Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of novel aminopiperidine derivatives for antibacterial activity against Gram-positive bacteria

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ARTICLE INFO

Article history:
Received 2 February 2016
Revised 6 April 2016
Accepted 29 April 2016
Available online xxxx

Keywords:
Peptide deformylase
Antibacterial
Gram-positive
Resistant
Aminopiperidine

ABSTRACT

We have previously reported amidopiperidine derivatives as a novel peptide deformylase (PDF) inhibitor and evaluated its antibacterial activity against Gram-positive bacteria, but poor pharmacokinetic profiles have resulted in low efficacy in in vivo mouse models. In order to overcome these weaknesses, we newly synthesized aminopiperidine derivatives with remarkable antimicrobial properties and oral bioavailability, and also identified their in vivo efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PRSP).

The discovery and development of antibiotics have successfully led to the drastic decrease in human mortality in the past decades, but the overuse of antibiotics has also resulted in hospital and community-acquired multi-drug resistant pathogens. In particular, a rapidly increasing number of cases of drug resistant Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PRSP), have been reported.[1,2] Therefore, there is a crucial need for the discovery of an antibiotics target and a new mechanism of action in order to overcome limitations of current antibiotics.

Peptide deformylase (PDF) is an attractive target, since it utilizes a ferrous ion (Fe^{2+}) to catalyze the deformylation of *N*-formylmethionine from newly synthesized polypeptide. It is also a critical pathway in bacterial cell survival but is not required in mammalian cells.[3]

Representative PDF inhibitors are listed in Figure 1. Actinonin, a naturally occurring antibacterial product, is a potent PDF inhibitor, with a well-established crystal structure that binds to the Ni-PDF of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).[4,5]

From the list of PDF inhibitors, clinical trial of **BB-83698** and **LBM-415** have been discontinued despite their effectiveness against respiratory infection.[6,7] Recently, lanopepden (**GSK-1322322**) is undergoing phase II clinical trial for acute bacterial skin and skin structure infections.[8]

Most PDF inhibitors are characterized by their metal chelator and peptidomimetic and these features of structure–activity relationship (SAR) were well built up in recent studies.[9–11] In particular, the *N*-formylhydroxylamine and hydroxamic acid are potent metal binding groups in PDF enzyme inhibition. Furthermore, P1′ is a hydrophobic methionine side chain which binds to the deep S1′ binding pocket where *n*-butyl or cyclopentylmethyl group are especially dominant. P2′ side chain, which is exposed to the solvent, has shown improved PDF enzyme inhibition and antibacterial activity, especially when the terminal residue of P2′ is *tert*-butyl. Finally, P3′ enables the regulation of antibacterial activity and pharmacokinetics.

In our previous study, we have focused on modifying the substituent of the amidopiperidine at the P3′ position while fixing the metal binding group, and we also have evaluated PDF enzyme inhibition and antibacterial activity on P1′ and P2′.[12] Despite moderate in vitro antibacterial activity, the compounds displayed low efficacy in animal models (data not disclosed). Based on these results, we hypothesized that poor oral absorption of amidopiperidine analogues was reason behind the low efficacy. Therefore, amidopiperidine was replaced with aminopiperidine in order to improve low pharmacokinetic profile (Fig. 2).

We introduced aryl or heteroaryl groups in P3′ positions of the PDF inhibitors in order to improve antibacterial activity. For the

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http://dx.doi.org/10.1016/j.bmcl.2016.04.086
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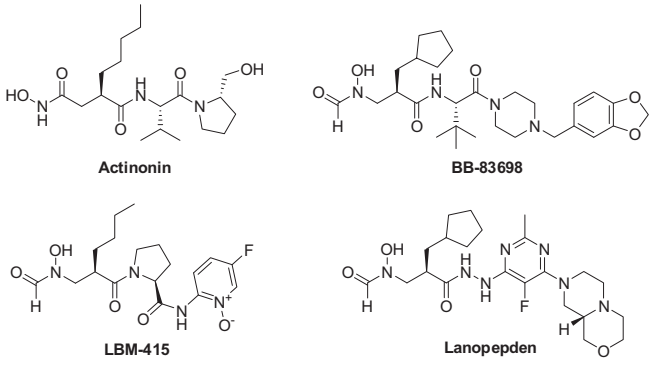


Figure 1. Representative PDF inhibitors.

