Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3595–3599

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationships of the Peptide Deformylase Inhibitor BB-3497: Modification of the Metal Binding Group

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Received 2 May 2002; revised 28 June 2002; accepted 11 September 2002

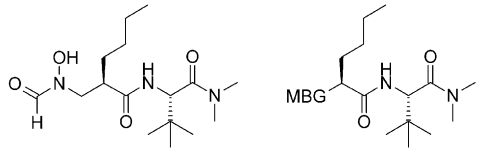
Abstract—A series of analogues of the potent peptide deformylase (PDF) inhibitor BB-3497 containing alternative metal binding groups was synthesised. Enzyme inhibition and antibacterial activity data for these compounds revealed that the bidentate hydroxamic acid and *N*-formyl hydroxylamine structural motifs represent the optimum chelating groups on the pseudopeptidic BB-3497 backbone.

Bacterial peptide deformylase (PDF) is a metalloenzyme responsible for the removal of an *N*-formyl group from the terminal methionine residue following protein synthesis.[1] PDF is essential for bacterial growth and, consequently, it has been widely recognised as an attractive novel target for antibacterial chemotherapy.[2]

We reported previously[3] that the *N*-formyl hydroxylamine BB-3497 (Fig. 1) is an effective inhibitor ($IC_{50} = 7$ nM) of the *Escherichia coli* PDF.Ni enzyme, exhibiting potent antibacterial activity both in vitro and in vivo. As part of an ongoing research programme, we undertook a comprehensive SAR study of BB-3497 observing the effects on enzyme inhibition and antibacterial activity. Herein, we describe the initial findings of this study in which analogues of BB-3497 containing alternative metal binding groups were synthesised and tested in our assays.

On the basis of our extensive knowledge of relevant metal binding groups for the inhibition of matrix metalloproteinases (MMPs),[4] we synthesised a variety of analogues of BB-3497 capable of either monodentate or bidentate chelation to a metal centre. In particular, we concentrated on functional groups that resembled the *N*-formyl hydroxylamine and hydroxamic acid structural motifs. These included an *N*-acetyl hydroxylamine, an *N*-formylamine, a hydrazide, an amidoxime and two *N*-hydroxyureas. Additionally, we elected to prepare other metal binding groups used in the MMP field such as a carboxylic acid, a thiol, an aminocarboxylate and some phosphorus-based chelating groups. During the course of this work, significant contributions in the area of PDF inhibitors containing hydroxamic acids[5] or alternative metal binding groups[5c,6] were reported from other laboratories, underlining the current interest in PDF as an antibacterial target.

For the synthesis of compounds related to *N*-formyl hydroxylamine, we were able to exploit a precursor used in our asymmetric route to BB-3497.[7] Thus, acetylation of **1**, followed by *O*-benzyl deprotection afforded the *N*-acetyl hydroxylamine **2** (Scheme 1). Alternatively, **1** was treated with benzyloxycarbonyl isocyanate (prepared from benzyl carbamate and oxalyl chloride[8]), followed by hydrogenolysis to give the *N*-hydroxyurea



BB-3497

Figure 1. BB-3497 and metal binding group (MBG) analogues.

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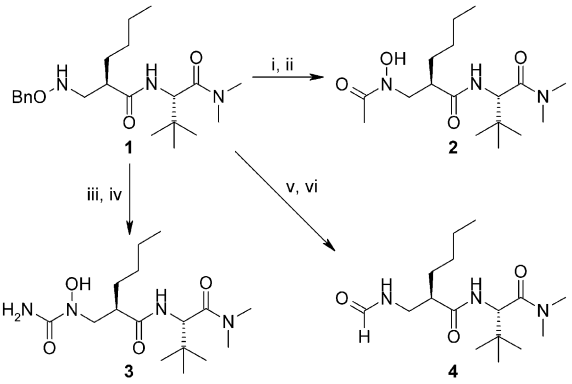


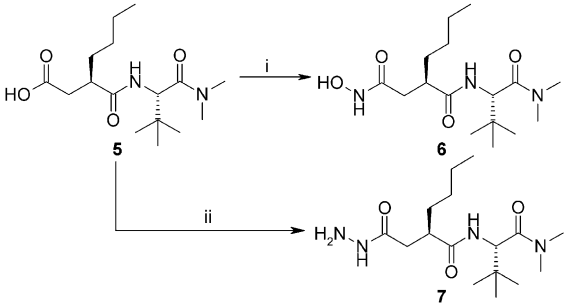
Scheme 1. (i) Ac_2O; (ii) H_2, 10% Pd/C, MeOH, 36% (2 steps); (iii) BnOCONCO, CH_2Cl_2, 76%; (iv) H_2, $Pd(OH)_2$/C, MeOH, 75%; (v) H_2, 10% Pd/C, EtOH, 32%; (vi) BtCHO, THF, 40%.

product **3**. Additionally, the *N*-formamide compound **4** was synthesised from **1** by reduction of the N–O bond and subsequent formylation with *N*-formylbenzotriazole[9] (BtCHO).

