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Design and Synthesis of Macrocyclic Peptidyl Hydroxamates as Peptide Deformylase Inhibitors

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Abstract

Macrocyclic peptidyl hydroxamates were designed, synthesized, and evaluated as peptide deformylase (PDF) inhibitors. The most potent compound exhibited tight, slow-binding inhibition of *Escherichia coli* PDF ($K_I^* = 4.4$ nM) and had potent antibacterial activity against Gram-positive bacterium *Bacillus subtilis* (MIC = 2–4 μ g/mL).

Peptide deformylase (PDF), an essential enzyme for bacterial protein biosynthesis and maturation, catalyzes the removal of *N*-formyl group from newly synthesized polypeptides.¹ PDF is being actively pursued as a target for designing novel antibiotics to treat antibiotic resistant pathogens.^{2, 3} Over the past decade, numerous PDF inhibitors have been reported; two of the inhibitors have already advanced to phase I clinical trials for treatment of upper respiratory tract infections.⁴ We have previously reported that macrocyclic *N*-formylhydroxylamine **1** (Figure 1) and its congeners are highly potent, selective inhibitors against PDF (e.g., $K_I^* = 0.33$ nM for **1** against *Escherichia coli* PDF) and have broad-spectrum antibacterial activity against clinically significant pathogens.⁵ However, synthesis of compound **1** was somewhat lengthy (11 steps), primarily due to an inefficient preparation of the *N*-formylhydroxylamine moiety (8 steps). In this work, we attempt to replace the *N*-formylhydroxylamine moiety with another metal chelating group, the synthetically more accessible hydroxamate. Thus, macrocyclic hydroxamates **2a–c** (Figure 1) were designed, synthesized, and evaluated as potential PDF inhibitors. Compound **2a** is isosteric to inhibitor **1** except that the *N*-formylhydroxylamine moiety in the latter is replaced by a hydroxamate. Compound **2b** contains a proline as the P₂' residue, because previous studies have shown that the proline confers excellent potency and pharmacokinetic properties.⁶ Compound **2c** should be synthetically very accessible because its chiral hydroxamate group can be readily prepared from *D*-aspartic acid.

Retrosynthetic analysis shows that the macrocycles can be conveniently prepared via olefin metathesis from diene **3**, which in turn can be prepared from acid **4** and amine **5** (Scheme 1). This synthetic strategy allows for the convergent synthesis of macrocycles of different ring sizes and specificity determinants by using the common acid **4** and varying the structure of the alkenyl amine **5**.

Synthesis of acid **4** started from the commercially available 5-hexenoic acid, which was stereoselectively alkylated at the C-2 position with a *t*-butoxycarbonylmethyl group using 4*S*-benzyloxazolidin-2-one as the chiral auxiliary group⁷ (Scheme 2). Removal of the auxiliary

group by hydrolysis produced the desired acid **4**, which was used as the common building block for the preparation of various cyclic hydroxamates.

The 6-heptenylamine needed for the preparation of amine **5** was obtained by reducing 5-hexenyl nitrile with LiAlH_4 (Scheme 3). The resulting amine was protected with a Boc group to facilitate its isolation. Removal of the Boc group by TFA, condensation with *N*-Boc-*tert*-leucine, and deprotection with TFA afforded amine **5**. Amine **5** was coupled to the common acid **4** to give diene **9**. The terminal alkenes were crosslinked using Grubbs's ruthenium catalyst⁸ followed by catalytic hydrogenation (Pd/C and H_2) to produce the 15-membered macrocycle **10**. The *t*-butyl ester was cleaved with TFA treatment and the resulting acid was coupled with *O*-*t*-butylhydroxylamine to give the *t*-butyl protected hydroxamate group. Finally, the *t*-butyl group on the hydroxamate was removed by treatment with TFA and aqueous HCl to give hydroxamate **2a**.⁹ Compound **2b** was synthesized in a similar manner except that *N*-Boc-proline was used instead of *N*-Boc-*tert*-leucine.