SAR of P3′, we introduced aminopiperidine substituents, such as five- or six-membered or bicyclic (hetero) aromatic group.

The general method for synthesis of the aminopiperidine moiety is described in Scheme 1. Reductive amination of the starting materials *N*-Boc-4-aminopiperidine or *N*-Boc-4-piperidone (**1**) and corresponding carboxaldehyde or amine by sodium triacetoxyborohydride were given to *N*-Boc-4-*N*-substituted aminopiperidine (**2**). The protection of **2** using benzyloxycarbonyl group under basic condition, followed by the deprotection of **3** under acidic condition, yielded amine hydrochloride intermediate **4**. For P2′ moiety, reaction of **4** with Boc-L-*tert*-leucine was performed to yield an amide intermediate **5**, through 4-(dimethylamino)pyridine (DMAP) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI). Subsequently, the removal of the protecting group under acidic condition yielded key intermediate (hetero) arylaminopiperidine hydrochloride **6**. All intermediates displayed higher yield and purity via solidification or acid/base workup than via column chromatography.

The *N*-formylhydroxylamine intermediate **7** was synthesized from commercially available acid through a sequence of reactions which were described in previous studies.[12] Intermediate **8** was prepared from (hetero) arylaminopiperidine hydrochloride **6** and intermediate **7** via amide coupling conditions without purification. Finally, through the removal of the protection group by palladium-catalyzed hydrogenolysis, we obtained quantitative and optically pure *N*-formylhydroxylamine **9a**–**9k** or **10a**–**10j**.

The antibacterial activity of these (hetero) arylaminopiperidine derivatives against selected resistant Gram-positive (MRSA, VRE (*faecalis*, *faecium*), PRSP) and Gram-negative pathogens (*E. coli*, *Pseudomonas aeruginosa*) (*P. aeruginosa*) were evaluated in comparison to that of linezolid and vancomycin. The in vitro results are summarized in Table 1.

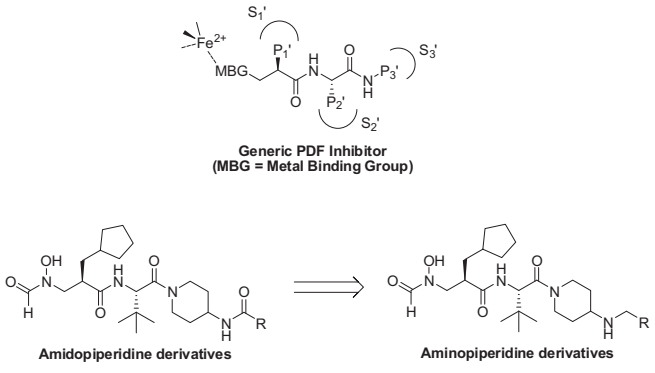


Figure 2. Generic PDF inhibitor and aminopiperidine derivatives.

