Bioorganic & Medicinal Chemistry Letters 21 (2011) 4032–4035

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Peptidomimetic inhibitors of bacterial peptide deformylase

Stephen P. East *, Andrew Ayscough, Ian Toogood-Johnson, Steven Taylor, Wayne Thomas

Vernalis (R&D) Ltd., Granta Park, Great Abington, Cambridge CB21 6GB, United Kingdom†

ARTICLE INFO

Article history:
Received 29 March 2011
Revised 26 April 2011
Accepted 27 April 2011
Available online 11 May 2011

Keywords:
Peptide deformylase
Peptidomimetic
Azapeptide
Antibacterial
Metalloprotease

ABSTRACT

A series of *N*-formyl hydroxylamine peptide deformylase inhibitors containing a cyclic azaamino acid moiety between the P1′ and P3′ substituents are presented. Selected compounds display antibacterial activity against pathogens associated with respiratory tract infections with representative compounds showing excellent MICs against *Haemophilus influenzae*.

Peptide deformylase (PDF) is an iron-containing metalloenzyme involved in post-translational modification of nascent polypeptides in bacterial cells.[1] Specifically PDF cleaves the *N*-formyl group from the terminal methionine residue following ribosomal protein synthesis. Deformylation is a critical step before further processing of the newly synthesized protein can occur and the *def* gene, which encodes PDF, is present in all sequenced bacteria. Gram-positive bacteria may have additional functional genes that encode other PDFs and eukaryotic homologues of PDF have also been identified. This has raised concerns over possible specificity issues but recently Meinnel and co-workers have demonstrated that selective inhibitors for bacterial PDF can be identified through exploiting differences in the S′ binding pocket.[2] As such, PDF remains an interesting molecular target for the development of new antibacterial agents and also antitubercular drugs.[3]

Nature has already provided examples of antibiotics that inhibit PDF including the peptidic hydroxamic acid actinonin (**1**).[4–6] Additionally, several groups have reported synthetic hydroxamic acids or *N*-formyl hydroxylamines related to the structure of **1** that display potent antibacterial activity via inhibition of PDF.[1,7–9] To date, these research efforts have culminated in the progression of two PDF inhibitors into Phase I clinical trials, LBM-415 (**2**)[10,11] and a compound from our laboratories, BB-83698 (**3**)[12,13] (Fig. 1).

As part of our research effort to identify a back-up to **3** we sought to mimic the peptidic structure by replacing the amino acid that links the P1′ and P3′ positions of our PDF inhibitors. This strategy and indeed attempts to identify non-peptidic PDF inhibitors has been adopted by others.[14–16] For example, in our previous publication we replaced the P2′-P3′ amide bond with a ketone and we found that we could retain good antibacterial activities against bacterial strains associated with respiratory tract infections (RTIs).[17] This communication describes alternative modifications to the peptidic backbone of our *N*-formyl hydroxylamine PDF inhibitors where the P2′ amino acid is replaced with a cyclic azaamino acid, generic formula **I**. The compounds presented here have been evaluated for their inhibitory activity against bacterial PDF isolated from *Escherichia coli*.

