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Original article

Synthesis, antibacterial activity, and biological evaluation of formyl hydroxyamino derivatives as novel potent peptide deformylase inhibitors against drug-resistant bacteria

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ABSTRACT

Peptide deformylase (PDF) has been identified as a promising target for novel antibacterial agents. In this study, a series of novel formyl hydroxyamino derivatives were designed and synthesized as PDF inhibitors and their antibacterial activities were evaluated. Among the potent PDF inhibitors (**1o**, **1q**, **1o′**, **1q′**, and **1x**), in vivo studies showed that compound **1q** possesses mild toxicity, a good pharmacokinetic profile and protective effects. The good in vivo efficacy and low toxicity suggest that this class of compounds has potential for development and use in future antibacterial drugs.

1. Introduction

The increased prevalence of multi drug-resistant (MDR) bacteria from clinical isolates has made the search for new antibacterial agents with novel modes of action even more important. One of the new targets currently receiving widespread attention from both academic and industrial research groups is peptide deformylase (PDF) [1–4]. PDF is an iron-containing metalloenzyme involved in the post-translational modification of nascent polypeptides in bacterial cells [4–8]. The catalytic mechanism of PDF enzymes containing zinc, iron, cobalt, and nickel dications was studied by Nino Russo in 2006 [9]. Protein synthesis in bacterial cells is initiated by ribosomal binding to a formyl methionine-charged transfer RNA, but most mature proteins do not retain the N-formyl group or the terminal methionine residue. Therefore, the removal of the N-formyl moiety catalyzed by PDF is a crucial step in bacterial protein biosynthesis and growth [10]. On the other hand, mammalian cytosolic protein synthesis does not produce N-formylated polypeptides and does not need the PDF enzyme [11]. The difference between bacterial and mammalian protein synthesis makes PDF an attractive and unique target for treating resistant bacteria [12,13].

Previous studies have shown that the potency of PDF inhibitors is closely linked to the additive effects of several chemical groups (Fig. 1): (a) metal-binding group: studies indicate that the best metal-binding group for most peptide deformylase inhibitors are hydroxamate or N-formyl hydroxylamine [14]; (b) P1′ group: substituents in the P1′ position that mimic the methionine residue in the natural substrate closely, such as n-butyl and cyclopentylmethyl, resulting in potent PDF inhibitors that display promising antibacterial activity [15]; (c) P2′ group: many active substituents have been described at the P2′ position and some inhibitors with proline at this position result in the desired combination of antibacterial activity and low toxicity [16–18], also the preliminary evaluation of our synthesized PDF inhibitors with proline derivatives at the P2′ position showed good to excellent antibacterial activity [19,20]; (d) P3′ group: the P3′ position is more amenable to different substitutions, and appropriate modifications

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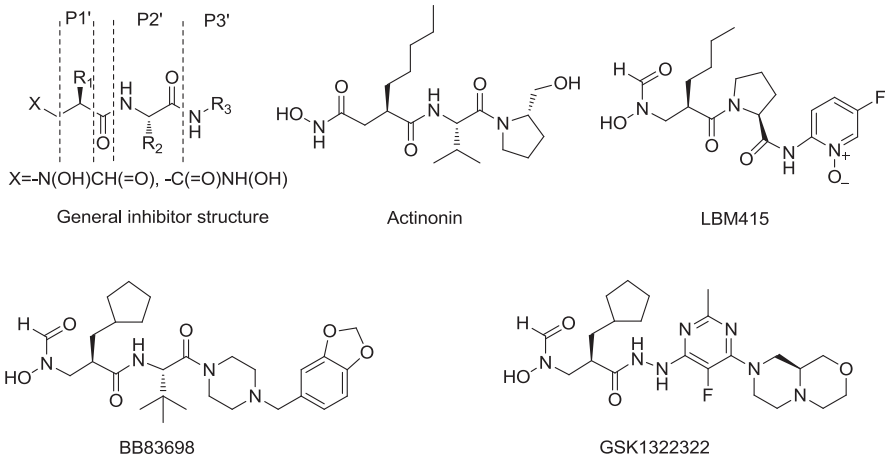


Fig. 1. Chemical structures of PDF inhibitors.

at the P3′ position could significantly improve the antibacterial activity of PDF inhibitors with only minor effects to the binding affinity for the enzyme. The first PDF inhibitor, actinonin (Fig. 1), was isolated from an actinomycete by Gordon in 1962 [21]. Actinonin exhibited moderate antibacterial activity against several Gram-positive and Gram-negative bacteria [22], but it did not show good in vivo antibacterial activity due to poor pharmacokinetic properties, which could be attributed to either poor absorption or quick clearance [23]. In order to overcome these drawbacks, novel PDF inhibitors were developed by many pharmaceutical companies and academic institutions [24–27]. LBM415 (Fig. 1, discovered by Vicuron Pharmaceuticals in collaboration with Novartis) [28,29] and BB83698 (Fig. 1, discovered by British Biotech in collaboration with Genesoft) [8,30] were the first two PDF inhibitors to undergo human clinical trials, and they exhibited much better in vitro and in vivo efficacies when compared to the original lead compound actinonin, but drawbacks of LBM415 were still founded in the further study [31]. GSK1322322 (Fig. 1, developed by GlaxoSmithKline, Brentford, UK) completed a phase II trial for acute bacterial skin and skin structure infections in April 2012 [32]. This compound not only possesses potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), but also exhibits activity against the respiratory pathogens *Haemophilus influenzae* and *Streptococcus pneumoniae* [33]. However, no PDF inhibitors are currently marketed.

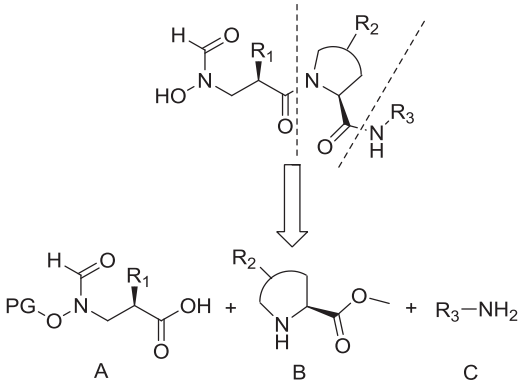
Among the PDF inhibitors that have undergone human clinical trials, LBM415 gained widespread attention for its good activity in vitro against a range of pathogens [34]. However, some concerns remain about its in vivo stability (e.g., proteolysis of the peptide bonds), solubility, and bioavailability, as these properties are closely linked to medical efficacy. In continuation of our previous study [19,20], we describe the design, synthesis, and biological evaluation of a series of PDF inhibitors based on the modification of LBM415. Five proline derivatives widely used in many biologically active products that usually play positive roles in promoting pharmacological activities and other medicinal properties, specifically (2*S*)-2,5-dihydro-1H-pyrrole-2-carboxylic acid, (2*S*)-4-methylene-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-methylpyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-fluoropyrrolidine-2-carboxylic acid, and (2*S*,3a*R*,7a*S*)-octahydro-1H-indole-2-carboxylic acid, were carefully selected to replace the proline at the P2′ position of LBM415 [35–37]. Novel PDF inhibitors were prepared by introducing various amines, such as aliphatic amines, aromatic amines, and heterocyclic aromatic amines, into the P3′ position. The in vitro antibacterial activities of all these compounds were evaluated for a primary selection, and the in vivo antibacterial activities, acute toxicity, solubility and stability, plasma protein binding rate, pharmacokinetic properties, and bioavailability of the selected representative compounds were evaluated to identify new compounds with an improved antibacterial profile and pharmacological properties.

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines the retrosynthetic analysis of the PDF inhibitors. The illustrated bond disconnection resulted in three fragments: **A**, **B**, and **C**. The target compound was assembled using the intermolecular amide coupling reaction of **A** and **B**, followed by hydrolysis of the ester group of the coupling product and subsequent coupling of the obtained carboxyl acids with amine **C**. The last step was deprotection to obtain the desired target compounds.

Following the route elucidated by Joel Slade [38], the synthesis of fragment **A** is illustrated in Scheme 2. Diethyl malonate was chosen as the starting material to synthesize fragment **A** bearing n-butyl (**A_1**) or cyclopentylmethyl (**A_2**) groups. Taking the synthesis of fragment **A_1** as an example, the reaction of diethyl malonate with 1-bromobutane in the presence of sodium ethoxide resulted in dimethyl 2-butylmalonate **6** (61% yield). The dimethyl 2-butylmalonate **6** was hydrolyzed with 25% aqueous sodium hydroxide to obtain 2-butylmalonic acid **7** (85% yield). Compound **7** was then converted to 2-methylenehexanoic acid **8** via treatment with formaldehyde in the presence of diethylamine. The reaction of



Scheme 1. Retrosynthetic analysis of PDF inhibitors.

8 with pivaloyl chloride gave an anhydride intermediate, which was treated with the anion of (*S*)-4-benzyl-2 -oxazolidinone at low temperature (−78 °C, THF) to yield conjugated amide **9** (63% yield). The diastereoselective Michael addition reaction of **9** with O-(4-methoxybenzyl)-hydroxyl amine resulted in **10** as a single diastereomer with a yield of 59% after recrystallization in t-butyl methyl ether. The chiral auxiliary was removed by LiOH and H_2O_2 to obtain the corresponding carboxylic acid derivative **11** (80% yield). Formylation of **11** using ethyl formate at 60 °C resulted in compound $\mathbf{A_1}$ (75% yield). $\mathbf{A_2}$ was prepared similarly from diethyl malonate and bromomethyl cyclopentane according to the same procedure as illustrated in Scheme 2, and the total yield was 9.6%.

Five proline derivatives (Scheme 3) were selected as fragment **B** and prepared according to known methods [39–43]: $\mathbf{B_1}$ ((*S*)-methyl 2,5-dihydro-1H-pyrrole-2-carboxylate), $\mathbf{B_2}$ ((*S*)-methyl 4-methylenepyrrolidine-2-carboxylate), $\mathbf{B_3}$ ((2*S*, 3a*R*, 7a*S*)-methyl octahydro-1H-indole-2-carboxylate), $\mathbf{B_4}$ ((2*S*, 4*S*)-methyl 4-fluoropyrrolidine-2-carboxylate), and $\mathbf{B_5}$ ((2*S*, 4*S*)-methyl 4-methylpyrrolidine-2-carboxylate).

The synthesis of PDF inhibitors **1a**–**2l** is outlined in Scheme 4. Coupling of the proline derivative **B** ($\mathbf{B_1}$–$\mathbf{B_5}$) with fragment **A** ($\mathbf{A_1}$–$\mathbf{A_2}$) using 1-hydroxy-benzotriazole monohydrate (HOBt) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI) in the presence of N-methylmorpholine (NMM) produced compound **18** (65–78% yield). Hydrolysis of **18** with lithium hydroxide gave **19** in moderate to good yield. Compound **20** was made from **19** and various amines (R_3NH_2) using the mixed anhydride coupling method. Finally, removal of the PMB protecting group by trifluoroacetic acid successfully provided the desired formyl hydroxyamino derivatives. In order to obtain pyridine N-oxide products, the PMB protected 5-fluoride pyridine derivatives were oxidized by urea hydrogen peroxide in the presence of phthalic anhydride (85–95% yield). Removal of the PMB protecting group with trifluoroacetic acid successfully provided the desired corresponding N-oxide products (41–53% yield). All new compounds were unambiguously characterized by ^1H, ^{13}C NMR spectroscopy and HR-MS.