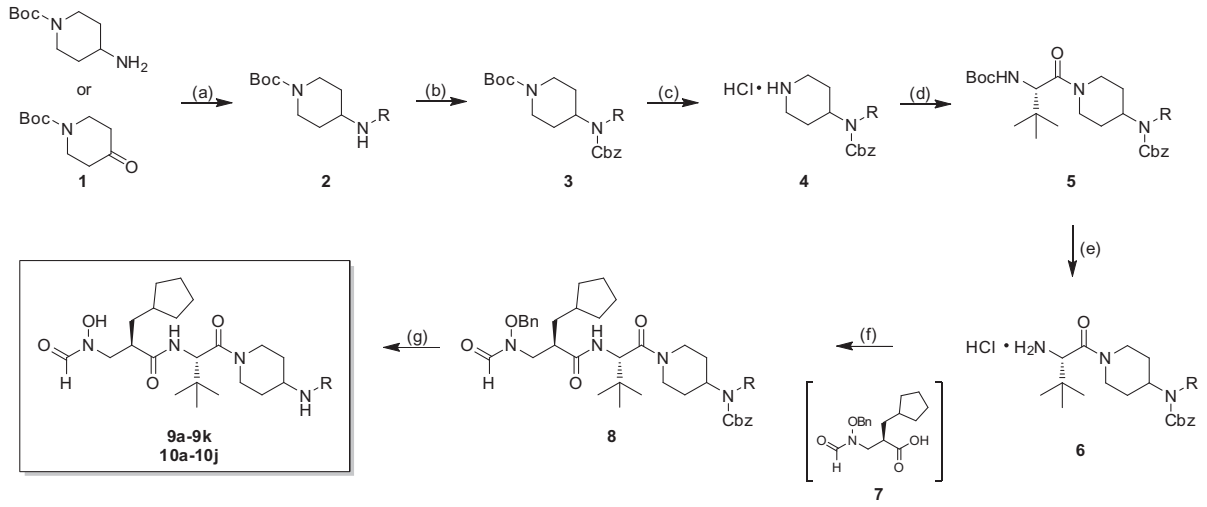
Our compounds were not potent against Gram-negative bacteria (MIC > 50 μg/mL) in minimum inhibitory concentration (MIC) assay. Possible explanations may include factors such as poor cell penetration or efflux pump of the bacteria. In the case of Gram-positive bacteria, aminopiperidines with unsubstituted or substituted five-membered pyrrole and furan group did not show antibacterial activity, with the exception of PRSP (**9a**, **9e**, **9f**), but six-membered aromatic group (**9b**, **9c**) showed results similar to that of vancomycin and linezolid. In contrast, the six-membered pyridine group (**9d**) was proven to be not an effective bacterial inhibitor compared to **9b**, since its hydrophobic deficiency had a negative effect on its potency. The antibacterial effect of the bond length of the linker between aminopiperidine and (hetero)aryl group did not affect potency (**9b**, **9c**, **9g**, **9h**), and other fused heteroaryl ring compounds did not show noticeable in vitro activity comparable to linezolid and vancomycin (**9i**, **9j**, **9k**). Consequently, six-membered aromatic substitutes **9b** and **9c** were similar to linezolid and vancomycin in terms of antibacterial activity. Based on our observation of the physical properties of **9b** and **9c**, benzyl group (**9b**) was more soluble than phenyl group (**9c**), so we selected **9b** for further SAR studies.

We have evaluated benzylaminopiperidine derivatives of compound **9b** since it may demonstrate different antibacterial activity when there is one or more substituent (Table 2). For improved antibacterial activity, we explored the electronic effects of functional group *N*-formylhydroxylamine. Assessing from the results shown in Table 2, electronic effects were not important, but it was apparent that proton donor groups completely lost their antibacterial activity. For example, antibacterial activity of **10a** was equivalent to that of **10e**, but proton donor groups, such as hydroxy (**10c**, **10h**) and amide (**10g**) did not display any activity against MRSA and VRE. Non-proton substituent groups (**10a**, **10b**, **10d**, **10e**, **10f**, **10i**, **10j**) showed good antibacterial activity (MIC < 1 μg/mL) against PRSP. Compounds **10b**, **10f** and **10i** were less active than unsubstituted **9b** when tested against all of the pathogens, but **10d** was twice as more potent than linezolid against VRE (*faecium*) and PRSP. Antibacterial activity of tri-substituted **10j** was higher than that of mono-substituted **10e** against MRSA and VRE (*faecalis*), and this supports the idea that the greater the compound's lipophilic structures are, the more permeable they are to bacterial cell. Compounds **10d** and **10j** were more potent than any other derivatives against Gram-positive pathogens, and **10j** especially showed potency equal to or higher than that of linezolid.

