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Peptide deformylase inhibitors with activity against respiratory tract pathogens

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Abstract—A series of analogues of the peptide deformylase (PDF) inhibitor BB-3497 where the P3′ amide bond was replaced with a ketone functionality is described. The in vitro antibacterial profiling of these compounds revealed that they demonstrate activity against pathogens associated with respiratory tract infections.

The emergence of multi-drug resistant bacterial isolates from clinical settings has made the search for new classes of antibacterial agents with novel modes of action of paramount importance. One of the new targets currently receiving widespread interest from both academic and industrial research groups is peptide deformylase (PDF).[1] PDF is an iron-containing metalloenzyme responsible for the removal of the *N*-formyl group from the terminal methionine residue following protein synthesis in bacteria.[2] As deformylation is necessary before further processing of the newly synthesised protein can occur, PDF is essential for bacterial growth. This, coupled with the observation that the gene encoding PDF (*def*) is present in all sequenced pathogenic bacterial genomes, has made PDF an attractive target for antibacterial chemotherapy.

Recently, we described a structure–activity relationship (SAR) analysis of the pseudopeptide PDF inhibitor, BB-3497 (Fig. 1).[3,4] Following this preliminary SAR evaluation, the P3′ methyl ketone analogue (**1**) of BB-3497 was selected as a lead compound for a new series of PDF inhibitors as it showed good inhibitory activity against the isolated PDF enzyme and retained some antibacterial activity despite the removal of the P2′-P3′ amide bond. At the outset, we considered that the reduced peptidic nature of **1** compared to BB-3497 was an attractive feature that may offer significant advantages over more peptidic compounds in terms of metabolic stability and development potential. In this communication, we present a focused SAR study on PDF inhibitors containing a ketone group in the P3′ position and report compounds with excellent in vitro antibacterial activity.

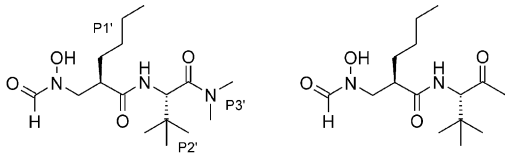

Figure 1. PDF enzyme inhibition and antibacterial activity of BB-3497 and the P3′ methyl ketone **1**.

From our earlier studies, we had established that the *N*-formyl hydroxylamine was the optimum metal-chelating group on the pseudopeptidic backbone of BB-3497.[3] Additionally, we had found that substituents in the P1′ position that mimicked the methionine residue in the natural substrate closely, such as *n*-butyl and cyclopentylmethyl, provided potent PDF inhibitors that displayed promising antibacterial activity.[4a] Consequently,

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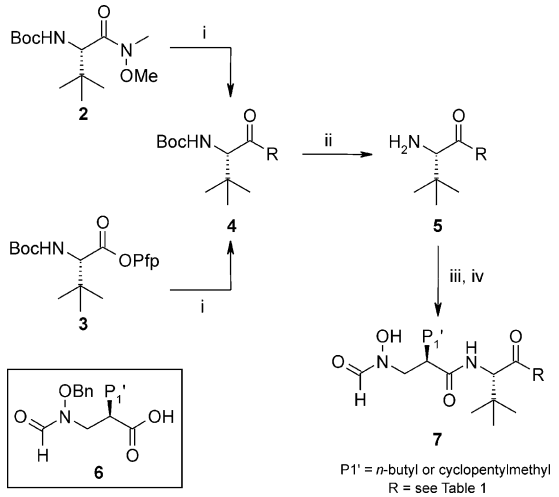
we elected to fix these optimised parameters, together with the *t*-butyl substituent in the P2′ position[4b] (which we anticipated might offer some protection to both the amide and the ketone), in the design of our P3′ ketone series.

To access compounds in this P3′ ketone series we developed convergent routes from either the Weinreb amide (**2**) or the pentafluorophenol (Pfp) ester (**3**) of *N*-Boc-*tert*-Leu (Scheme 1). Thus, Grignard or alkyl/aryl lithium addition to **2** or **3**[5] provided ketone **4**. Removal of the *N*-Boc group with HCl/dioxane gave amine **5**, which, in our preferred synthetic route, was coupled to carboxylic acid **6**[6] (P1′ = *n*-butyl or cyclopentylmethyl) activated using EDC and HOBt. The product from this reaction (not shown) was then subjected to palladium-catalysed hydrogenation to reveal the *N*-formyl hydroxylamine **7**.[7]