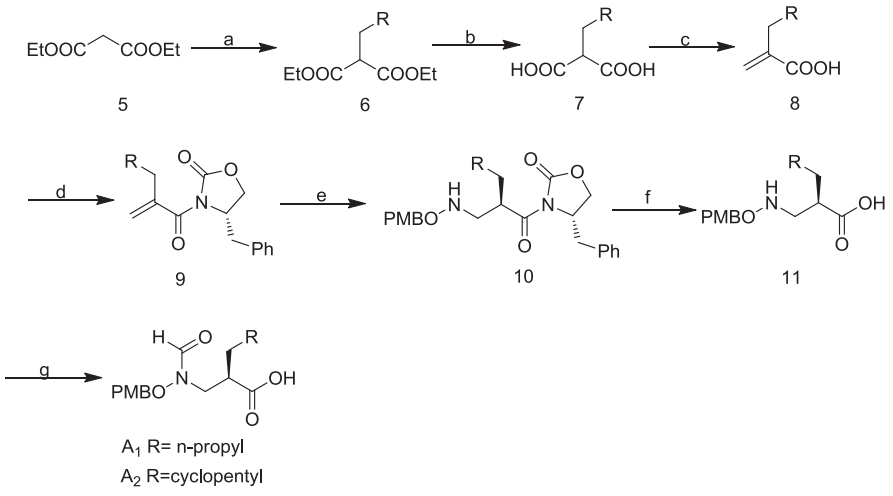
2.2. Pharmacology

2.2.1. Structure–activity relationship study

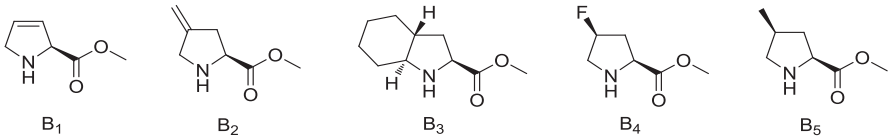
As the binding potency of a PDF inhibitor depends on the additive effects of several chemical groups, we decided to search for more efficacious PDF inhibitors through structural modification of LBM415. N-Butyl and cyclopentylmethyl groups were selected as part of the P1′ group to mimic the methionine residue present in the natural substrate. In order to improve stability and enhance antibacterial activity, significant modification at the P2′ position was carried out by choosing five widely used L-proline derivatives to replace the pyrrolidine functionality of LBM415. By introducing various aliphatic amines, aromatic amines, and heterocyclic aromatic amines at the P3′ position, 43 PDF inhibitor candidates were designed and synthesized. The in vitro antibacterial activities were evaluated and the results summarized in Tables 1–4.

Eighteen 2,5-dihydropyrrole formyl hydroxyamino derivatives with n-butyl at the P1′ position (**1a**–**r**) were synthesized initially for in vitro evaluation of antibacterial activity (Table 1). Among these PDF inhibitor candidates, compounds **1a** and **1b** containing aliphatic amines at the P3′ position exhibited unsatisfactory antibacterial activity against all tested bacterial strains. Moderate antibacterial activity was observed when using aliphatic 1-phenylethylamines containing an aromatic ring at the P3′ position (compounds **1c** and **1d**). Taking these results into account, we decided to replace the aliphatic amines at the P3′ position with aromatic amines. Compounds **1e**–**m**, each bearing aromatic amide moieties, were synthesized, and they exhibited moderate to good antibacterial activity against the Gram-positive bacterial strains. Finally, heterocyclic aromatic amines were introduced at the P3′ position, resulting in compounds **1n**–**q**, and they exhibited comparable or better antibacterial activities than the positive controls. Because compound **1f**, which contains an electron-withdrawing group, exhibited favorable antibacterial activity, we incorporated the electron-withdrawing trifluoromethyl group in a heterocyclic amine to synthesize compound **1r**. Unfortunately, a dramatic decrease in antibacterial activity was observed with **1r**.

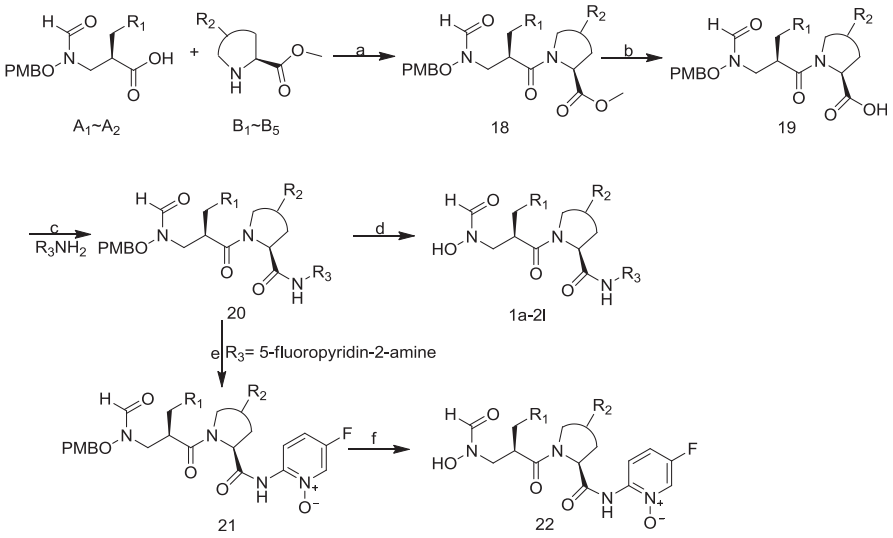
To further confirm the structure–activity relationship (SAR) of the P3′ position, several derivatives of formyl hydroxyamino 3-methylenepyrrolidine with n-butyl at the P1′ position were designed and synthesized (**1s**–**x**) for evaluation of antibacterial activity. Unlike compound **1a**, which exhibited almost no antibacterial activity, compound **1s** bearing an aliphatic amino morpholine group at the P3′ position exhibited moderate antibacterial activity. Compounds bearing aromatic amines (**1t**, **1u**) at the P3′ position typically resulted in moderate to good antibacterial activity. Good to excellent antibacterial activities were observed when



Scheme 2. Synthesis of intermediate A.
Reagents and conditions: (a) R = n-propyl: 1-bromobutane, EtOH, EtONa, reflux overnight, 61%; R = cyclopentyl: bromomethyl cyclopentane, EtOH, EtONa, reflux overnight, 60%. (b) NaOH, H_2O, reflux, 4 h, 85%. (c) HCHO, Et_2NH, EtOH, reflux overnight, 84%. (d) i, Pivaloyl chloride, Et_3N, THF, −78 °C, 2 h; ii, (S)-4-benzyl-2-oxazolidinone, BuLi, THF, −78 °C, overnight, 63%. (e) i, $PMBONH_2$, 45 °C, 24 h; ii, EtOAc, p-TsOH; iii, EtOAc, aqNa_2CO_3, 59.6%. (f) H_2O_2, LiOH, THF, H_2O, 1 h, 80%. (g) Ethyl formate, 60 °C, overnight, 75%.



Scheme 3. Chemical structures of intermediates $\mathbf{B_1}$–$\mathbf{B_5}$.



Scheme 4. Synthesis of PDF inhibitors **1a**–**2l**.
Reagents and conditions: (a) HOBT, EDCI, NMM, CH_2Cl_2, rt, 12 h, 65–78%. (b) LiOH, THF, H_2O, 2 h, rt, 85–95%. (c) i, Et_3N, $ClCO_2Et$, THF, 0–25 °C, 0.5 h; ii, R_3NH_2, 12 h, rt, 55–75%. (d) TFA, DCM, 0–25 °C, 2 h, 90%. (e) $CO(NH_2)_2$–H_2O_2, phthalic anhydride, EA, rt, 85–95%. (f) TFA, DCM, 0–25 °C, 2 h, 41–53%.

heterocyclic aromatic amines were introduced at the P3′ position in compounds **1v**–**x**. Among the screened compounds, compound **1x** bearing a 5-methylthiazol-2-amino group at the P3′ position exhibited the best antibacterial activity and was approximately 2–8-fold more potent than the positive control LBM415.

In summary, 3-methylenepyrrolidine formyl hydroxyamino derivatives (**1s**–**x**) and 2,5-dihydropyrrole formyl hydroxyamino derivatives (**1a**–**r**) exhibited the same tendency of SAR in the P3′ position. In addition, aliphatic amine derivatives are poor antibacterial agents, whereas compounds bearing aromatic amines at the P3′ position typically exhibit moderate to good antibacterial activity. Introducing heterocyclic aromatic amines into the P3′ position results in the best antibacterial activity.

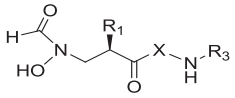
To explore the SAR at the P1′ position, several 2,5-dihydropyrrole formyl hydroxyamino derivatives with a cyclopentylmethyl group at the P1′ position were synthesized (1a′–q′). The cyclopentylmethyl compounds exhibited better in vitro antibacterial activity (2–4 times) than the corresponding n-butyl analogs (**1m**′ vs. **1m**, **1o**′ vs. **1o**, **1p**′ vs. **1p**, and **1q**′ vs. **1q**). Preliminary SAR at the P3′ position of the cyclopentylmethyl compounds was also studied, and the tendency of SAR in the P3′ position was almost identical to that of the n-butyl derivatives. The cyclopentylmethyl compounds containing aliphatic amines, such as morpholine (1a′), exhibited unsatisfactory antibacterial activity. Moderate antibacterial activities were observed when aromatic rings were introduced at the P3′ position. Notably, compounds containing heterocyclic aromatic amines at the P3′ position exhibited better antibacterial activity than existing drugs, such as penicillin, ciprofloxacin, linezolid, and vancomycin. In particular, compounds with 5-methylthiazol-2-amine at the P3′ position (**1o**, **1o**′) were exceedingly potent PDF inhibitors and 4–8-fold more potent than the positive control LBM415.

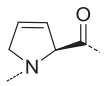
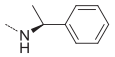
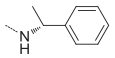
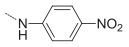
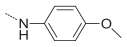
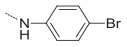
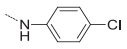
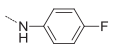
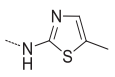
In order to obtain more information regarding the SAR at the P2′ position, three other proline derivatives, specifically (2*S*, 3a*R*, 7a*S*)-octahydro-1H-indole-2-carboxylic acid, (2*S*, 4*S*)-4-fluoropyrrolidine-2-carboxylic acid, and (2*S*, 4*S*)-4-methylpyrrolidine-2-carboxylic acid, were selected at the P2′ position to make new PDF inhibitors (**2a**–**l**). The P3′ position of these new PDF inhibitors consisted mainly of heterocyclic aromatic amines, as the SAR study of both 2,5-dihydropyrrole and 3-methylenepyrrolidine formyl hydroxyamino derivatives showed that heterocyclic aromatic amines, such as 5-methylthiazol-2-amine and 5-fluoropyridin-2-amine, are the best candidates for the P3′ position. The new compounds (**2a**–**l**) were screened against a wide range of bacteria, including *S. aureus*, MSSA, PRSP, MRSA, and MRSE, and the results are summarized in Table 2. Compounds bearing octahydroindole at the P2′ position (**2a**, **2b**, **2f**, **2g**, **2h**) exhibited only moderate antibacterial activity, which may be attributed to the large steric hindrance of the octahydroindole. The 3-fluoropyrrolidine formyl hydroxyamino derivatives (**2c**, **2d**, **2i**, **2j**) exhibited moderate to good antibacterial activity. Good to excellent antibacterial activities were observed when 3-methylpyrrolidine was introduced into the P2′ position. Notably, compound **2e**, which contains 2-amino-5-fluoropyridine-N-oxide at the P3′ position and an n-butyl group at the P1′ position exhibited better antibacterial activity than the positive control LBM415. The 3-methylpyrrolidine formyl hydroxyamino derivatives with n-butyl at the P1′ position were more potent than the corresponding cyclopentylmethyl derivatives (**2e** vs **2l**), which is different from the 2,5-dihydropyrrole and other formyl hydroxyamino analogs.