Compound **2c** was synthesized from *N*-Fmoc-Asp(*t*-Bu)-OH, which was condensed with amine **5** (Scheme 4). After removal of the N-terminal Fmoc group, the resulting amine **11** was acylated with 4-pentenoic acid and EDC. Cyclization of the diene **12** was achieved with the second-generation Grubbs's catalyst, which afforded better yields than the first-generation catalyst. The configuration of the ring C=C bond in intermediate **13** was not determined. To install the hydroxamate group, alkene **13** was treated with TFA to cleave the *t*-butyl ester and the resulting acid was coupled with *O*-benzylhydroxylamine to give cycloalkene **14**. Catalytic hydrogenation of **14** reduced the ring C=C bond and simultaneously removed the benzyl group from the hydroxamate moiety. Unfortunately, the hydroxamate was unstable under the hydrogenolysis condition and even under carefully controlled conditions, substantial reduction of the hydroxamate to the corresponding amide was observed. As a result, the hydrogenation step had rather poor yields (24%).

Compounds **2a–c** were tested for inhibition of cobalt(II)-substituted *E. coli* PDF (Co-EcPDF)¹⁰ using a continuous assay and peptide *N*-formyl-Met-Leu-*p*-nitroanilide (f-ML-*p*NA) as substrate.¹¹ Compounds **2a** and **2b** exhibited slow-binding inhibition¹² that can be described by equation

where K_1 is the equilibrium constant for the formation of the initial $\text{E} \cdot \text{I}$ complex, whereas k_5 and k_6 are the forward and reverse rate constants for the slow interconversion of $\text{E} \cdot \text{I}$ and $\text{E} \cdot \text{I}^*$, respectively. The overall potency of the inhibitor is described by the equilibrium constant $K_1^* = K_1 \cdot k_6 / (k_5 + k_6)$. The equilibrium and rate constants for **2a** and **2b** are listed in Table 1. Compound **2a** is a highly potent inhibitor, having a K_1^* value of 4.4 nM against EcPDF. The corresponding *N*-formylhydroxylamine (compound **1**) is also a slow-binding inhibitor and has a K_1^* value of 0.33 nM.⁵ Compound **2b**, which has a proline at the P_2' position, is also a slow-binding but significantly weaker inhibitor ($K_1^* = 1400$ nM). Compound **2c**, which was synthetically most accessible, was a very poor inhibitor ($K_1 > 20,000$ nM). This is not unexpected, because previous studies have shown that PDF has stringent requirement for a hydrophobic residue such as methionine and norleucine at the P_1' site.¹³ Thus, both *N*-formylhydroxylamine and hydroxamate are effective metal-chelating groups when attached to proper macrocycles.

Next, compounds **2a** and **2b** were tested for antibacterial activities in vitro. *Bacillus subtilis* and *E. coli* overnight cultures were diluted 6-fold into fresh LB medium containing 0–24 $\mu\text{g/mL}$ inhibitor and cell growth was monitored at 600 nm. Compound **2a** had potent antibacterial activity against *B. subtilis*, with a minimal inhibitory concentration (MIC) of 2–4 $\mu\text{g/mL}$.

Interestingly, in contrast to most of the reported PDF inhibitors, which are usually bacteriostatic, compound **2a** exhibited bactericidal activity. When added to cell cultures in the exponential phase, the cell density continued to increase for several hours and then gradually decreased to near zero as a result of cell lysis (Figure 2a). It had only weak activity against the Gram-negative *E. coli* cells (MIC > 24 µg/mL) (Figure 2b). Compound **2b** showed weak activity against *B. subtilis* (MIC > 24 µg/mL) and was inactive against *E. coli* cells (not shown).

In summary, a novel class of macrocyclic peptidyl hydroxamates has been prepared from commercially available 5-hexenoic acid (9 steps). One of the inhibitors showed potent inhibition of EcPDF and bactericidal activity against Gram-positive bacteria. Further optimization of the ring size and P₂' side chain may lead to highly potent, selective PDF inhibitors.