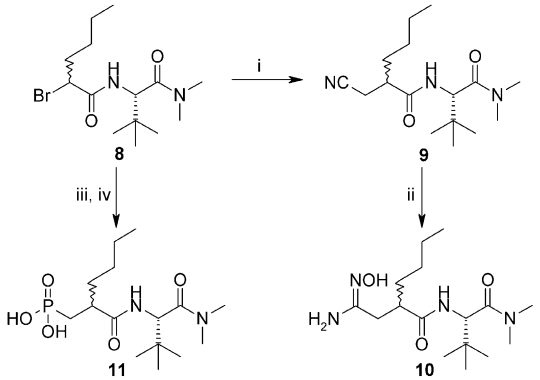
The hydroxamic acid analogue **6** of BB-3497 and the related hydrazide **7** were prepared from the succinate-derived carboxylic acid **5**[10] under standard peptide coupling conditions (Scheme 2).

The amidoxime **10** and phosphonic acid **11** were prepared as mixtures of separable diastereoisomers from the α-bromo derivative **8**[11] via nucleophilic displacement (Scheme 3). Target **10** was synthesised through reaction of **8** with the anion of acetonitrile to provide the cyano derivative **9**. Subsequent treatment of **9** with hydroxylamine under basic conditions gave the required amidoxime **10**. Alternatively, reaction of the anion of dimethyl methylphosphonate with **8** followed by removal of the methyl groups using TMSBr-bis(-trimethylsilyl)trifluoroacetamide (BSTFA)[12] gave phosphonic acid **11**.

L-Norleucine was used as the chiral building block to access analogues of BB-3497 where the methylene unit adjacent to the metal binding group was replaced with a nitrogen atom. This strategy generated peptide **12**, which was treated with 4-nitrophenyl chloroformate followed by reaction with hydroxylamine to give *N*-hydroxyurea **13** (Scheme 4). Additionally, alkylation of



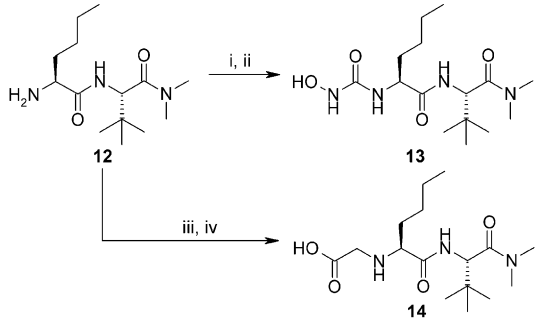
Scheme 2. (i) NH_2OH, EDC, HOBt, DMF, 42%; (ii) $N_2H_4.H_2O$, EDC, HOBt, DMF, 40%.



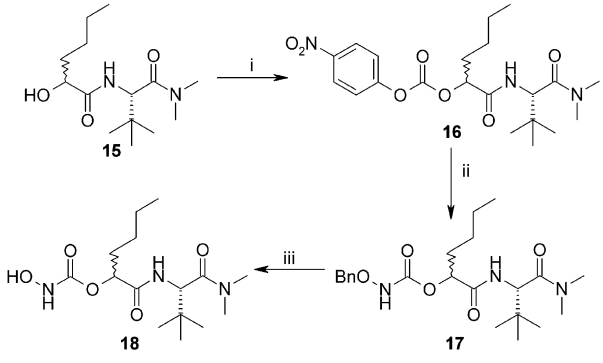
Scheme 3. (i) MeCN, LDA, THF; (ii) $NH_2OH.HCl$, K_2CO_3, MeOH:H_2O 54% (2 steps), *then* separate diastereoisomers (preparative HPLC); (iii) $CH_3P(O)(OMe)_2$, *n*-BuLi, THF, 26%; (iv) TMSBr, BSTFA, CH_2Cl_2, 41%, *then* separate diastereoisomers (preparative HPLC).

12 with benzyl bromoacetate, and subsequent hydrogenolysis provided the aminocarboxylate **14**.

To draw analogy with compounds **6** and **13**, we synthesised **18**, which contained an oxygen atom adjacent to the metal binding group. Compound **15**, derived from racemic 2-hydroxycaproic acid, was reacted with 4-nitrophenyl chloroformate to give the activated carbonate **16**. This was treated with *O*-benzyl hydroxylamine to furnish **17**. Hydrogenolysis under standard conditions afforded the target compound **18** as a mixture of diastereoisomers (Scheme 5).