2.2.2. Broad-spectrum antimicrobial activity

After preliminary evaluation of the antimicrobial activities of the 43 synthesized novel compounds, seven candidates (**1q**, **1e**′, **1o**

Table 1
In vitro minimum inhibitory concentration (MIC, μg/ml)[a] values for **1a**–**q**′ in various strains.



No.	R_1	X	R_3	*S. aureus*	MSSA[b]	MRSA[c]	*S. epidermidis*	*E. coli*.1	*E. coli*.2
1a				>64	>64	32	>64	>64	>64
1b				64	32	2	4	64	32
1c				4	16	8	8	>64	>64
1d				4	16	2	4	>64	>64
1e				4	8	1	2	>64	64
1f				0.5	2	0.5	0.5	>64	32
1g				0.5	2	1	2	>64	>64
1h				32	4	1	8	>64	>64
1i				8	1	0.125	1	64	16
1j				4	1	0.5	2	>64	>64
1k				0.5	2	0.25	1	>64	64
1l				8	2	1	4	>64	>64
1m				2	2	1	4	>64	64
1n				0.125	1	0.125	0.5	>64	32
1o				0.0625	0.25	0.125	0.25	32	16

(*continued on next page*)

Table 1 (*continued*)

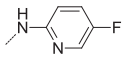
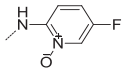
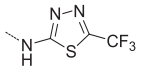
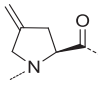
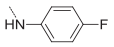
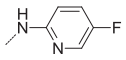
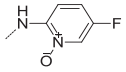
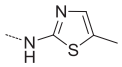
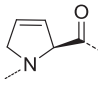
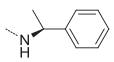
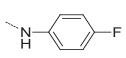
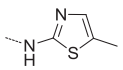
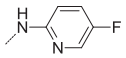
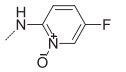
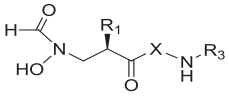
No.	R_1	X	R_3	*S. aureus*	MSSA[b]	MRSA[c]	*S. epidermidis*	*E. coli*.1	*E. coli*.2
1p				0.5	1	0.25	0.5	>64	64
1q				0.5	1	0.5	1	>64	64
1r				32	16	8	16	>64	>64
1s				4	64	4	8	>64	>64
1t				1	4	0.5	1	64	>64
1u				0.5	2	0.25	0.5	64	64
1v				0.5	1	0.125	0.25	32	>64
1w				1	4	0.5	1	>64	>64
1x				0.25	0.5	0.0312	0.0625	64	32
1a′				64	64	32	64	>64	>64
1c′				4	16	4	8	>64	>64
1e′				4	4	1	0.5	32	32
1m′				0.25	0.5	0.25	1	32	64

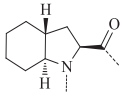
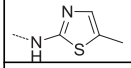
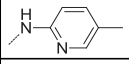
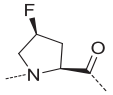
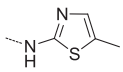
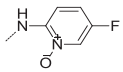
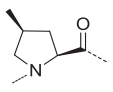
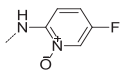
Table 1 (*continued*)

No.	R_1	X	R_3	*S. aureus*	MSSA[b]	MRSA[c]	*S. epidermidis*	*E. coli*.1	*E. coli*.2
1o′				0.03125	0.25	0.125	0.0625	16	16
1p′				0.25	0.5	0.125	0.125	32	16
1q′				0.25	0.5	0.25	0.25	64	32
LBM415				0.25	1	0.5	0.5	64	64
Linezolid				2	2	4	0.5	>64	>64
Penicillin				0.0625	1	64	2	>64	>64
Ciprofloxacin				0.5	0.5	1	0.25	<0.0312	<0.0312
Vancomycin				1	1	1	2	>64	>64

[a] MIC was determined using the broth microdilution technique. The MICs of **1a**–**r** in this table were reported previously by our group [18].
[b] MSSA, methicillin-susceptible *S. aureus*.
[c] MRSA, methicillin-resistant *S. aureus*.

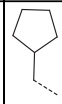
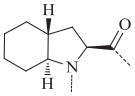
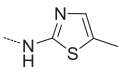
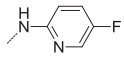
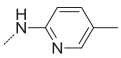
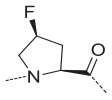
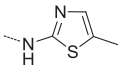
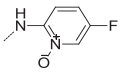
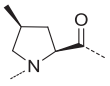
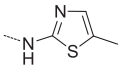
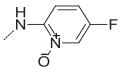
Table 2
In vitro minimum inhibitory concentration (MIC, μg/ml)[a] values for **2a**–**l** in various strains.



No.	R_1	X	R_3	*S. aureus*	MSSA[b]	MSSE[c]	*Enterococcus faecalis*	MRSA[d]	MRSE[e]	*Moraxella catarrhalis*	PRSP[f]
2a				8	8	0.5	>16	8	16	0.03	0.03
2b				8	8	0.5	>16	4	16	0.03	0.03
2c				8	8	4	>16	8	8	0.25	0.06
2d				4	4	4	>16	4	16	0.25	0.06
2e				0.25	0.25	0.0625	4	0.25	0.5	0.03	0.03

(*continued on next page*)

Table 2 (*continued*)

No.	R_1	X	R_3	*S. aureus*	MSSA[b]	MSSE[c]	*Enterococcus faecalis*	MRSA[d]	MRSE[e]	*Moraxella catarrhalis*	PRSP[f]
2f				4	4	0.5	>16	4	8	0.5	0.03
2g				4	4	0.25	>16	4	4	0.03	0.03
2h				4	4	0.5	>16	4	8	0.5	0.03
2i				1	0.5	0.125	>16	0.5	1	0.125	0.03
2j				2	0.5	0.25	8	2	4	0.25	0.03
2k				1	0.5	0.25	4	1	2	0.5	0.03
2l				0.5	0.5	1	2	0.5	1	0.5	0.03
LBM415				0.5	0.5	0.25	4	0.5	2	0.25	0.03

[a] MIC was determined using the broth microdilution technique.
[b] MSSA, methicillin-susceptible *S. aureus*.
[c] MSSE, methicillin-sensitive *Staphylococcus epidermidis*.
[d] MRSA, methicillin-resistant *S. aureus*.
[e] MRSE, methicillin-resistant *S. epidermidis*.
[f] PRSP, penicillin-resistant *Streptococcus pneumoniae*.

′, **1q**′, **1m**′, **1v**, and **1x**) were selected as representative compounds based on their in vitro antibacterial activity. The seven representative compounds, along with the control compound amoxicillin, were screened against a panel of 14 isolates representing a broad spectrum of activity. The results are summarized in Table 3. Compounds **1q**, **1o**′, **1q**′, **1m**′, and **1x** exhibited good activity against all isolates except *Escherichia coli*. However, compounds **1v** and **1e**′ exhibited unsatisfactory antibacterial activity against several tested strains, such as Entc.faecalis I and Haem.influenzae H128.

Compound **1q** was selected for further in vitro exploration of antibacterial activity by screening against 31 clinical isolates of *S. aureus*. The screening results are summarized in Table 4. Nineteen clinical isolates exhibited resistance to amoxicillin, and nine strains exhibited resistance to mupirocin. However, only one strain exhibited moderate resistance to **1q**. The Gmean values of **1q**, amoxicillin, and mupirocin were 0.625 μg/mL, 5.72 μg/mL, and 0.855 μg/mL, respectively. The results indicate that **1q** has better antibacterial potency and spectrum than amoxicillin and mupirocin.

2.2.3. Pharmacokinetic study

The outstanding in vitro antibacterial activities of compounds **1o**, **1q**, **1o**′, **1q**′, and **1x** encouraged us to evaluate their further medicinal properties. Thus, in vivo pharmacokinetic study was carried out with compounds **1o**, **1q**, **1o**′, **1q**′, **1x**, and LBM415. Rats were dosed with 5 mg/kg via intravenous (i.v.) routes and 25 mg/kg via oral (p.o.) routes. Blood samples were collected from anesthetized rats via cardiac puncture at 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h after dosing, and the concentrations of the compounds were determined by HPLC analysis. The results are summarized in Table 5 and Fig. 2. The tested compounds were rapidly absorbed after oral administration and reached maximum concentrations

Table 3
In vitro minimum inhibitory concentration (MIC, μg/mL)[a] values for seven typical compounds against 14 bacterial strains.[a]

Isolate	Compound MIC (μg/mL)							
Genus_Species_Strain	Amoxicillin	**1q**	**1o′**	**1e′**	**1q′**	1m′	**1v**	**1x**
Staph. aureus OXFORD	0.125	0.5	0.25	1	0.5	1	4	0.5
Staph. aureus WCUH29	64	0.125	≤0.06	0.125	0.125	0.125	0.5	0.125
Entc.faecalis I	0.5	4	2	>64	2	2	32	4
Entc.faecium X7501	16	2	0.5	1	1	1	4	2
Haem.influenzae Q1	0.25	1	2	2	1	2	16	4
Haem.influenzae H128	32	2	2	2	2	2	16	4
Haem.influenzae H128 Acr A-	64	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.5	≤0.06
Morax.catarrhalis 1502	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.25	≤0.06
Strep.pneumoniae 1629	≤0.06	1	0.5	2	0.5	1	16	2
Strep.pneumoniae N1387	2	0.5	0.25	0.5	0.125	0.5	4	1
Strep.pneumoniae ERY2	≤0.06	1	0.5	1	0.25	1	8	2
E. coli 3	1	64	32	64	64	32	>64	64
Strep.pyogenes 1307006P	≤0.06	2	0.25	0.25	0.25	0.25	4	2
Strep.pyogenes 1308007P	≤0.06	2	0.5	0.5	0.5	0.25	4	2

[a] MIC was determined using the broth microdilution technique.

22.5–41.3 min after dosing (Table 5a). In contrast, the T_{max} for LBM415 is 52 min after dosing, which is 11–30 min slower than the other testing compounds. The oral bioavailability seems to be closely related to the P1′ group of the tested compounds. PDF inhibitors with n-butyl in the P1′ position exhibited better oral bioavailability (**1o** vs. **1o′**, **1q** vs. **1q′**) than the corresponding cyclopentylmethyl analogs (Table 5b). When the P3′ group was taken into consideration, we found that PDF inhibitors with 5-methylthiazol-2-amine at the P3′ position exhibited much higher bioavailability than the corresponding 5-fluoropyridin-2-amino analogs (**1o** vs. **1q**, **1o′** vs. **1q′**). The absolute oral bioavailability of **1o** was calculated to be 88%, which was much higher than that of the other PDF inhibitors and LBM415.

Table 4
In vitro minimum inhibitory concentration (MIC, μg/mL)[a] values for **1q** against *Staphylococcus aureus* isolates.[a]

Isolate	Compound MIC (μg/mL)		
Genus_Species_Strain	Amoxicillin	Mupirocin	**1q**
ATCC 29213	1	0.25	1
A53	0.125	0.125	0.5
CARTER 37	0.25	>16	0.5
CL 939	2	0.25	0.125
Ealing 23	0.5	>16	0.5
Ealing 32	1	>16	0.25
F89	2	>16	1
MILES HALL	0.25	>16	0.5
OXFORD	0.25	0.125	0.5
RN4220 pmz1	0.125	>16	0.5
RUSSELL	>16	0.25	0.5
Smith ATCC 13709	0.125	0.06	0.25
SWEETING	0.125	0.125	0.125
CL 1033	>16	0.25	1
CL 938	>16	0.25	1
MN 1255	>16	0.25	0.25
NEQAS 4026	>16	>16	1
OGA833	>16	>16	0.5
PAV 5	>16	0.125	1
RN1024	>16	0.25	0.5
RN1030	>16	0.25	0.5
WCUH29	>16	0.125	0.125
306	>16	>16	2
68/8684	>16	0.125	>16
929003	>16	0.25	2
929035	>16	0.25	1
934324	>16	0.25	1
934334	>16	0.25	1
934335	>16	0.25	0.25
934387	>16	0.25	0.5
NEQAS 4158	>16	0.25	1
MIC 90	>16	>16	1
MIC 50	>16	0.25	0.5
GMEAN	5.720	0.855	0.625
MIC Min	0.125	0.06	0.125
MIC Max	>16	32	>16

[a] MIC was determined using the broth microdilution technique.