As a result, antibacterial activities were observed in compounds **10d** and **10j**. Additionally, enzyme inhibitory test of these compounds against *S. aureus* PDF was evaluated according to the literature.[13] The half maximal inhibitory concentration (IC_{50}) values were similar to that of **BB-83698**, we were able to identify aminopiperidine derivatives **10d** and **10j** to have excellent antibacterial properties (Table 3).

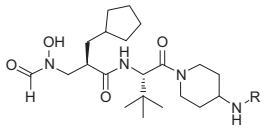
The pharmacokinetics of **10d** and **10j** was studied using mice models. Our amidopiperidine derivatives were previously confirmed to have shown a low pharmacokinetic profile. As shown in Table 4, for amidopiperidine derivatives having the same substituents as **10d**, although they exhibit similar clearance, a low therapeutic efficacy in in vivo animal models is expected as a result of low oral absorption. On the other hand, **10d** and **10j** displayed high absolute oral bioavailability (%*F*) in mice models, with rapid absorption of these compounds after oral administration. In the case of intravenous administration, **10d** was quickly eliminated, with total plasma clearance (CL) of 3.09 L/h/kg, with an apparent terminal elimination half-life ($t_{1/2}$) of 0.36 h. However, **10j** showed a 2.71-fold lower clearance and 2.91-fold longer half-life when compared to **10d** in mice.

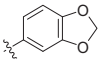
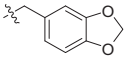
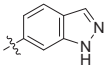
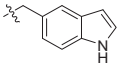
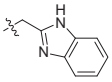
Please cite this article in press as: Lee, H.-Y.; et al. *Bioorg. Med. Chem. Lett.* (2016), http://dx.doi.org/10.1016/j.bmcl.2016.04.086



Scheme 1. Reagents and conditions: (a) carboxaldehyde (or amine), $NaBH(OAc)_3$, acetic acid, 1,2-dichloroethane, rt; (b) aq NaOH, benzyl chloroformate, THF, rt; (c) 6 N HCl, EtOAc, rt; (d) Boc-L-*tert*-leucine, DMAP, EDCI, CH_2Cl_2, rt; (e) 6 N HCl, EtOAc, rt; (f) **7**, DMAP, EDCI, CH_2Cl_2, rt; (g) H_2, Pd/C, EtOH, rt.

Table 1
Antibacterial activity of (hetero) arylaminopiperidine derivatives

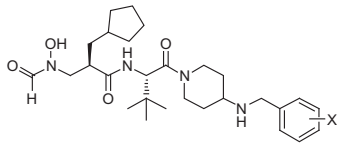


Compound	R	MIC[a] (μg/mL)					
		MRSA	VRE (*faecalis*)	VRE (*faecium*)	PRSP	*E. coli*	*P. aeruginosa*
9a	–H	>50	50	>50	1.6	>50	>50
9b		3.1	1.6	0.8	0.8	>50	>50
9c		6.3	3.1	0.8	0.8	>50	>50
9d		25	12.5	12.5	1.6	>50	>50
9e		50	25	50	1.6	>50	>50
9f		>50	>50	>50	6.3	>50	>50
9g		6.3	3.1	1.6	0.4	>50	>50
9h		6.3	3.1	0.8	1.6	>50	>50
9i		12.5	6.3	1.6	0.4	>50	>50
9j		6.3	3.1	6.3	0.8	>50	>50
9k		6.3	6.3	12.5	1.6	>50	>50
Linezolid[b]	–	1.6	0.8	1.6	0.8	>50	>50
Vancomycin[c]	–	3.1	>50	>50	0.8	>50	>50

[a] MIC values determination was performed in duplicate.
[b,c] MIC values were measured from our experimental conditions.