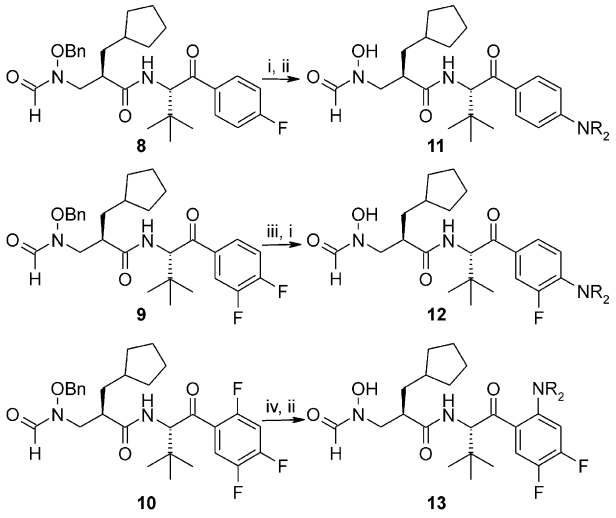
Scheme 1. (i) RLi or RMgBr, Et_2O or THF, −78 °C to rt, 40–85%; (ii) HCl, 1,4-dioxane, rt, 90–95%; (iii) **6**, EDC, HOBt, DMF, 0 °C to rt, 50–75%; (iv) H_2, 10% Pd/C, MeOH, rt, 90–95%.

Compounds were tested in an *Escherichia coli* PDF.Ni enzyme[8] assay and in primary microbiology screens against in-house strains of *E. coli* and *Staphylococcus capitis* (*S. capitis*).[9] Data from these assays on selected compounds is recorded in Table 1.

An immediate improvement in PDF inhibition and antibacterial activity was observed when the methyl group in **1** was replaced with an aromatic or heteroaromatic group. Compounds **7a–s** show excellent potency against the isolated PDF enzyme with nearly all examples inhibiting at levels below 10 nM, irrespective of the aromatic substituent in the P3′ position. Interestingly, both electron-donating (e.g., **7i**) and electron-withdrawing (e.g., **7r**) substituents were tolerated on the aryl ring without conferring a significant difference in antibacterial activity. Additionally, we concluded that the cyclopentylmethyl group in the P1′ position provided compounds that demonstrate at least a 2-fold improvement in *S. capitis* MIC's compared with the corresponding *n*-butyl analogues.

Following this study, we were particularly intrigued by the encouraging microbiological data for the P3′ aryl ketones **7o** (4-morpholinophenyl) and **7q** (4-fluorophenyl). We recognised that the combination of a fluoro and an amino substituent on an aromatic ring had been used to develop SAR in other classes of antibacterials (notably the oxazolidinones[10] and the quinolones[11]), with the effect of improving antibacterial activity and/or reducing metabolic liability. With this in mind, we were eager to investigate their combined potential as antibacterial fragments in our PDF inhibitors.

A series of P3′ 4-aminoaryl ketones containing 0 (general formula **11**) and 1 (general formula **12**) fluorine substituent in the aromatic ring were synthesised in parallel from **8** and **9** respectively using S_NAr chemistry (Scheme 2). Although the route that was used to access compounds **12** allowed diversification at the final step, the two-step route used for compounds **11** was preferred as it suffered from fewer side processes. A series of P3′ 2-aminoaryl ketones (general formula **13**) using the same set of amines was also prepared via S_NAr displacements on the corresponding trifluoroaryl ketone **10**.[12] All of the compounds prepared by these routes were purified by preparative HPLC at the final step.



Scheme 2. (i) HNR_2, DMSO, 60 °C, 16 h; (ii) 10% Pd/C, cyclohexene, MeOH, reflux, 4 h; (iii) H_2, 10% Pd/C, MeOH, rt; (iv) HNR_2, DMSO, 40 °C, 16 h.

Concomitant to this medicinal chemistry programme, experiments to explore the mechanisms of resistance against PDF inhibitors were undertaken in our research group[1d] and by Margolis et al.[13] These studies suggested that the *fmt* gene that encodes the enzyme responsible for *N*-formylation of methionine prior to protein synthesis is essential in *Streptococcus pneumoniae* and *Haemophilus influenzae*. Consequently, deformylation is a necessary process for maturation of proteins in these pathogens. As *S. pneumoniae* and *H. influenzae* are two of the major pathogens associated with respiratory tract infections (RTI's), we concluded that the PDF inhibitors were best suited to combat these types of infection. Our in vitro screening panel of bacteria was altered to reflect these observations and the P3′ aminoaryl ketones

were tested against strains of *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis*, which is also responsible for some RTI's (Table 2).[14]

From these microbiological results, we observe that the incorporation of a focused series of amines in the 2- or 4-position of the aromatic ketones is well tolerated by the enzyme. These compounds exert an excellent antibacterial effect against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, although additional basic residues (**11c**–**13c** and **11e**–**13e**) in the amino substituent may cause a slight drop in potency against Gram -ve strains. The presence of fluorine substituents in the aromatic ring does not appear to have a detrimental effect on the in vitro antibacterial profile of these PDF inhibitors.