2.2.4. *hERG K^+ inhibition study*

Human ether-a-go-go-related gene1 (hERG) has received a tremendous amount of attention since its discovery in 1994 because inherited mutations or drug-induced blockade of channels increases the risk of lethal arrhythmia [44]. The outstanding in vitro antibacterial activities and good pharmacokinetics of **1o**, **1q**, **1o′**, **1q′**, and **1x** prompted us to carry out the hERG K^+ inhibition study (Table 6). Except for compound **1o′**, which exhibited 15.86 ± 1.27% inhibition at a concentration of 10 μM, the PDF inhibitors exhibited only very mild or no hERG K^+ inhibition, making them unlikely to cause serious lethal arrhythmia.

2.2.5. *Solubility and stability*

Solubility and stability of a medicine are closely linked to its absorption and dosage. Thus, the solubility and stability of the three most promising compounds (**1o**, **1p**, and **1q**) and the positive control LBM415 were studied in artificial gastric juice and artificial intestinal juice. As shown in Fig. 3, all tested compounds exhibited better solubility than the positive control LBM415. Compound **1q** was 2-fold and 3-fold more soluble than LBM415 in artificial gastric juice and artificial intestinal juice, respectively. Samples were taken every 2 h for 12 h and evaluated by HPLC to investigate the stability of these compounds (Fig. 3B and C). All compounds had similar or better stability than LBM415.

2.2.6. *In vivo efficacy study*

2-Amino-5-fluoropyridine-N-oxide derivative **1q** and 5-methylthiazol-2-amino derivative **1x** were selected as representative candidates to evaluate in vivo efficacy based on their good pharmacokinetic properties and robust activities in broader antibacterial profiling. The in vivo efficacy of **1q** was evaluated in a mouse MRSA08-12 infection model. Compound **1q** and the reference inhibitor LBM415 were administered by i.v. to the infected mice with single dose, and the results are summarized in Table 7a. It is gratified to find that compound **1q** had comparable protective effects as LBM415. The 50% effective dose (ED_{50}) of compound **1q** indicated good in vivo efficacy against MRSA (Table 7a).

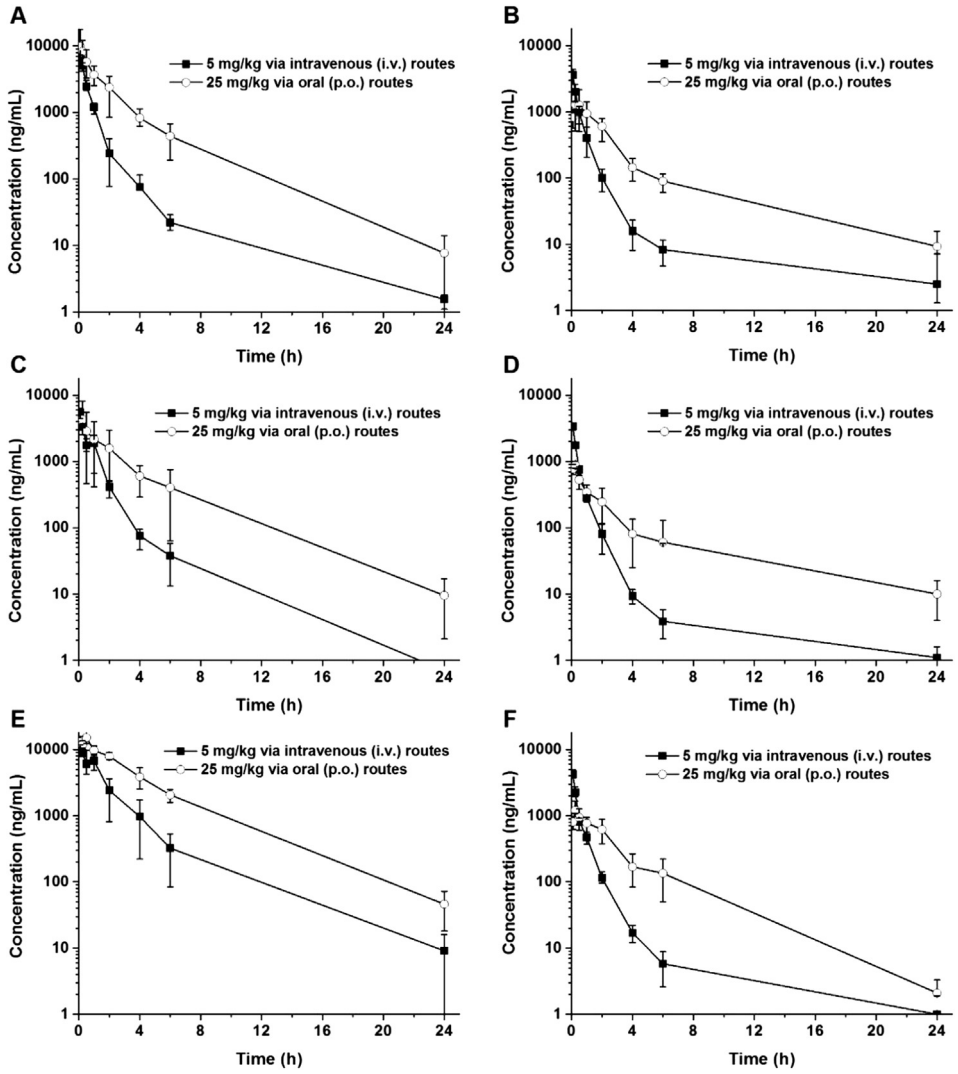


Fig. 2. Mean plasma concentration–time curve of (A) **1o**, (B) **1q**, (C) **1o′**, (D) **1q′**, (E) **1x**, and (F) LBM415 in male Sprague Dawley mice. The mice received a 5 mg/kg injection or 25 mg/kg oral dose. Data are given as mean ± SD. $n = 4$ mice in each group.

The in vivo efficacy of **1x** was evaluated in a mouse MRSA (ATCC 33591) infection model with intragastric administration of single doses (Table 7b). Although compound **1x** was still efficacious against MRSA, it had lower efficacies than the marketed drug linezolid. The inconsistency between the in vitro potency and in vivo efficacy of compound **1x** was surprising. As the in vivo efficacy may be affected by the degree to which the drug binds to the proteins within blood plasma, we decided to conduct a plasma protein binding (PPB) rate test for compounds **1q** and **1x**.

2.2.7. Plasma protein binding rate study

PPB is an important biological property with implications on a number of toxicological, pharmacological, and pharmacokinetic parameters. Therefore, we evaluated the PPB rate of compounds **1q** and **1x** with warfarin as a positive control agent, and the results are summarized in Table 8. The high PPB rate of compound **1x** (99% PPB rate) may be the responsible factor for the lower efficacy in vivo despite its good in vitro activity and pharmacokinetic properties.

Table 5a
The pharmacokinetics parameters following PDF inhibitor administration to male Sprague Dawley mice (5 mg/kg intravenous).

Parameter (mean)	**1o**	**1q**	**1o′**	**1q′**	**1x**	LBM415
$T_{1/2}$ (h)	4.08	1.17	2.68	0.807	3.21	0.784
$MRT_{0\text{-}t}$ (h)	0.952	0.564	1.24	0.503	2.03	0.562
$AUC_{0\text{-}t}$ (μg.h/L)	4924	1949	4701	1611	19,752	2239
$AUC_{0\text{-}\infty}$ (μg.h/L)	4931	1960	4704	1615	19,795	2244
CLz (mL/h/kg)	1019	2810	1115	3101	290	2265
Vss (mL/kg)	1007	1715	1373	1601	571	1305

Table 5b
The pharmacokinetics parameters following PDF inhibitor administration to male Sprague Dawley mice (25 mg/kg oral).

Parameter (mean)	**1o**	**1q**	**1o′**	**1q′**	**1x**	LBM415
C_{max} (ng/mL)	12,365	1393	4565	956	15,700	1302
t_{max} (h)	0.625	0.688	0.625	0.375	0.688	0.875
$t_{1/2}$ (h)	2.56	1.82	3.31	2.58	3.02	2.66
AUC_{0-t} (μg h/L)	21,834	3315	12,243	1626	58,108	3689
$AUC_{0-\infty}$ (μg h/L)	21,862	3390	12,295	1808	58,312	3788
MRT_{0-t} (h)	2.45	2.34	3.39	2.01	3.41	2.71
F (%)	88.67	34.59	52.27	22.39	58.92	33.76

Table 6
The inhibition ratio of PDF inhibitors in various concentrations on hERG K^+ channels.

Concentration	Fractional block %					
	1o	**1q**	**1o′**	**1q′**	**1x**	LBM415
1 μM	5.70 ± 2.65	4.55 ± 4.24	5.38 ± 1.14	1.15 ± 1.02	−0.35 ± 2.16	1.78 ± 1.26
10 μM	5.50 ± 2.42	6.71 ± 2.55	15.86 ± 1.27	0.89 ± 1.49	−2.98 ± 3.10	2.14 ± 1.81

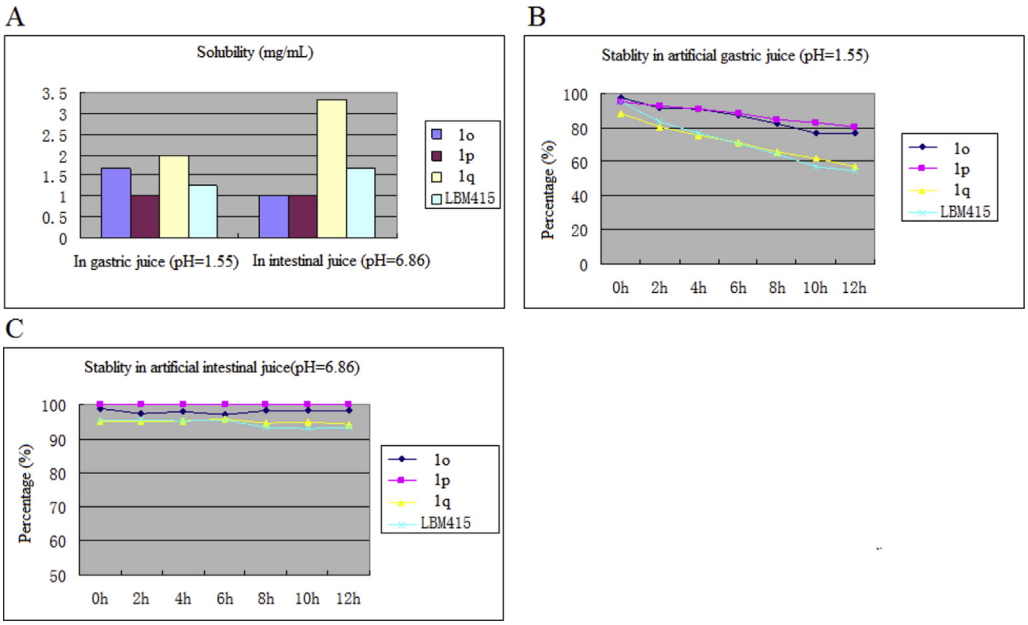


Fig. 3. The solubility and stability of **1o**, **1p**, **1q**, and LBM415 in (A, B) artificial gastric juice and (A, C) artificial intestinal juice.