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- 2a**: ¹H NMR (400 MHz, CD₃OD): δ 8.04 (brs, 0.8H), 4.32–4.27 (m, 1H), 3.69–3.64 (m, 1H), 2.89–2.81 (m, 2H), 2.38–2.32 (m, 1H), 2.17–2.11 (m, 1H), 1.59–1.22 (m, 16H), 1.02 (s, 4.5H), 0.99 (s, 4.5H). ¹³C NMR (100 MHz, CD₃OD): δ 176.6, 172.6, 170.7, 62.1, 43.7, 38.9, 35.5, 29.0, 28.7 (d), 28.6 (d), 28.2, 27.8, 27.1, 26.8, 26.5. ESI-HRMS: Calcd for C₁₉H₃₅N₃O₄Na⁺ ([M + Na]⁺), 392.2520; found, 392.2537. **2b**: ¹H NMR (400 MHz, CD₃OD): δ 8.37 (brs, 0.4H), 4.25 (brs, 1H), 3.40–3.24 (m, 3H), 2.85–2.81 (m, 2H), 2.45–2.37 (m, 2H), 2.06–1.83 (m, 4H), 1.54–1.33 (m, 14H). ¹³C NMR (100 MHz, CD₃OD): δ 177.4, 174.2, 169.4, 61.3, 47.5, 40.8, 38.2, 32.5, 32.0, 31.3, 30.4, 30.3 (d), 30.2, 27.8, 27.3. ESI-HRMS: Calcd for C₁₈H₃₁N₃O₄H⁺ ([M + H]⁺), 354.2387; found, 354.2399.
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Figure 1.
Structures of PDF inhibitors.

Figure 2.
Antimicrobial activity of compound **2a** against *B. subtilis* (a) and *E. coli* (b).

Scheme 1.
Retrosynthetic analysis of **2a**.

Scheme 2.

(a) pivaloyl chloride, TEA, THF; (b) LiCl, 86% (two steps); (c) LDA, THF, *t*-butylbromoacetate, 50%; (d) H₂O₂, LiOH, H₂O, THF, 96%.

Scheme 3.

(a) LiAlH_4 , Et_2O ; (b) $(\text{Boc})_2\text{O}$, NaOH aq, 85% (2 steps); (c) TFA, CH_2Cl_2 ; (d) *N*-Boc-L-*tert*-Leu-OH, EDC, HOAt, Et_3N , CH_2Cl_2 , 78%; (e) TFA, CH_2Cl_2 ; (f) **4**, EDC, Et_3N , CH_2Cl_2 , 42%; (g) 2nd-generation Grubbs's catalyst, CH_2Cl_2 , reflux, 55%; (h) H_2 , 10% Pd/C, MeOH, EtOAc; (i) TFA; (j) *t*-BuONH $_2$ •HCl, EDC, Et_3N , HOAt, CH_2Cl_2 , 40%; (k) TFA, 37% HCl, 48%.

Scheme 4.

(a) *tert*-Leu-NH(CH₂)₅CH=CH₂, EDC, DIPEA, CH₂Cl₂, 64%; (b) 20% piperidine; (c) CH₂=CHCH₂CH₂CO₂H, EDC, Et₃N, CH₂Cl₂, 71%; (d) 2nd-generation Grubbs's catalyst, 76%; (e) TFA; (f) BnONH₂, EDC, Et₃N, CH₂Cl₂, 54%; (g) H₂, 10% Pd/C, MeOH, EtOAc, 24%.

Table 1Inhibition constants of Co-EcPDF by compounds **1** and **2a–c**.

Compound	K_I , nM	K_I^* , nM	k_5 , min ⁻¹	k_6 , min ⁻¹
1	109 ± 5^a	0.33 ± 0.15^a	1.2^a	0.0038 ± 0.0010^a
2a	210 ± 30	4.4 ± 0.7	12	0.26 ± 0.02
2b	16000 ± 4000	1400 ± 200	3.1	0.30 ± 0.01
2c	>20,000	ND ^b	ND	ND

^aData from ref. 5^bND, no slow binding detected.