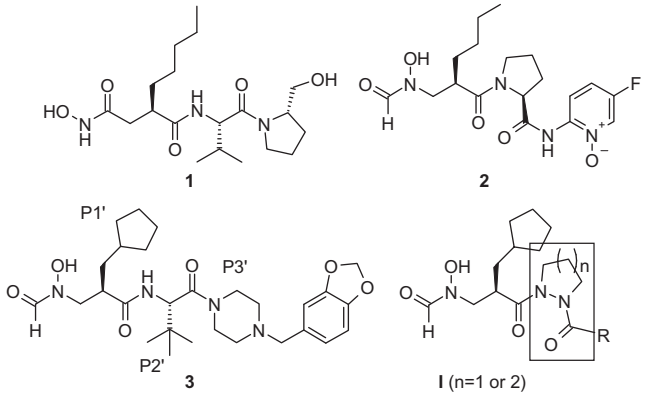
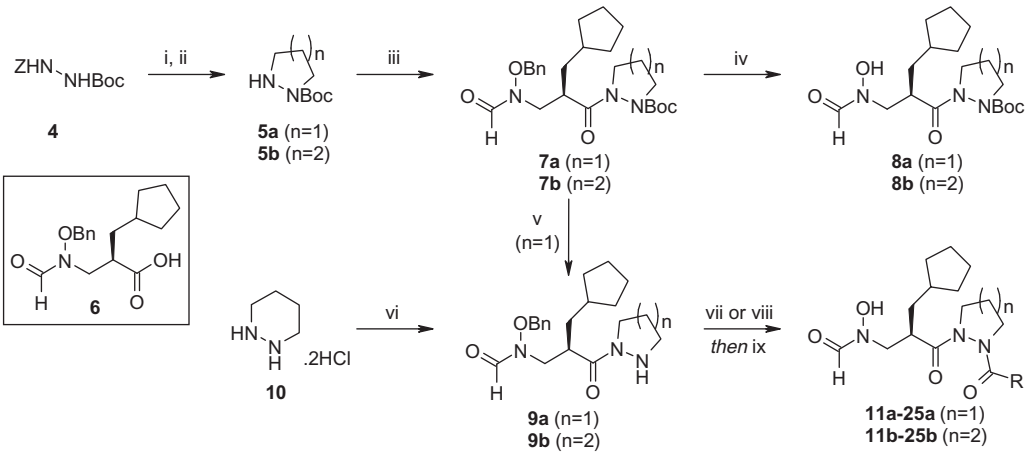


Figure 1. PDF inhibitors

* Corresponding author at present address: Evotec (UK) Ltd, 114 Milton Park, Abingdon, Oxon, OX14 4SA, United Kingdom. Tel.: +44 1235 441735.
E-mail address: stephen.east@evotec.com (S.P. East).
† This work was performed at British Biotech Pharmaceuticals Limited.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.132



Scheme 1. Reagents and conditions: (i) 1,3-dibromopropane, NaH, DMF, rt, 89% (*n* = 1) *or* 1,4-dibromobutane, NaH, DMF, rt, 94% (*n* = 2); (ii) H_2, 10% Pd/C, MeOH, rt, 78–100%; (iii) **6**, HATU, DIPEA, DMF 0 °C to rt, 52% (*n* = 1) or **6**, HATU, DIPEA, DMF 0–60 °C, 7% (*n* = 2); (iv) H_2, 10% Pd/C, MeOH, rt, 90%; (v) $BF_3{\cdot}Et_2O$, CH_2Cl_2, AcOH, rt, 95%; (vi) **6**, HATU, DIPEA, DMF 0 °C to rt, 65%; (vii) acid chloride, DIPEA, CH_2Cl_2, 0 °C to rt; (viii) isocyanate, EtOAc, rt; (ix) H_2, 10% Pd/C, MeOH, rt, yields typically >75% (2 steps).

Bioisosteric replacement of amino acids with azaamino acids is a peptidomimetic strategy[18] which has been applied previously to a number of protease inhibitor programmes.[19] Changing the asymmetric carbon centre for a nitrogen atom usually cause minimal disruption to the conformation of the molecule and so binding affinities of compounds containing an azaamino acid are expected to be comparable to the corresponding amino acid containing analogues. Additionally, azaamino acids may be expected to be more stable, particularly to hydrolytic cleavage by proteolytic enzymes. We,[20] and others,[21] reported *N*-formyl hydroxylamine and hydroxamic acid PDF inhibitors containing cyclic amino acids in the P2′ position (e.g., **2**). As an extension of this work, we have investigated *N*-formyl hydroxylamines containing azaproline (azPro) and azapipcolinic acid (azPip). From our previous SAR analysis,[20] together with available crystallographic information,[22] the major point of diversity for this investigation was the P3′ position.