Furthermore, compound **10j** was tested in murine infection models against strains of MRSA, VRE and PRSP. The ED_{50} values of **10j** for each strain are shown in Table 5. In the case of systemic MRSA and pulmonary PRSP infection, **10j** was less effective than linezolid, with ED_{50} values of 16.6 mg/kg (MRSA) and 3.0 mg/kg (PRSP), compared to 5.0 mg/kg (MRSA) and 1.5 mg/kg (PRSP) for

Table 2
Antibacterial activity of substituted arylaminopiperidine derivatives



Compound	X	MIC[a] (μg/mL)			
		MRSA	VRE (*faecalis*)	VRE (*faecium*)	PRSP
9b	–H	3.1	1.6	0.8	0.8
10a	4-Methyl	6.3	3.1	0.4	0.4
10b	4-Methoxy	12.5	6.3	1.6	0.8
10c	4-Hydroxy	>50	>50	50	3.1
10d	4-Cyano	3.1	0.8	0.8	0.4
10e	4-Fluoro	6.3	3.1	0.4	0.4
10f	4-Methoxycarbonyl	12.5	6.3	3.1	0.8
10g	4-Acetamido	>50	12.5	25	1.6
10h	3,4-Dihydroxy	>50	>50	>50	12.5
10i	2,4-Dimethoxy	25	6.3	3.1	0.8
10j	2,4,5-Trifluoro	1.6	0.8	0.8	0.4
Linezolid[b]	—	1.6	0.8	1.6	0.8
Vancomycin[c]	—	3.1	>50	>50	0.8

[a] MIC values determination was performed in duplicate.
[b,c] MIC values were measured from our experimental conditions.

Table 3
PDF enzyme activity of compounds **10d** and **10j**

Compound	**BB-83698**	**10d**	**10j**
IC_{50} (nM)	30–100	30–100	30–100

linezolid. Against systemic VRE infection, ED_{50} of **10j** was 13.3 mg/kg, which was similar to that of linezolid (11.4 mg/kg). Oral administration of **10j** was shown to have activity against MRSA, VRE and PRSP infection.

In conclusion, we have designed and identified novel aminopiperidine analogues to overcome the limitations of the pharmacokinetic profiles of the amidopiperidine at P3′ position. Among them, compounds **10d** and **10j** were promising enzyme inhibitors and displayed high antibacterial activity, and compound **10j** exhibited especially good oral bioavailability, moderate clearance and half-life. Additionally, **10j** has effectively treated infected models against drug resistant Gram-positive pathogens, including MRSA, VRE and PRSP. Since compound **10j** was evaluated for GLP authentication toxicity test, we selected compound **10j** as a clinical candidate, and is currently undergoing phase I clinical studies.

Acknowledgment

This research was supported by grant from the Ministry of Trade, Industry & Energy, Republic of Korea.

Table 4
Pharmacokinetic properties of compounds **10d** and **10j** (n = 3–5, per group)

Compound	CL (L/h/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	T_{max} (h)	F (%)
Amidopiperidine(4-cyanophenyl)	2.64	2.54	0.92	0.67	55.5
10d	3.09	3.49	0.36	0.5	103.0
10j	1.14	1.71	1.05	0.5	95.8

Table 5
In vivo oral efficacy of **10j** in murine infection model (n = 6, per group)

Bacterial strain	Infection	Treatment	Compound	ED_{50} (mg/kg)	95% CI
MRSA[a]	2×10^8 CFU/200 μL/mouse, ip	BID for 3 days, po	Linezolid	5.0	1.4–7.9
			10j	16.6	8.1–24.8
VRE[b]	1×10^9 CFU/200 μL/mouse, ip	BID for 3 days, po	Linezolid	11.4	6.4–18.6
			10j	13.3	4.9–23.1
PRSP[c]	1×10^6 CFU/50 μL/mouse, in	BID for 3 days, po	Linezolid	1.5	0.3–2.5
			10j	3.0	0.0–18.6

ED_{50} and 95% confidence interval (95% CI) was calculated from the survival data at day 7 by probit analysis using the SPSS19 program.
[a] Methicillin-resistant *Staphylococcus aureus* standard strain systemic infection; normal mice.
[b] Vancomycin-resistant *Enterococcus faecium* standard strain systemic infection; immunosuppressed mice.
[c] Penicillin-resistant *Streptococcus pneumoniae* Yonsei15 lung infection; immunosuppressed mice.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.086.

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