | 0.195 | 0.39 | >6.25 | >6.25 |
| **1b** | 0.78 | 0.195 | 0.78 | >6.25 | >6.25 |
| **1c** | 0.39 | 0.195 | 0.39 | >6.25 | >6.25 |
| **1d** | 0.39 | 0.195 | 0.39 | >6.25 | >6.25 |
| **1e** | 0.39 | 0.195 | 0.78 | >6.25 | >6.25 |
| **1f** | 0.78 | 0.098 | 0.78 | >6.25 | >6.25 |
| **1g** | 0.39 | 0.098 | 0.39 | 6.25 | 6.25 |
| **1h** | 1.56 | 0.39 | 0.78 | 6.25 | >6.25 |
| **1i** | 0.78 | 0.098 | 0.39 | 3.13 | 3.13 |
| **1j** | 1.56 | 0.195 | 1.56 | >6.25 | >6.25 |
| **1k** | 0.78 | 0.78 | 1.56 | >6.25 | >6.25 |
| **1l** | 1.56 | 0.098 | 0.78 | >6.25 | >6.25 |
| **1m** | 1.56 | 0.195 | 0.78 | 3.13 | >6.25 |
| **1n** | 0.78 | 0.195 | 1.56 | >6.25 | >6.25 |
| **1o** | 0.78 | 0.098 | 0.39 | >6.25 | >6.25 |
| **1p** | 1.56 | 0.39 | 0.39 | 3.13 | >6.25 |
| **1q** | 0.78 | 0.195 | 0.78 | 3.13 | >6.25 |
| **1r** | 1.56 | 0.195 | 0.78 | >6.25 | >6.25 |

est concentration resulting in inhibition of visible bacterial growth after incubation at 37 °C for 24 h.

Shown in Table 2, all the oxazolidine formyl hydroxyamino derivatives **1a–1r** demonstrated significantly better antibacterial activity against Gram-positive bacteria than Gram-negative. The antibacterial spectrum was similar with LBM-415. Among the most potent ones, **1a**, **1c** and **1d** exhibited the same inhibitory effect on the tested Gram-positive bacteria as LBM-415. On the other hand, **1g**, **1i**, **1m** and **1q** showed better activities than LBM-415 to the Gram-negative bacteria. There are no substantial differences in the activity against *Streptococcus pneumoniae* between the serine-based oxazolidine derivatives and the threonine-based oxazolidine. But against the *Staphylococcus aureus*, the serine-based oxazolidine compounds showed better activities. Overall, the compound **1g** with (3-chloro-4-morpholinyl)phenyl substitution exhibited highest activity and its potency is comparable to or even slightly better than reference agent LBM-415.

In summary, a series of oxazolidine formyl hydroxyamino derivatives were synthesized and evaluated for antibacterial activity. Most of the new compounds showed high antibacterial activity in vitro. Among them, compound **1g** exhibited excellent activity against tested Gram-positive and Gram-negative organisms.

## Acknowledgments

The authors thank Mr. Weiliang Zhou for MIC test and Dr. Zhenfa Zhang for the preparation of manuscript. The work was supported by 'New Drug Innovation 2009X09301-007' from Ministry of Science and Technology of China.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.097.

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