2.2.8. Acute toxicity study

Ideal antibacterial agents kill selectively and without being harmful to the host. Therefore, we evaluated the acute toxic behavior of our representative compounds (**1q**) toward KM mice in SPF grade. During the 14 successive days of the acute toxicity study, 10 male and 9 female mice were injected with different concentrations of **1q** and their behavior change, weight, coat color, anal temperature, daily food intake, daily water intake, and daily weight of urine and stool were checked to judge toxicity. The mice lived without apparently abnormal behavior when **1q** was given at a dose of 100 mg/kg. When the dosage was increased to 250 mg/kg, three male mice died after the injection, and three female mice exhibited decreased activity but appeared normal 2 h later. No adverse effects were observed for the remaining mice that received **1q** at a dose of 250 mg/kg. On day 14, all animals were sacrificed and gross necropsy performed for the skin, lungs, heart, liver, kidneys, and spleen. No abnormalities were observed except dark red plaques in the lungs of one of the three dead mice in the 250 mg/kg group. In general, the PDF inhibitors studied in this work exhibited only mild toxicity toward the laboratory mice, with an LD_{50} > 250 mg/kg.

3. Conclusion

In summary, 43 formyl hydroxyamino derivatives were rationally designed and synthesized, and an SAR study of the P1′, P2′, and P3′ positions carried out to provide crucial information for the development of novel and potent PDF inhibitors. A preliminary SAR

Table 7a
In vivo efficacy of 1q and LMB415 in a rat MRSA infection model.

Strain (CFU/mL)	Drug	Route	Dose (mg/kg)	Rat amount	Death amount	Death rate (%)	ED_{50}(mg/kg) (95% CI)
MRSA08-12 (5×10^7)	1q	i.v.	20	10	1	10	6.253 (4.307–9.077)
			10	10	3	30	
			5	10	6	60	
			2.5	10	8	80	
			1.25	10	10	100	
	LBM415	i.v.	20	10	0	0	4.269 (2.934–6.211)
			10	10	2	20	
			5	10	5	50	
			2.5	10	7	70	
			1.25	10	9	90	
	Control group	–	–	10	10	100	–
	Blank control (normal saline)	i.v.	–	10	0	0	–

Table 7b
In vivo efficacy of **1x** and linezolid in a rat MRSA infection model.

Strain (CFU/mL)	Drug	Route	Dose (mg/kg)	Rat amount	Death amount	Death rate (%)	ED_{50}(mg/kg) (95% CI)
MRSA (ATCC 33591)	**1x**	i.g.	20	6	3	50	19.58 (9.11–42.09)
			10	6	4	66	
			5	6	5	83	
	Linezolid	i.g.	20	6	1	16	11.90 (8.02–17.66)
			10	6	4	66	
			5	6	5	83	
	Control group	–	–	10	10	100	–
	Blank control	i.g.	–	10	0	0	–

Table 8
Protein binding test for **1q**, **1x**, and warfarin in plasma using equilibrium dialysis.[a]

Drug	Species/matrix	%Unbound ($n = 3$)	SD	% Bound	% Recovery ($n = 3$)	SD
1q	CD-1 mouse	46.7	4.0	53.3	91.2	2.4
1x	plasma	1.0	0.2	99.0	93.7	1.8
Warfarin		5.9	0.9	94.1	104.3	5.2

[a] The concentration was calculated using the peak area ratio of analyte and internal standard.

study of the P1′ position found that the cyclopentylmethyl group provided better in vitro antibacterial activity than the corresponding n-butyl analogs. When the P2′ group was taken into consideration, we found that large steric hindrance and electron-withdrawing groups in the P2′ position decreases the antibacterial activity of the PDF inhibitors, and ingenious modification of the proline in P2′ position can maximize antibacterial activity. The SAR study of the P3′ position indicated that compounds with heterocyclic aromatic amines typically result in favorable antibacterial activity, and the optimum groups in the P3′ position were 5-methylthiazol-2-amine and 2-amino-5-fluoropyridine-N-oxide. The studies of solubility, stability, hERG K^+ channel-inhibiting ability, in vivo pharmacokinetics, and acute toxicity of the representative compounds showed that most of the representative compounds possess very mild toxicity and good pharmacokinetic profiles. We also noted that the 2-amino-5-fluoropyridine-N-oxide compounds, such as compound **1q**, exhibited comparable antibacterial activities in vitro and in vivo with known compound LBM415. The 5-methylthiazol-2-amino derivative, compound **1x**, which had a 99% PPB rate, still exhibited reasonable efficacy in vivo. Studies on reducing the PPB rate of compound **1x** by structural modification are currently in progress. We believe that derivatives of **1x** with appropriate PPB rate will have robust activities against drug-resistant bacteria both in vitro and in vivo.

4. Experimental

4.1. Chemistry

Unless otherwise described, all commercial reagents and solvents were purchased from commercial suppliers and used without further purification. Flash column chromatography was carried out using silica gel 60 (200–400 mesh), and preparative thin layer chromatography was carried out with glass-backed silica gel plates (1 mm). Thin layer chromatography was performed to monitor reactions. All ^{1}H NMR spectra are reported in δ units ppm relative to tetramethylsilane (TMS) in $CDCl_3$. All chemical shift values are reported with multiplicity, coupling constants, and proton count. All ^{13}C NMR spectra are reported in δ units ppm relative to the central line of the triplet at 77.23 ppm in $CDCl_3$. Coupling constants (J values) are reported in hertz.

4.1.1. Diethyl 2-butylmalonate (**6**)

Diethyl malonate (8.3 g, 51.8 mmol) was added to a solution of sodium ethoxide (3.52 g, 51.8 mmol) in EtOH (75 mL) and the reaction mixture heated to reflux for 30 min. N-BuBr (8.64 g, 63.2 mmol) was added over an hour and the reaction mixture refluxed overnight. The solvent was removed under reduced pressure and then water (23 mL) added. The mixture was extracted with ethyl acetate (43 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by flash chromatography using the solvent system hexane/ethyl acetate (5:1) to yield compound **6** (6.9 g, 61.2%) as a colorless oil. The NMR and MS data were the same as previously reported [45].

4.1.2. 2-Methylenehexanoic acid (**8**)

Compound **6** (8.6 g, 39.8 mmol) was added to a solution of NaOH (8.6 g, 215 mmol) in water (25 mL) at room temperature. The reaction mixture was heated to reflux for 4 h, and then cooled to ambient temperature and the pH adjusted to 1–2 with concentrated hydrochloric acid. The mixture was extracted with ethyl acetate (86 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Ether (35 mL) was added to the residue petroleum and stirred for 3 h. The product was isolated by filtration and dried at 50 °C to give 5.5 g (85%) of **7**.

Et_2NH (0.6 mL, 5.88 mmol), HCHO (aq) (4.7 mL, 58.8 mmol), and **7** (4.7 g, 29.4 mmol) were mixed in EtOH (90 mL) and the reaction mixture heated to reflux overnight. Volatiles were removed under reduced pressure, the residue dissolved in water (23 mL), and the pH adjusted to 3–4 with hydrochloric acid. The mixture was extracted with ethyl acetate (40 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield **8** (3.2 g, 84%) as a colorless oil. The NMR data were the same as reported [46].

4.1.3. (S)-3-(2-methylenehexanoyl)-4-benzyloxazolidin-2-one (**9**)

Et_3N (5.5 mL, 38.0 mmol) was added to a solution of **8** (3.9 g, 30.5 mmol) in THF (90 mL) at −78 °C under nitrogen atmosphere. Pivaloyl chloride (3.8 mL, 31.7 mmol) was then added dropwise below −60 °C. The reaction mixture was stirred at −78 °C for 30 min, warmed to room temperature and stirred for another 2 h, then cooled to −78 °C for further use (**9a**). In another flask, THF (90 mL) and (*S*)-4-benzyloxazolidin-2-one (4.9 g, 27.6 mmol) were combined and the mixture cooled to −78 °C under nitrogen atmosphere. N-BuLi (13.2 mL, 32.8 mmol) was added dropwise at −78 °C and then warmed to room temperature and stirred for another 30 min (**9b**).

The two solutions (**9a** and **9b**) were mixed together at −78 °C and then warmed to room temperature and stirred overnight. Aqueous $KHCO_3$ solution (40 mL, 40 mmol) was added to quench the reaction and the solvent removed under reduced pressure. Ethyl acetate (100 mL) and water (100 mL) were added to the residue and stirred for 10 min. The water layer was extracted with ethyl acetate (100 mL). The organic layer was combined and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to yield a yellow residue. Petroleum ether (20 mL) was added to the residue and stirred for 2 h. The product was isolated by filtration and dried to yield **9** (5.5 g, 63%) as a white power. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 5H), 5.40 (d, $J = 7$ Hz, 2H), 4.44 (m, 1H), 4.22 (m, 2H), 3.37 (m, 1H), 2.82 (m, 1H), 2.39 (m, 2H), 1.43 (m, 4H), 0.93 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1,

152.8, 144.3, 135.1, 129.4, 128.9, 127.4, 118.9, 66.4, 55.2, 37.6, 32.6, 29.9, 22.21, 13.8.

4.1.4. (S)-3-((R)-2-((4-methoxybenzyloxyamino)methyl)hexanoyl)-4-benzyloxazolidin-2-one (**10**)

O-(4-Methoxybenzyl) hydroxylamine (30 g, 0.196 mol) and **9** (23 g, 0.08 mol) were mixed together under nitrogen atmosphere. The mixture was warmed to 45 °C and stirred for 24 h. The resulting mixture was diluted with ethyl acetate (50 mL). p-Toluene sulphonic acid (68 g, 0.394 mol) in ethyl acetate (120 mL) was added to this solution. The resulting mixture was stirred for 1.5 h, filtered, and concentrated under reduced pressure. MTBE (550 mL) was added to this residue and stirred overnight. The resulting mixture was filtered and the filter cake added to a mixture solution of ethyl acetate (150 mL) and aqueous sodium carbonate (0.36 mol/L, 150 mL). The reaction mixture was stirred for 30 min and the aqueous layer separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **10** (21 g, 59.6%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (m, 5H), 7.19 (d, J = 8.5, 2H), 6.82 (d, J = 8.5, 2H), 5.75(br, 1H), 4.63 (m, 3H), 4.14 (m, 3H), 3.70(s, 3H), 3.36 (m, 1H), 3.20 (m, 2H), 2.43 (m, 1H), 1.75 (m, 1H), 1.55 (m, 1H), 1.31 (m, 4H), 0.89 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.9, 159.3, 153.4, 135.7, 130.1, 129.8, 129.4, 128.8, 127.1, 113.7, 75.6, 65.9, 55.7, 55.1, 54.3, 42.0, 37.3, 30.6, 29.2, 22.7, 13.8.

4.1.5. (R)-2-((4-methoxybenzyloxyamino)methyl)hexanoic acid (**11**)

Hydrogen peroxide (4.9 mL, 43 mmol) was added to a solution of **10** (8.6 g, 19.5 mmol) in THF (50 mL) and water (12 mL) at 0 °C, and then lithium hydroxide monohydrate (0.9 g, 21.5 mmol) in water (10 mL) was added. The mixture was stirred at 0 °C for 1 h. Sodium sulfite aqueous solution (0.42 mol/L, 70 mL) was added to this solution and the resulting solution stirred for another hour. Volatiles were distilled off and the resulting residue extracted with ethyl acetate (30 mL). The pH of the water layer was adjusted to 4–5 with hydrochloric acid and then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to yield **11** (4.4 g, 80%) as a yellow residue. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (d, J = 7 Hz, 2H), 6.88 (d, J = 7 Hz, 2H), 4.68 (m, 2H), 3.80 (s, 3H), 3.14 (m, 2H), 2.72 (m, 1H), 1.70 (m, 1H), 1.53 (m, 1H), 1.33 (m, 4H), 0.91 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.3, 159.4, 130.1, 129.4, 113.8, 75.8, 55.2 53.1, 43.0, 29.6, 29.2, 22.5, 13.8; HR-MS(ESI) calc. for $C_{15}H_{24}NO_4$ $(M+H)^+$: 282.1700, found: 282.1745.