AzPro analogues were synthesized according to the route illustrated in Scheme 1. Orthogonally protected hydrazine **4** (prepared from commercially available *N*-Boc hydrazine in quantitative yield) was cycloalkylated using 1,3-dibromopentane and sodium hydride. The Z-protecting group was then selectively removed via standard hydrogenation conditions and the resulting *N*-Boc protected pyrazolidine **5** was coupled to carboxylic acid **6**[23] using HATU to give intermediate **7a**. Deprotection of the *O*-benzyl group in **7a** provided *N*-formyl hydroxylamine **8a**. Alternatively, the Boc group in **7a** could be removed using boron trifluoride etherate and the resultant analogue **9a** was capped with acid chlorides, to give compounds **11a**–**20a** or isocyanates to afford compounds **21a**–**25a** following *O*-benzyl deprotection by a palladium-catalyzed hydrogenation reaction.

A similar route was investigated for the azPip analogues however the coupling of **5b** with **6** gave compound **7b** in an unacceptably poor yield of <10%. This route did lead to analogue **8b** following deprotection of the *O*-benzyl group but it did not represent a viable synthesis to investigate the capping step. Fortunately the unprotected cyclic hydrazine **10**, prepared according to literature procedures,[24] underwent coupling with **6** using HATU to provide adduct **9b** ready for treatment with acid chlorides and isocyanates. These reactions were followed by hydrogenation of the *O*-benzyl group to give compounds **11b**–**25b**.

All of the compounds were tested in a PDF inhibition assay and their minimum inhibitory concentrations (MICs) were determined against Gram-positive (*Streptococcus pneumoniae*) and Gram-negative bacteria (*Haemophilus influenzae* and *Moraxella catarrhalis*) responsible for RTIs (Table 1).

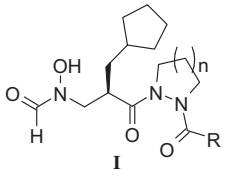
Most of the compounds examined are potent (sub μM) PDF inhibitors indicating that the presence of the azPro or azPip were tolerated by the enzyme. In general, the IC_{50}'s for 5- or 6-membered ring P2′ matched pairs are broadly similar (typically 1–5× difference) suggesting that the enzyme does not have a preference for either ring size. In line with our previous observations[20], the enzyme accepts a variety of changes in the P3′ position. Compounds with a 6-membered aryl/heteroaryl ring directly attached to the azPro/Pip carbonyl generally have higher IC_{50}'s (**13**–**17**) with the 3-pyridyl substituent (**17**) being the least-favoured of the modifications investigated, particularly on the azPip scaffold. In contrast the 5-membered furan containing compounds (**18** and **19**) provided some of the most potent PDF inhibitors in this study. The N-linked analogues (**21**–**25**) also show low IC_{50}'s against the enzyme.

Aliphatic (**8**, **11**, **12**, **20**) and aryl/heteroaryl (**13**–**19**) compounds demonstrate good antibacterial activity against Gram-positive and Gram-negative organisms. The compounds containing the azPip P2′ are consistently more active when compared with the corresponding azPro compounds, even when the IC_{50}'s are worse, for example, **13b** versus **13a**. The explanation for this is not clear but we can speculate that they are possibly less susceptible to efflux mechanisms. The most interesting compound in terms of a good balance between Gram-positive and Gram-negative activity is the 2-furyl derivative **18b**.

Compounds **20–25** containing the P2′P3′ NH display good antibacterial activity against *S. pneumoniae* (MICs 2–16 μg/ml). They also show excellent activity against *M. catarrhalis* and *H. influenzae*. Some examples **21**, **23** and **24** showed MICs that are amongst the best PDF inhibitors that we have reported against *H. influenzae*.[17] The data for the azPro and azPip compounds was similar.