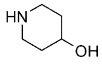
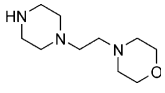
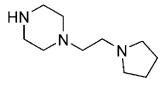
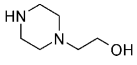
Further to this study, **7o** and **13a** were progressed for additional in vitro profiling[15] against a panel of 104 organisms (Table 3). These compounds displayed an improved spectrum of activity compared with BB-3497 with **13a** having an antibacterial profile comparable to amoxicillin-clavulanate (AMC).

Table 1. PDF and in vitro susceptibility profile for P3′ ketones

Compds	P1′	R	*E. coli* PDF.Ni IC_{50} (nM)	*E. coli* MIC[a] (μM)	*S. capitis* MIC[a] (μM)
1	*n*-Butyl	Me	20	25	100
7a	*n*-Butyl	Ph	3	12.5	12.5
7b	*n*-Butyl	2-Pyridyl	3	12.5	50
7c	*n*-Butyl	2-Furyl	5	12.5	12.5
7d	*n*-Butyl	2-Benzofuryl	4	6.25	1.5
7e	Cyclopentylmethyl	2-Benzofuryl	4	6.25	0.7
7f	*n*-Butyl	4-(OH)Ph	8	6.25	12
7g	Cyclopentylmethyl	4-(OH)Ph	2	6.25	3.1
7h	*n*-Butyl	4-(MeO)Ph	1	12.5	6.25
7i	Cyclopentylmethyl	4-(MeO)Ph	3	3.1	1.6
7j	*n*-Butyl	4-(NH_2)Ph	8	6.25	12
7k	*n*-Butyl	4-(NHCOMe)Ph	8	12	3.1
7l	*n*-Butyl	4-($NHCOCF_3$)Ph	20	6.25	6.25
7m	*n*-Butyl	4-($NHSO_2Me$)Ph	4	50	3.1
7n	*n*-Butyl	4-(morpholino)Ph	5	6.25	3.1
7o	Cyclopentylmethyl	4-(morpholino)Ph	6	12.5	1.5
7p	*n*-Butyl	4-(F)Ph	3	12.5	25
7q	Cyclopentylmethyl	4-(F)Ph	3	3.1	3.1
7r	*n*-Butyl	4-(CN)Ph	7	6.25	3.1
7s	Cyclopentylmethyl	4-(SO_2Me)Ph	10	25	6.25

[a] MIC = minimum inhibitory concentration.

Table 2. PDF and in vitro susceptibility profile for P3′ aminoaryl ketones

Compds	HNR_2	*E. coli* PDF.Ni IC_{50} (nM)	*S. pneumoniae* (6) MIC (μg/mL)	*H. influenzae* (4) MIC (μg/mL)	*M. catarrhalis* (3) MIC (μg/mL)
7o		2	0.25–0.5	0.5–1	0.125
12a		0.6	0.25	0.5–4	<0.125
13a		0.5	0.25–0.5	0.25–2	<0.125
11b		2	0.25	0.25–2	<0.125
12b		2	0.25	0.125–2	<0.125
13b		1	0.25–0.5	0.25–2	<0.125
11c		2	0.25–0.5	0.5–2	<0.125
12c		6	0.25–0.5	0.125–4	0.125
13c		2	0.25–1	2–4	0.125–0.25
11d		7	<0.125–0.25	0.25–2	<0.125
12d		2	0.25	0.125–2	<0.125
13d		2	0.25	0.5–2	<0.125
11e		3	0.25–0.5	0.5–2	0.125–0.25
12e		9	0.25–0.5	0.5–8	0.125–0.25
13e		2	0.25–0.5	0.5–4	0.125–0.25
11f		2	1–2	0.25–2	<0.125
12f		1	0.5–1	0.125–2	<0.125
13f		2	0.5–1	0.25–2	<0.125

Number of strains tested against in parentheses

Table 3. Extended in vitro susceptibility profile for **7o** and **13a**

Compds	*S. pneumoniae* MIC (μg/mL) (40)		*H. influenzae* MIC (μg/mL) (35)		*M. catarrhalis* MIC (μg/mL) (29)	
	90%	Range	90%	Range	90%	Range
BB-3497	≥16	2–>8	2	0.06–8	0.12	0.015–0.12
7o	2	0.25–2	4	0.06–≥16	0.12	0.015–0.12
13a	1	0.06–2	2	0.5–4	0.12	0.03–0.12
Penicillin	1	0.008–2	ND	ND	ND	ND
Ciprofloxacin	8	0.5–128	0.015	0.004–1	0.06	0.03–0.06
AMC	1	0.008–1	1	0.12–16	0.12	0.008–0.12

Number of strains tested against in parentheses.
ND = not determined.

In conclusion we have identified a new series of potent PDF inhibitors that display in vitro antibacterial activity against pathogens commonly associated with respiratory tract infections. Efforts to translate this in vitro activity to in vivo efficacy are ongoing in our research laboratories.

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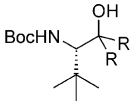

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