4.1.6. (R)-2-[(4-methobenzyloxy-formyl-amino)-methyl]-hexanoic acid (**A_1**)

Compound **11** (4.5 g, 16 mmol) and ethyl formate (30 mL) were mixed at room temperature, and then warmed to 60 °C and stirred overnight. The solvents were removed in vacuo and the crude product purified by flash chromatography using the solvent system hexane/ethyl acetate (1:1) to yield compound **12** (3.7 g, 75%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09 (br, 1H), 7.35 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.89–4.69 (m, 2H), 3.81–3.64 (s, 5H), 2.72 (m, 1H), 1.62 (m, 1H), 1.48 (m, 1H), 1.24 (m, 4H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 180.0, 163.4, 160.2, 131.2, 130.0, 114.1, 55.2, 45.1, 43.4, 29.6, 29.3, 28.9, 22.4, 13.7; HR-MS(ESI) calc. for $C_{16}H_{23}NNaO_5$ $(M+Na)^+$: 332.1468, found: 332.1542.

4.1.7. (R)-3-cyclopentyl-2-((N-((4-methoxybenzyl)oxy)formamido)methyl)propanoic acid(**A_2**)

Prepared following the same procedure as **A_1**, the total yield of **A_2** was 9.6%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (bs, 1H), 7.34–7.23 (m, 2H), 6.89 (d, J = 8.5 Hz, 2H), 5.00–4.71 (m, 2H), 3.79 (s, 5H), 2.76 (m, 1H), 1.81–1.75 (m, 4H), 1.60–1.50 (m, 4H), 1.05–1.07 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) 180.0, 163.3, 160.2, 131.3, 128.9, 126.0, 114.1, 55.2, 45.3, 43.0, 37.7, 36.0, 32.7, 32.3, 25.1, 25.0; HR-MS(ESI) calc. for $C_{18}H_{25}NNaO_5$ $(M+Na)^+$: 358.1625, found: 358.1617.

4.1.8. General procedure for intermolecular amide coupling of ***A*** and ***B***

NMM (1.94 mL, 17.6 mmol) was added to a solution of **A** (8.8 mmol), HOBt (1.1 g, 8.8 mmol), and EDCI (1.7 g, 8.8 mmol) in DCM (20 mL) at room temperature, and then compound **B** (**B_1**–**B_5**, 8.8 mmol) in DCM (10 mL) was added and stirred for 12 h. The mixture was washed with 10% citric acid aqueous solution (20 mL), followed with saturated aqueous sodium carbonate (20 mL), the organic layer was dried with $MgSO_4$, and concentrated under reduced pressure to give **18** in 65–78% yield.

4.1.9. General procedure for the hydrolysis of ***18***

Lithium hydroxide monohydrate (0.28 g, 6.8 mmol) aqueous solution (15 mL) was added to a solution of **18** (6.8 mmol) in THF (15 mL) and the reaction mixture stirred for 1 h at room temperature and extracted with ethyl acetate (20 mL). The pH of the water layer was adjusted to 4–5 and extracted with ethyl acetate (20 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to obtain **19** (85–95% yield).

4.1.10. General procedure for intermolecular amide coupling of ***C*** (R_3NH_2) and ***19***

Et_3N (0.72 mL, 5.0 mmol) and ethyl chlorocarbonate (0.54 g, 5.0 mmol) were added to a solution of **19** (5.0 mmol) in THF (20 mL) at 0 °C and the reaction mixture stirred for 1 h at room temperature. R_3NH_2 (6 mmol) was added and the reaction mixture stirred for another 12 h and concentrated under reduced pressure. Ethyl acetate (20 mL) was added to the residue and stirred for 10 min. The mixture was washed with 10% citric acid aqueous solution (20 mL), followed with saturated sodium carbonate (20 mL) solution. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to obtain **20** (55–75% yield). The product was used for the next step without further purification.

4.1.11. General procedure for oxidation of ***20***

Urea-hydrogen peroxide (1.05 g, 12 mmol) was added to a solution of **20** (4 mmol) in ethyl acetate (20 mL) at 0 °C, followed by the addition of phthalic anhydride, and the reaction mixture stirred for 10 h at room temperature and diluted with sodium sulfite (2.48 g, 20 mmol) aqueous solution. The water layer was separated, the organic layer dried with $MgSO_4$, and then concentrated under reduced pressure to obtain oxynitride (85–95% yield).

4.1.12. General procedure for deprotection reaction of ***20*** or ***21***

TFA (5 mL, 67.5 mmol) was added to a solution of **20** or **21** (3.6 mmol) in DCM (10 mL) at 0 °C and the reaction mixture stirred for 2 h at room temperature. The mixture was diluted with DCM (10 mL) and water (10 mL), stirred for 10 min, and the water layer separated. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residue was subjected to chromatography on silica gel, eluting with 10% methanol/DCM to obtain **1a**–**2l** (41–53% yield).

4.1.13. N-hydroxy-N-((R)-2-((S)-2-(morpholine-4-carbonyl)-2,5-dihydro-1H-pyrrole-1-carbonyl)hexyl)formamide (***1a***)

Prepared from **A_1**, **B_1**, and morpholine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1a** (21% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.41 (s, 1H), 6.04 (m, 1H), 5.71 (m,

1H), 5.61 (m, 1H), 4.39 (m, 2H), 4.15 (m, 1H), 3.88 (m, 1H), 3.75–3.65 (m, 5H), 3.61 (m, 2H), 3.39 (m, 1H), 3.02 (m, 1H), 1.48 (m, 1H), 1.31–1.24 (m, 4H), 0.88 (t, J = 3.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 168.6, 163.0, 129.54, 123.3, 66.6, 66.4, 64.1, 54.4, 52.3, 46.0, 43.1, 42.4, 29.6, 29.5, 22.7, 13.8; HR-MS(ESI) calc. for $C_{17}H_{27}N_3NaO_5$ $(M+Na)^+$: 376.1843, found: 376.1849.

*4.1.13.1. (S)-N-cyclopropyl-1-((R)-2-((N-hydroxyformamido)methyl) hexanoyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1b**).* Prepared from **A_1**, **B_1**, and cyclopropanamine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1b** (23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50–9.75 (bs, 1H), 7.59 (s, 1H), 7.06 (s, 1H), 6.00–5.97 (m, 1H), 5.86–5.85 (m, 1H), 5.24 (m, 1H), 4.76 (m, 1H), 4.34 (m, 1H), 3.88 (m, 1H), 3.38 (m, 1H), 3.13 (m, 1H), 2.75 (m, 1H), 1.60 (m, 1H), 1.49 (m, 1H), 1.35–1.30 (m, 4H), 0.92 (t, J = 6 Hz, 3H), 0.75 (m, 2H), 0.52 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 171.4, 127.4, 126.2, 67.7, 53.9, 51.4, 40.5, 29.8, 28.9, 22.7, 22.5, 13.8, 6.5, 6.2; HR-MS(ESI) calc. for $C_{16}H_{25}N_3NaO_4$ $(M+Na)^+$: 346.1737, found: 346.1728.

*4.1.13.2. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-((S)-1-phenylethyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1c**).* Prepared from **A_1**, **B_1**, and (*S*)-1-phenylethanamine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1c** (18% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.18 (bs, 1H), 7.60 (s, 1H), 7.25 (m, 5H), 5.94 (d, J = 5 Hz, 1H), 5.81 (d, J = 5 Hz, 1H), 5.29 (t, J = 5 Hz, 1H), 5.02 (t, J = 10 Hz, 1H), 4.75 (d, J = 10 Hz, 1H), 4.28 (d, J = 10 Hz, 1H), 3.84 (t, J = 10 Hz, 1H), 3.35 (d, J = 10 Hz, 1H), 3.13 (m, 1H), 1.55 (m, 1H), 1.44 (m, 5H), 1.30 (m, 4H), 0.86 (t, J = 10 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 169.1, 158.1, 143.2, 128.3, 127.1, 126.9, 126.3, 125.9, 67.9, 53.8, 51.3, 48.9, 40.4, 29.8, 28.9, 22.5, 21.9, 13.6; HR-MS(ESI) calc. for $C_{21}H_{29}N_3NaO_4$ $(M+Na)^+$: 410.2050, found: 410.2063.

*4.1.13.3. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-((R)-1-phenylethyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1d**).* Prepared from **A_1**, **B_1**, and (*R*)-1-phenylethanamine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1d** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (bs, 1H), 7.58 (s, 1H), 7.21 (m, 5H), 5.89 (d, J = 5 Hz, 1H), 5.81 (d, J = 5 Hz, 1H), 5.32 (s, 1H), 4.99 (t, J = 10 Hz, 1H), 4.67 (d, J = 15 Hz, 1H), 4.24 (d, J = 10 Hz, 1H), 3.83 (t, J = 10 Hz, 1H), 3.32 (d, J = 15 Hz, 1H), 3.05 (m, 1H), 1.49–1.19 (m, 9H), 0.78 (t, J = 10 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 165.3, 154.2, 139.4, 124.8, 124.7, 124.5, 123.2, 122.6, 122.2, 64.0, 50.1, 47.5, 45.2, 36.8, 26.1, 25.1, 18.8, 18.1, 9.95; HR-MS(ESI) calc. for $C_{21}H_{29}N_3NaO_4$ $(M+Na)^+$: 410.2050, found: 410.2064.

*4.1.13.4. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-phenyl-2,5-dihydro-1H-pyrrole-2-carboxamide (**1e**).* Prepared from **A_1**, **B_1**, and aniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1e** (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.02 (s, 1H), 9.54 (s, 1H), 8.50 (s, 1H), 7.40 (d, J = 5 Hz, 2H), 7.03 (t, J = 5 Hz, 2H), 6.90 (t, J = 5 Hz, 1H) 5.99 (d, J = 5 Hz, 1H), 5.83 (d, J = 5 Hz, 1H), 5.48 (s, 1H), 4.49 (t, J = 15 Hz, 1H), 4.37 (d, J = 10 Hz, 1H), 4.17 (dd, J = 10 Hz, 5 Hz, 1H), 3.48 (t, J = 10 Hz, 1H), 3.10 (m, 1H), 1.70 (m, 1H), 1.46 (m, 1H), 1.29 (m, 4H), 0.88 (t, J = 5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 168.6, 163.4, 137.2, 128.4, 128.3, 125.4, 124.3, 119.7, 68.4, 54.8, 52.2, 42.4, 29.6, 29.3, 22.6, 13.7; HR-MS(ESI) calc. for $C_{19}H_{25}N_3NaO_4$ $(M+Na)^+$: 382.1737, found: 382.1715.

*4.1.13.5. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(4-nitrophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1f**).* Prepared from **A_1**, **B_1**, and 4-nitroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1f** (13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.05 (m, 4H), 7.70 (s, 1H), 5.99 (m, 1H), 5.83 (m, 1H), 5.22 (m, 1H), 4.58 (m, 1H), 4.21 (m, 1H), 3.89 (m, 1H), 3.43 (m, 1H), 3.20 (m, 1H), 1.68 (m, 1H), 1.50–1.26 (m, 5H), 0.89 (t, J = 5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.3, 169.3, 164.4, 140.1, 139.1, 125.0, 121.0, 120.7, 115.2, 114.9, 64.5, 50.9, 47.6, 37.0, 27.0, 26.3, 25.0, 18.8; HR-MS(ESI) calc. for $C_{19}H_{24}N_4NaO_6$ $(M+Na)^+$: 427.1588, found: 427.1620.