We also examined the antibacterial activity of two related hydroxamic acids containing the azPip P2′ group with two of the more interesting P3′ substituents. These compounds, **29** and **30**, were prepared according to the synthetic route illustrated in Scheme 2. Advanced intermediate **27**, which was prepared from **26**,[25] was coupled to **10** using EDAC and HOAt to provide compound **28**. Subsequent capping with phenyl isocyanate or 2-furoyl chloride gave **29** and **30** respectively after acid-promoted removal of the hydroxamic acid protecting group.

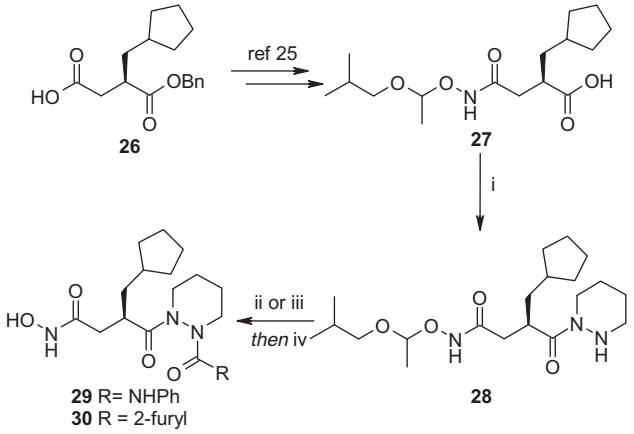
Table 1
PDF IC_{50}'s and MICs for compounds **8**, **11**–**25**



I

Compd	R	*n*	PDF Ni *E. coli* IC_{50}[a] (nM)	MIC[b] (μg/ml)		
				S. pneumoniae (4)	*H. influenzae* (3)	*M. catarrhalis* (1)
8a	OtBu	1	30	8-16	0.5–2	<0.125
8b		2	9	4–16	1–4	<0.125
11a	CH_2OMe	1	60	8	1–8	0.125
11b		2	200	1–8	0.25–8	0.25
12a	Cyclohexyl	1	40	8	4–16	0.06
12b		2	200	0.5–2	4–8	0.06
13a	Ph	1	70	16	2–16	0.06
13b		2	500	0.5–2	1–4	0.125
14a	4-MeOPh	1	50	4–16	16–32	0.06
14b		2	100	0.5–4	1–>32	0.5
15a	4-FPh	1	20	16	4–8	0.125
15b		2	60	0.5–4	0.5–2	0.5
16a	4-Pyridyl	1	60	16–32	2–>32	0.125
16b		2	300	2–16	2–8	0.25
17a	3-Pyridyl	1	300	8	2–>32	0.125
17b		2	40%@1 μM	nd	nd	nd
18a	2-Furyl	1	30	4–8	<0.125–1	<0.125
18b		2	5	0.5–2	<0.125–0.25	<0.125
19a	3-Furyl	1	10	4	1–>32	0.06
19b		2	6	0.5–2	0.125–8	<0.03
20a	Bn	1	30	4–8	2–16	0.06
20b		2	60	0.5–4	0.25–2	0.25
21a	NHPh	1	10	4–16	<0.125–2	<0.125
21b		2	20	2–4	<0.125–4	<0.125
22a	NH(2-MeOPh)	1	7	8–16	1–2	0.25
22b		2	10	4–8	0.5–2	<0.03
23a	NH(3-MeOPh)	1	10	4–8	<0.125–2	<0.125
23b		2	20	2–4	<0.125–4	<0.125
24a	NH(4-FPh)	1	10	8–16	<0.125–2	<0.125
24b		2	10	4–8	<0.125–4	<0.125
25a	NH(4-CF_3Ph)	1	30	2–8	2–8	0.06
25b		2	9	4	0.25–16	<0.125

[a] The natural ferrous-containing PDF enzyme oxidises readily to the inactive ferric form. For ease of experimentation, enzyme inhibition was tested routinely using the stable nickel-containing enzyme, which retains full catalytic activity.
[b] Minimum number of strains tested against in parentheses; nd: no data.