Scheme 4. (i) 4-Nitrophenyl chloroformate, pyridine, CH_2Cl_2; (ii) $NH_2OH.HCl$, Et_3N, DMF, 30% (2 steps); (iii) $BrCH_2CO_2Bn$, K_2CO_3, acetone, 25%; (iv) H_2, 10% Pd/C, MeOH, 85%.



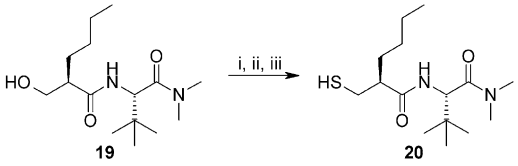
Scheme 5. (i) 4-Nitrophenyl chloroformate, pyridine, CH_2Cl_2; (ii) NH_2OBn, Et_3N, CH_2Cl_2, 28% (2 steps); (iii) H_2, 10% Pd/C, MeOH, 98%.

The thiol analogue of BB-3497 was prepared from the β-hydroxy carboamide **19**[13] (Scheme 6). Displacement of the mesylate derived from alcohol **19** with potassium thioacetate was followed by saponification with aqueous LiOH to give thiol **20**.

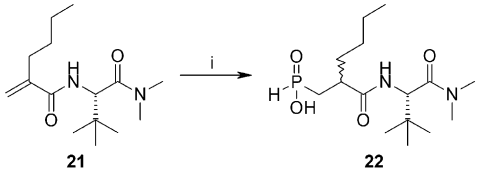
Finally, the phosphinic acid **22** was prepared as a mixture of separable diastereoisomers from the α,β-unsaturated amide **21**[7] by a modification of a procedure described by Regan et al.[14] (Scheme 7). Thus, conjugate addition of bis(trimethylsilyl)phosphonite (prepared in situ from ammonium phosphinate and BSTFA) to **21** provided phosphinic acid **22**.

All compounds were screened for activity against the *E. coli* PDF.Ni enzyme and Gram-positive and Gram-negative in-house bacterial strains (Table 1).[15]

Replacement of the formyl proton with a methyl group as in **2** or an amino substituent as in **3** greatly reduced PDF activity. Examination of the *E. coli* PDF X-ray structure[16] and associated molecular modelling of the PDF enzyme active site suggested that these substrates were too bulky to bind effectively to the metal centre.



Scheme 6. (i) MsCl, Et_3N, CH_2Cl_2, 86%; (ii) HSAc, K_2CO_3, DMF, 44%; (iii) LiOH, H_2O, 31%.



Scheme 7. (i) $H_2PO_2NH_4$, BSTFA, 17%, *then* separate diastereoisomers (preparative HPLC).

Deletion of a chelating *N*-hydroxyl group as in **4** completely abolished activity against PDF.

The hydroxamic acid **6** is an excellent inhibitor of the enzyme and also exerted a strong antibacterial effect. However, replacement of the hydroxyl group in **6** with an amino functionality gave a hydrazide **7** which was 200 times less active against PDF and did not show antibacterial activity. The amidoxime **10** was inactive against the enzyme. The parent carboxylic acid **5** showed moderate activity against the enzyme but no antibacterial activity. Substituting heteroatoms (oxygen and nitrogen) in place of the methylene spacer in **6** provided compounds **13** and **18**, with weak activity against PDF. This result is consistent with observations by Thorarensen et al.[5c] where an *N*-hydroxyurea was identified as a weak PDF inhibitor ($IC_{50} = 2.2\,\mu M$), except that our inhibitor **13** did not exhibit antibacterial activity.

The thiol compound **20**, which would be expected to chelate in a monodentate fashion to the Ni centre, showed only weak activity against the PDF enzyme and no antibacterial activity. Meinnel et al.[6b] described a related thiol PDF inhibitor **23** (Fig. 2) that also demonstrated weak activity against PDF (K_i (*E. coli* PDF.Ni) = 2.5 μM), however no microbiological data were provided. Additionally, Pei and co-workers[6c,d] prepared compounds containing a thiol metal binding group (e.g., **24**), which inhibited PDF in the low nanomolar range (K_i (*E. coli* PDF.Fe) = 19 nM) and exerted a moderate antibacterial effect (*E. coli* MIC

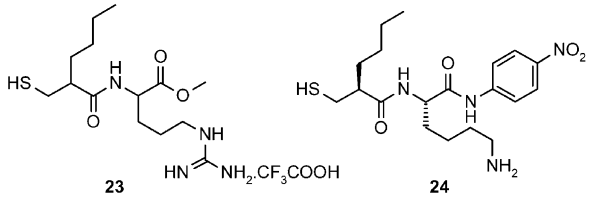


Figure 2. Thiol PDF inhibitors.