*4.1.13.6. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(4-methoxyphenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1g**).* Prepared from **A_1**, **B_1**, and 4-methoxyaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1g** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.66 (s, 1H), 7.56 (m, 2H), 6.72 (s, 2H), 6.02–5.93 (m, 2H), 5.48 (s, 1H), 4.76 (s, 1H), 4.42 (m, 1H), 3.86–3.73 (m, 4H), 3.42 (m, 1H), 3.18 (m, 1H), 1.66–1.19 (m, 6H), 0.89 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 167.6, 155.8, 131.2, 127.8, 125.9, 120.9, 113.6, 68.3, 55.2, 54.1, 51.4, 29.8, 29.5, 28.7, 22.6, 13.7; HR-MS(ESI) calc. for $C_{20}H_{27}N_3NaO_5$ $(M+Na)^+$: 412.1843, found: 412.1865.

*4.1.13.7. (S)-1-((R)-2-((Formyl-hydroxy-amino)methyl)hexanoyl)-N-(2-bromophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1h**).* Prepared from **A_1**, **B_1**, and 2-bromoaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1h** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.20 (bs, 1H), 8.77 (s, 1H), 8.23 (d, J = 8 Hz, 1H), 7.65 (s, 1H), 7.49 (d, J = 8 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 6.95 (t, J = 7.5 Hz, 1H), 6.06 (d, J = 5 Hz, 1H), 6.01 (d, J = 5 Hz, 1H), 5.60 (s, 1H), 4.83 (d, J = 13.5 Hz, 1H), 4.43 (dd, J = 10 Hz, 2.5 Hz, 1H), 3.92 (t, J = 10 Hz, 1H), 3.39 (dd, J = 10 Hz, 2.5 Hz, 1H), 3.18 (m, 1H), 1.66 (m, 1H), 1.50 (m, 1H), 1.31 (m, 4H), 0.83 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 168.4, 158.2, 135.5, 132.1, 128.0, 127.9, 125.9, 125.4, 122.9, 114.0, 68.6, 53.8, 51.3, 40.5, 29.8, 28.9, 22.6, 13.7; HR-MS (ESI) calc. for $C_{19}H_{24}N_3BrNaO_4$ $(M+Na)^+$: 460.0842, found: 460.0840.

*4.1.13.8. (S)-1-((R)-2-((Formyl-hydroxy-amino)methyl)hexanoyl)-N-(3-bromophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1i**).* Prepared from **A_1**, **B_1**, and 3-bromoaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1i** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H), 7.71 (s, 1H), 7.59 (s, 1H), 7.28 (d, J = 9 Hz, 1H), 7.08 (d, J = 8 Hz, 1H), 6.99 (t, J = 8 Hz, 1H), 6.06 (d, J = 5 Hz, 1H), 5.90 (d, J = 5 Hz, 1H), 5.44 (s, 1H), 4.79 (d, J = 14 Hz, 1H), 4.45 (d, J = 14 Hz, 1H), 3.80 (t, J = 13 Hz, 1H), 3.45 (dd, J = 10 Hz, 3 Hz, 1H), 3.24 (m, 1H), 1.70 (m, 1H), 1.68–1.25 (m, 5H), 0.90 (*t*, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 164.2, 153.6, 135.4, 126.0, 124.5, 123.0, 121.7, 118.5, 118.3, 113.9, 64.6, 50.6, 47.4, 36.8, 27.0, 25.0, 18.9, 10.0; HR-MS(ESI) calc. for $C_{19}H_{25}N_3BrO_4$ $(M+H)^+$: 438.1023, found: 438.1050.

*4.1.13.9. (S)-1-((R)-2-((Formyl-hydroxy-amino)methyl)hexanoyl)-N-(4-bromophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1j**).* Prepared from **A_1**, **B_1**, and 4-bromoaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1j** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.71 (bs, 1H), 9.35 (s, 1H), 7.57 (s, 1H), 7.26 (d, J = 5 Hz, 2H), 7.20 (d, J = 5 Hz, 2H), 6.02 (s, 1H), 5.87 (s, 1H), 5.42 (s, 1H), 4.74 (d, J = 12.5 Hz, 1H), 4.43 (d, J = 13.5 Hz, 1H), 3.77 (t,

J = 12 Hz, 1H), 3.45 (d, J = 13 Hz, 1H), 3.21 (s, 1H), 1.67 (m, 1H), 1.49–1.42 (m, 5H), 0.91 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 167.8, 157.5, 137.2, 131.5, 128.4, 125.4, 12.7, 116.3, 68.3, 54.4, 51.4, 40.6, 30.0, 28.8, 22.7, 13.8; HR-MS(ESI) calc. for $C_{19}H_{24}N_3BrNaO_4$ $(M+Na)^+$: 460.0842, found: 460.0855.

*4.1.13.10. (S)-1-((R)-2-((Formyl-hydroxy-amino)methyl)hexanoyl)-N-(2-chlorophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1k**).* Prepared from **A_1**, **B_1**, and 2-chloroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1k** (18% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (bs, 1H), 9.29 (s, 1H), 7.61 (s, 1H), 7.38 (d, J = 8 Hz, 2H), 7.20 (d, J = 7.5 Hz, 2H), 6.06 (s, 1H), 5.91 (s, 1H), 5.46 (s, 1H), 4.77 (d, J = 14 Hz, 1H), 4.44 (d, J = 13.5 Hz, 1H), 3.79 (d, J = 12 Hz, 1H), 3.45 (d, J = 12 Hz, 1H), 3.23 (m, 1H), 1.69 (m, 1H), 1.55–1.36 (m, 5H), 0.91 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 167.9, 157.6, 136.6, 128.7, 128.5, 128.3, 125.5, 120.4, 68.4, 54.4, 51.3, 40.5, 29.9, 28.8, 22.7, 13.8; HR-MS(ESI) calc. for $C_{19}H_{24}N_3ClNaO_4$ $(M+Na)^+$: 416.1384, found: 416.1374.

*4.1.13.11. (S)-1-((R)-2-((Formyl-hydroxy-amino)methyl)hexanoyl)-N-(4-chlorophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1l**).* Prepared from **A_1**, **B_1**, and 4-chloroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1l** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.27 (d, J = 8 Hz, 1H), 7.66 (s, 1H), 7.34 (m, 2H), 7.20 (m, 1H), 6.04 (s, 1H), 6.00 (s, 1H), 5.59 (s, 1H), 4.83 (d, J = 14 Hz, 1H), 4.41 (d, J = 13 Hz, 1H), 3.94 (q, J = 12 Hz, 1H), 3.42 (d, J = 13 Hz, 1H), 3.19 (m, 1H), 1.69 (m, 1H), 1.55 (m, 1H), 1.35 (m, 4H), 0.86 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 168.4, 158.2, 134.4, 129.0, 127.8, 127.4, 125.9, 124.8, 123.3, 122.2, 68.5, 53.9, 51.3, 40.5, 29.8, 28.9, 22.6, 13.7; HR-MS(ESI) calc. for $C_{19}H_{24}N_3ClNaO_4$ $(M+Na)^+$: 416.1348, found: 416.1357.

*4.1.13.12. (S)-N-(4-Fluorophenyl)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1m**).* Prepared from **A_1**, **B_1**, and 4-fluoroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1m** (14% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 (bs, 1H), 9.20 (s, 1H), 7.60 (s, 1H), 7.41 (t, J = 5 Hz, 2H), 6.85 (t, J = 8.5 Hz, 2H), 6.05 (s, 1H), 5.91 (s, 1H), 5.44 (s, 1H), 4.83 (d, J = 14 Hz, 1H), 4.41 (d, J = 13 Hz, 1H), 3.94 (q, J = 12 Hz, 1H), 3.44 (d, J = 13 Hz, 1H), 3.21 (m, 1H), 1.69 (m, 1H), 1.55 (m, 1H), 1.35 (m, 4H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 167.8, 157.7, 134.9, 128.1, 125.7, 121.0, 120.9, 115.3, 115.0, 68.4, 54.3, 51.3, 40.6, 29.9, 28.8, 22.6, 13.7; HR-MS(ESI) calc. for $C_{19}H_{24}N_3FNaO_4$ $(M+Na)^+$: 400.1643, found: 400.1653.

*4.1.13.13. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(pyridin-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1n**).* Prepared from **A_1**, **B_1**, and pyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1n** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50–9.75 (bs, 1H), 9.48 (s, 1H), 8.19 (m, 2H), 7.75 (s, 1H), 7.65 (t, J = 8 Hz, 1H), 6.98 (m, 1H), 5.97 (s, 1H), 5.94 (s, 1H), 5.47 (s, 1H), 4.67 (d, J = 14 Hz, 1H), 4.46 (d, J = 14 Hz, 1H), 3.94 (t, J = 13.7 Hz, 1H), 3.45 (d, J = 11.7 Hz, 1H), 3.20 (m, 1H), 1.69 (m, 1H), 1.42 (m, 1H), 1.33 (m, 4H), 0.88(t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 168.3, 157.6, 151.3, 147.4, 138.3, 127.9, 125.8, 119.6, 114.3, 68.4, 54.0, 51.4, 40.9, 29.9, 28.9, 22.7, 13.7; HR-MS(ESI) calc. for $C_{18}H_{24}N_4NaO_4$ $(M+Na)^+$: 383.1690, found: 383.1692.

*4.1.13.14. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(5-methylthiazol-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1o**).* Prepared from **A_1**, **B_1**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1o** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.48 (bs, 1H), 7.69 (s, 1H), 5.95 (s, 1H), 5.73 (s, 1H), 4.92 (s, 1H), 4.37 (d, J = 14 Hz, 1H), 4.07 (m, 1H), 3.37 (d, J = 13.5 Hz, 1H), 3.17 (s, 1H), 2.37–2.28 (m, 4H), 1.69 (m, 1H), 1.54(m, 2H), 1.38 (m, 3H), 0.95 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 168.5, 158.7, 154.3, 128.6, 127.1, 125.0, 66.9, 53.2, 51.4, 40.2, 29.6, 28.8, 22.7, 14.1; HR-MS (ESI) calc. for $C_{17}H_{24}N_4NaO_4S$ $(M+Na)^+$: 403.1410, found: 403.1409.

*4.1.13.15. (S)-1-((R)-2-(((formyl-hydroxyamino)methyl)hexanoyl)-N-(5-fluoropyridin-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1p**).* Prepared from **A_1**, **B_1**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1p** (15% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50–9.75 (bs, 1H), 9.66 (s, 1H), 8.23 (m, 1H), 8.03 (s, 1H), 7.70 (s, 1H), 7.41 (m, 1H), 6.01 (m, 1H), 5.94 (m, 1H), 5.51 (m, 1H), 4.74 (m, 1H), 4.45 (m, 1H), 3.94 (m, 1H), 3.44 (m, 1H), 3.21 (m, 1H), 1.75 (m, 1H), 1.55 (m, 1H), 1.42–1.25 (m, 4H), 0.89 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 168.2, 157.8, 155.0, 147.6, 135.2, 134.9, 128.1, 125.6, 115.1, 68.4, 54.0, 51.4, 40.7, 29.9, 28.9, 22.7, 13.7; HR-MS(ESI) calc. for $C_{18}H_{23}FN_4NaO_4$ $(M+Na)^+$: 401.1596, found: 401.1607.