Scheme 2. Reagents and conditions: (i) **10**, EDAC, HOAt, DIPEA, CH_2Cl_2, 0 °C to rt, 50%; (ii) phenyl isocyanate, EtOAc, rt, 100%; (iii) 2-furoyl chloride, DIPEA, CH_2Cl_2, rt, 91%; (iv) HCl, MeOH, rt, 75%.

Table 2
PDF IC_{50}'s and MICs for hydroxamic acids

Compd	PDF Ni *E. coli* IC_{50}[a] (nM)	MIC[b] (μg/ml)		
		S. pneumoniae (4)	*H. influenzae* (3)	*M. catarrhalis* (1)
29	20	4–8	1–4	nd
21b	20	2–4	<0.125–4	<0.125
30	8	2–4	0.06–0.125	0.06
18b	5	0.5–2	<0.125–0.25	<0.125

[a] The natural ferrous-containing PDF enzyme oxidises readily to the inactive ferric form. For ease of experimentation, enzyme inhibition was tested routinely using the stable nickel-containing enzyme, which retains full catalytic activity.
[b] Minimum number of strains tested against in parentheses; nd: no data.

The PDF inhibitory activity and antibacterial activity for **29** and **30** are shown in Table 2. Results from these assays suggest that both the hydroxamic acid and *N*-formyl hydroxylamine metal binding group provide similarly potent PDF inhibitors with comparable antibacterial activity against Gram-positive and

Gram-negative organisms. This observation is consistent with our previous findings.[26]

In conclusion, we have presented a new series of *N*-formyl hydroxylamines containing an azPro or azPip residue. Compounds such as **18b** are less peptidic than our previously disclosed PDF inhibitors and significantly, they retain good enzymatic potency and the potential for broad-spectrum activity against pathogens associated with RTIs. The antibacterial activity against Gram-negative organisms, in particular *H. influenzae*, is amongst the most promising data we have reported.