Table 1. PDF enzyme inhibition and antibacterial activity of BB-3497 and its metal binding group analogues

Compd	MBG	*E. coli* PDF.Ni IC_{50} (nM)	*E. coli* MIC (μM)	*S. capitis* MIC (μM)
BB-3497	$CH_2N(OH)CHO$	7	12.5	25
2	$CH_2N(OH)COCH_3$	60%@1 μM[a]	>200	>200
3	$CH_2N(OH)CONH_2$	>1000	>200	>200
4	CH_2NHCHO	>1000	>200	>200
6	$CH_2CONHOH$	1	25	100
7	$CH_2CONHNH_2$	200	>200	>200
10[b]	$CH_2C(NH_2)=NOH$	>1000	>200	>200
5	CH_2COOH	200	>200	>200
13	NHCONHOH	40%@1 μM[a]	>200	>200
18[c]	OCONHOH	20%@1 μM[a]	>200	>200
14	$NHCH_2CO_2H$	>1000	>200	>200
20	CH_2SH	10%@1 μM[a]	>200	>200
22[b]	$CH_2PO(OH)H$	>1000	>200	>200
11[b]	$CH_2PO(OH)_2$	>1000	>200	>200

Staphylococcus capitis (*S. capitis*).
[a]IC_{50} expressed as percentage inhibition at a concentration of 1 μM.
[b]Both diastereoisomers were inactive.
[c]Mixture of diastereoisomers.

75–100 μM). These data suggest that the nature of the peptidic backbone contributes to inhibitory activity.

The phosphinic acid **11** and phosphinic acid **22**, which could potentially bind in a bidentate fashion to the metal centre thus forming a 4-membered ring, or alternatively behave as a transition-state mimic, as proposed by Pei et al.[6a] with their phosphonates, were inactive in our assays.

Overall, the results provided in Table 1 demonstrate that the *N*-formyl hydroxylamine and hydroxamic acid represent the optimum metal chelating groups for the pseudopeptidic backbone of BB-3497 in terms of PDF inhibition and antibacterial activity. Other metal binding group analogues such as the hydrazide **7** and the carboxylic acid **5**, which were weak PDF inhibitors, were not potent enough to exert an antibacterial effect.

A diagrammatic representation of the X-ray structure for BB-3497 bound to the active site of the *E. coli* PDF enzyme is shown in Figure 3.[3] Based on this and our structure of the PDF-actinonin complex,[3] we predict that **6** binds in a similar bidentate mode.

In both BB-3497 and **6**, the two oxygen atoms of the metal binding group establish a bidentate coordination to the metal centre. Additionally, they can hydrogen bond with amino acid residues in the active site, enhancing their binding potential to the enzyme. These observations correlate well with the structure reported for the PDF.Ni enzyme bound to the substrate, Met-Ala-Ser,[17] in which two water molecules at the active site occupy similar positions to the oxygen atoms in our inhibitors. Metal binding groups other than the *N*-formyl hydroxylamine or the hydroxamic acid are weak PDF inhibitors. In some cases this may be explained by a relatively weak monodentate interaction whilst in other examples the steric requirements for backbone and sidechain interactions may not be compatible with the orientation necessary for efficient bidentate chelation to the metal.

In conclusion, a series of BB-3497 analogues with alternative metal binding groups have been synthesised. The nature of the metal chelating group was found to be critical for PDF inhibitory activity and the optimum groups on the pseudopeptidic backbone of BB-3497 were the *N*-formyl hydroxylamine and the hydroxamic acid. These two compounds also demonstrated the best antibacterial activity but we have shown elsewhere[3] that improvements in antibacterial activity cannot necessarily be attributed to improvements in enzyme inhibition. Other factors that affect drug distribution such as bacterial membrane permeability, efflux and metabolism are clearly important in determining antibacterial activity.

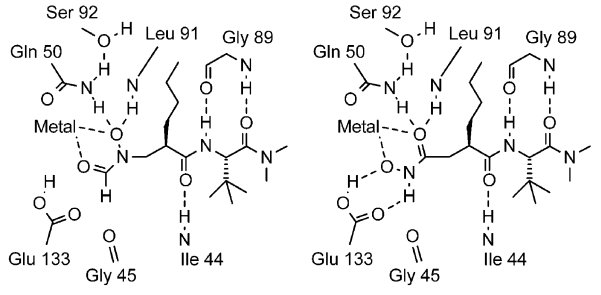


Figure 3. Binding mode of BB-3497 to the active site of *E. coli* PDF from X-ray data and predicted binding of hydroxamic acid **6**.

Acknowledgements

We thank Drs. Andy Ayscough, Mario Lobell and Jac Wijkmans of British Biotech for useful discussions.

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