References and notes

1. (a) Pei, D. *Emerging Ther. Targets* **2001**, *5*, 23; (b) Clements, J. M.; Ayscough, A.; Keavey, K.; East, S. P. *Curr. Med. Chem. Anti Infect. Agents* **2002**, *1*, 239; (c) Johnson, K. W.; Lofland, D.; Moser, H. E. *Curr. Drug Targets Infect. Disord.* **2005**, *5*, 39; (d) Jain, R.; Chen, D.; White, R. J.; Patel, D. V.; Yuan, Z. *Curr. Med. Chem.* **2005**, *12*, 1607; (e) Leeds, J. A.; Dean, C. R. *Curr. Opin. Pharmacol.* **2006**, *6*, 445.
2. Boularot, A.; Giglione, C.; Petit, S.; Duroc, Y.; Alves de Sousa, R.; Larue, V.; Cresteil, T.; Dardel, F.; Artaud, I.; Meinnel, T. *J. Med. Chem.* **2007**, *50*, 10.
3. Sharma, A.; Khuller, G. K.; Sharma, S. *Expert Opin. Ther. Targets* **2009**, *13*, 753.
4. Chen, D. Z.; Patel, D. V.; Hackbarth, C. J.; Wang, W.; Dreyer, G.; Young, D. C.; Margolis, P. S.; Wu, C.; Ni, Z. J.; Trias, J.; White, R. J.; Yuan, Z. Y. *Biochemistry* **2000**, *39*, 1256.
5. Chu, M.; Mierzwa, R.; He, L.; Xu, L.; Gentile, F.; Terracciano, J.; Patel, M.; Miesel, L.; Bohanon, S.; Kravec, C.; Cramer, C.; Fischman, T. O.; Hruza, A.; Ramanathan, L.; Shipkova, P.; Chan, T. M. *Tetrahedron Lett.* **2001**, *42*, 3549.
6. Yoo, J. S.; Zheng, C. J.; Lee, S.; Kwak, J. H.; Kim, W. G. *Bioorg. Med. Chem. Lett.* **2006**, *15*, 4889.
7. Shi, W.; Duan, Y.; Qian, Y.; Li, M.; Yang, L.; Hu, W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3592.
8. Lee, S. K.; Choi, K. H.; Lee, S. J.; Suh, S. W.; Kim, B. M.; Lee, B. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4317.
9. Shi, W.; Ma, H.; Duan, Y.; Aubart, K.; Fang, Y.; Zonis, R.; Yang, L.; Hu, W. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1060.
10. Fritsche, T. R.; Sader, H. S.; Cleeland, R.; Jones, R. N. *Antimicrob. Agents Chemother.* **2005**, *49*, 1468.
11. Osborne, C. S.; Neckermann, G.; Fischer, E.; Pecanka, R.; Yu, D.; Manni, K.; Goldovitz, J.; Amaral, K.; Dzink-Fox, J.; Ryder, N. S. *Antimicrob. Agents Chemother.* **2009**, *53*, 3777.
12. Lofland, D.; Difuntorum, S.; Waller, A.; Clements, J. M.; Weaver, M. K.; Karlowsky, J. A.; Johnson, K. J. *Antimicrob. Chemother.* **2004**, *53*, 664.
13. Ramanathan-Girish, S.; McColm, J.; Clements, J. M.; Taupin, P.; Barrowcliffe, S.; Hevizi, J.; Safrin, S.; Moore, C.; Patou, G.; Moser, H.; Gadd, A.; Hoch, U.; Jiang, V.; Lofland, D.; Johnson, K. W. *Antimicrob. Agents Chemother.* **2004**, *48*, 4835.
14. Aubart, K.; Zalacain, M. *Prog. Med. Chem.* **2006**, *44*, 109.
15. Lee, S. K.; Choi, K. H.; Lee, S. J.; Lee, J. S.; Park, J. Y.; Kim, B. M.; Lee, B. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 133.
16. Yu, L.; Zhou, W.; Wang, Z. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1541.
17. East, S. P.; Beckett, R. P.; Brookings, D. C.; Clements, J. M.; Doel, S.; Keavey, K.; Pain, G.; Smith, H. K.; Thomas, W.; Thompson, A. J.; Todd, R. S.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 59.
18. Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699.
19. Zega, A. *Curr. Med. Chem.* **2005**, *12*, 589.
20. Davies, S. J.; Ayscough, A. P.; Beckett, R. P.; Clements, J. M.; Doel, S.; Pratt, L. M.; Spavold, Z. M.; Thomas, S. W.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2715.
21. Jain, R.; Sundram, A.; Lopez, S.; Neckermann, G.; Wu, C.; Hackbarth, C.; Chen, D.; Wang, W.; Ryder, N. S.; Weidmann, B.; Patel, D.; Trias, J.; White, R.; Yuan, Z. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4223.
22. Clements, J. M.; Beckett, R. P.; Brown, A.; Catlin, G.; Lobell, M.; Palan, S.; Thomas, W.; Whittaker, M.; Wood, S.; Salama, S.; Baker, P. J.; Rodgers, H. F.; Barynin, V.; Rice, D. W.; Hunter, M. G. *Antimicrob. Agents Chemother.* **2001**, *45*, 563.
23. Hunter, M. G.; Beckett, R. P.; Clements, J. M.; Whittaker, M.; Davies, S. J.; Pratt, L. M.; Spavaold, Z. M.; Launchbury, S. B. *Int. Pat. Appl.* WO01010835, **2001**.
24. Stetter, H.; Spangenberger, H. *Chem. Ber.* **1958**, *91*, 1982.
25. Pratt, L. M.; Keavey, K. N.; Pain, G. D.; Mounier, L. F. *Int. Pat. Appl.* WO01010834, **2001**.
26. Smith, H. K.; Beckett, R. P.; Clements, J. M.; Doel, S.; East, S. P.; Launchbury, S. B.; Pratt, L. M.; Spavold, Z. M.; Thomas, W.; Todd, R. S.; Whittaker, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3595.