*4.1.13.16. (S)-1-((R)-2-(((Formyl-hydroxyamino)methyl)hexanoyl)-N-5-fluoro-1-oxido-pyridin)-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1q**).* Prepared from **A_1**, **B_1**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1q** (13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.58 (s, 1H), 8.47 (m, 1H), 8.17 (m, 1H), 7.65 (s, 1H), 7.15 (m, 1H), 6.08 (m, 1H), 6.00 (m, 1H), 5.67 (m, 1H), 4.85 (m, 1H), 4.44 (m, 1H), 3.94 (m, 1H), 3.40 (m, 1H), 3.18 (m, 1H), 1.75 (m, 1H), 1.53 (m, 1H), 1.42 (m, 4H), 0.90 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 168.5, 158.3, 156.0, 141.5, 128.7, 127.4, 127.0, 125.3, 114.9, 68.5, 53.8, 51.2, 40.4, 29.6, 28.9, 22.6, 13.8; HR-MS(ESI) calc. for $C_{18}H_{23}FN_4NaO_5$ $(M+Na)^+$: 417.1545, found: 417.1567.

*4.1.13.17. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1r**).* Prepared from **A_1**, **B_1**, and 5-(trifluoromethyl)-1,3,4-thiadiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1r** (11% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (bs, 1H), 6.09 (s, 1H), 5.89 (s, 1H), 5.56 (m, 1H), 4.72 (d, J = 12.8 Hz, 1H), 4.55 (d, J = 12.8 Hz, 1H), 3.90 (m, 1H), 3.48 (m, 1H), 3.23 (s, 1H), 1.65–1.51(m, 2H), 1.33 (m, 4H), 0.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 168.8, 161.8, 158.1, 129.7, 123.8, 120.6, 118.4, 67.4, 54.1, 51.5, 40.5, 29.7, 28.8, 22.6, 13.8; HR-MS (ESI) calc. for $C_{16}H_{20}F_3N_5NaO_4S$ $(M+Na)^+$: 458.1080, found: 458.1086.

*4.1.13.18. N-hydroxy-N-((R)-2-((S)-4-methylene-2-(morpholine-4-carbonyl)pyrrolidine-1-carbonyl)hexyl)formamide (**1s**).* Prepared from **A_1**, **B_2**, and morpholine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1s** (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 5.10–5.05 (m, 3H), 4.45 (t, J = 14 Hz, 1H), 4.32 (t, J = 14 Hz, 1H), 3.78 (m, 1H), 3.65 (m, 7H), 3.53 (m, 2H), 3.18 (m, 1H), 2.93 (m, 2H), 1.72 (m, 1H), 1.45–1.25 (m, 5H), 0.92 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 169.5, 157.2, 142.5, 108.4, 66.7, 66.5, 56.2, 51.5, 51.2, 45.9, 42.3, 40.7, 35.1, 29.6, 28.9, 22.6, 13.8; HR-MS(ESI) calc. for $C_{18}H_{29}N_3NaO_5$ $(M+Na)^+$: 390.1999, found: 390.2024.

*4.1.13.19. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylene-N-phenylpyrrolidine-2-carboxamide (**1t**).* Prepared from **A_1**, **B_2**, and aniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1t** (22% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (s, 1H), 7.76 (s, 1H), 7.48 (m, 2H), 7.24 (m, 2H), 7.04 (m, 1H), 5.12–5.08 (d, J = 24 Hz, 2H), 4.92 (m, 1H), 4.51 (d, J = 14 Hz, 1H), 4.22 (d, J = 14 Hz, 1H), 3.91 (t, J = 12 Hz, 1H), 3.46 (d, J = 14 Hz, 1H), 3.18 (m, 1H), 2.97 (m, 1H), 2.84 (m, 1H), 1.66 (m, 1H), 1.52 (m, 1H), 1.31 (m, 4H), 0.84 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 169.2, 157.7, 142.8, 138.0, 128.7, 123.9, 119.5, 108.5, 60.4, 51.6, 51.4, 40.8, 33.9, 30.0, 28.8, 22.7, 13.7; HR-MS (ESI) calc. for $C_{20}H_{27}N_3NaO_4$ $(M+Na)^+$: 396.1894, found: 396.1914.

*4.1.13.20. (S)-N-(4-fluorophenyl)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylenepyrrolidine-2-carboxamide (**1u**).* Prepared from **A_1**, **B_2**, and 4-fluoroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1u** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 7.73 (s, 1H), 7.38 (m, 2H), 6.87 (t, J = 8 Hz, 2H), 5.10–5.06 (d, J = 19 Hz, 2H), 4.88 (m, 1H), 4.50 (d, J = 14 Hz, 1H), 4.23 (d, J = 14 Hz, 1H), 3.88 (t, J = 12 Hz, 1H), 3.45 (d, J = 14 Hz, 1H), 3.17 (m, 1H), 2.93–2.81 (m, 2H), 1.66 (m, 1H), 1.51 (m, 1H), 1.30 (m, 4H), 0.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 169.2, 159.9, 157.9, 142.6, 134.1, 120.9, 115.2, 115.0, 108.5, 60.3, 51.7, 51.4, 40.8, 34.1, 29.9, 28.8, 22.6, 13.7; HR-MS (ESI) calc. for $C_{20}H_{26}N_3NaO_4$ $(M+Na)^+$: 414.1800, found: 414.1799.

*4.1.13.21. (S)-N-(5-fluoropyridin-2-yl)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylenepyrrolidine-2-carboxamide (**1v**).* Prepared from **A_1**, **B_2**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1v** (14% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (s, 1H), 8.20 (m, 1H), 8.07 (s, 1H), 7.78 (s, 1H), 7.41 (m, 1H), 5.09 (m, 2H), 4.96 (m, 1H), 4.55 (d, J = 13.8 Hz, 1H), 4.26 (d, J = 13.8 Hz, 1H), 3.95 (t, J = 12 Hz, 1H), 3.45 (d, J = 12 Hz, 1H), 3.20 (m, 1H), 2.88 (m, 2H), 1.67 (m, 1H), 1.51 (m, 1H), 1.32 (m, 4H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 169.7, 157.2, 155.2, 147.6, 142.6, 135.0, 125.3, 115.0, 108.5, 60.2, 51.5, 40.8, 34.3, 29.8, 28.8, 22.6, 13.7; HR-MS (ESI) calc. for $C_{19}H_{25}FN_4NaO_4$ $(M+Na)^+$: 415.1752, found: 415.1832.

*4.1.13.22. 5-Fluoro-2-((S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylenepyrrolidine-2-carboxamido)pyridine 1-oxide (**1w**).* Prepared from **A_1**, **B_2**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1w** (12% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.60 (s, 1H), 8.45 (m, 1H), 8.22 (s, 1H), 7.72 (s, 1H), 7.14 (m, 1H), 5.29 (m, 3H), 4.63 (d, J = 12.5 Hz, 1H), 4.23 (d, J = 12.5 Hz, 1H), 3.95 (t, J = 12.5 Hz, 1H), 3.42 (d, J = 11.5 Hz, 1H), 3.16 (m, 1H), 2.99 (m, 1H), 2.86 (m, 1H), 1.68 (m, 1H), 1.50 (m, 1H), 1.32–1.30 (m, 4H), 0.84–0.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 170.2, 157.2, 155.2, 147.6, 142.6, 135.0, 127.6, 114.9, 108.5, 60.2, 51.4, 51.2, 40.6, 34.7, 29.7, 28.9, 22.6, 13.8; HR-MS (ESI) calc. for $C_{19}H_{25}FN_4NaO_5$ $(M+Na)^+$: 431.1701, found: 431.1755.

*4.1.13.23. (S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylene-N-(5-methylthiazol-2-yl)pyrrolidine-2-carboxamide (**1x**).* Prepared from **A_1**, **B_2**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1x** (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (s, 1H), 7.79 (m, 1H), 5.31 (m, 2H), 4.96 (m, 1H), 4.77 (m, 1H), 4.26 (d, J = 14.0 Hz, 1H), 4.11 (m, 1H), 3.42 (d, J = 12.5 Hz, 1H), 3.14 (m, 2H), 2.62 (d, J = 15.0 Hz, 1H), 2.27 (s, 3H), 1.91 (m, 1H), 1.50–1.44 (m, 5H), 0.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 168.2, 158.4, 157.2, 142.8, 126.9, 117.5, 108.5, 59.3, 51.4, 40.3, 29.0, 28.8, 22.6, 13.9, 11.4; HR-MS(ESI) calc. for $C_{18}H_{26}N_4NaO_4S$ $(M+Na)^+$: 417.1572, found: 417.1573.

*4.1.13.24. N-((R)-2-(cyclopentylmethyl)-3-((S)-2-(morpholine-4-carbonyl)-2,5-dihydro-1H-pyrrol-1-yl)-3-oxopropyl)-N-hydroxyformamide (**1a′**).* Prepared from **A_2**, **B_1**, and morpholine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1a′** (18% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.43 (s, H), 6.06 (m, 1H), 5.73 (m, 1H), 5.62 (s, 1H), 4.42 (m, 2H), 4.13 (m, 1H), 3.86 (m, 1H), 3.75–3.65 (m, 5H), 3.62 (m, 2H), 3.38 (m, 1H), 3.15 (m, 1H), 1.78 (m, 4H), 1.61 (m, 5H), 1.26 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 168.6, 163.0, 129.5, 123.3, 66.5, 66.3, 64.1, 54.3, 52.5, 45.9, 43.0, 41.5, 37.7, 35.9, 33.0, 32.5, 25.0; HR-MS(ESI) calc. for $C_{19}H_{29}N_3NaO_5(M+Na)^+$: 402.1999, found: 402.1999.

*4.1.13.25. (S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-((S)-1-phenylethyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1c′**).* Prepared from **A_2**, **B_1**, and (*S*)-1-phenylethanamine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1c′** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.06 (bs, 1H), 7.61 (s, 1H), 7.26 (m, 4H), 7.12 (d, J = 5 Hz, 1H), 5.96 (d, J = 5 Hz, 1H), 5.84 (d, J = 5 Hz, 1H), 5.29 (s, 1H), 5.03 (m, 1H), 4.75 (d, J = 15 Hz, 1H), 4.33 (d, J = 10 Hz, 1H), 3.83 (t, J = 10 Hz, 1H), 3.42 (d, J = 10 Hz, 1H), 3.15 (m, 1H), 1.80–1.76 (m, 4H), 1.65–1.50 (m, 5H), 1.26 (m, 3H), 1.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 169.1, 158.0, 143.2, 128.5, 127.1, 126.3, 126.0, 67.9, 53.9, 51.2, 48.9, 40.1, 37.4, 36.4, 33.0, 32.7, 24.9, 21.8; HR-MS (ESI) calc. for $C_{23}H_{31}N_3NaO_4$ $(M+Na)^+$: 436.2207, found: 436.2221.

*4.1.13.26. (S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-phenyl-2,5-dihydro-1H-pyrrole-2-carboxamide (**1e′**).* Prepared from **A_2**, **B_1**, and aniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1e′** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 9.52 (s, 1H), 8.50 (s, H), 7.32 (d, J = 10 Hz, 2H), 7.04 (t, J = 10 Hz, 2H), 6.91 (t, J = 10 Hz, 1H), 6.02 (s, 1H), 5.85 (s, 1H), 5.49 (s, 1H), 4.51 (m, 1H), 4.40 (d, J = 15 Hz, 1H), 4.21 (dd, J = 10 Hz, 5 Hz, 1H), 3.48 (t, J = 10 Hz, 1H), 3.15 (m, 1H), 1.78–1.76 (m, 4H), 1.61–1.45 (m, 5H), 1.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 168.8, 163.4, 137.2, 128.5, 128.3, 125.4, 124.4, 119.7, 68.5, 54.8, 52.4, 41.6, 38.1, 36.0, 32.8, 25.0; HR-MS(ESI) calc. for $C_{21}H_{27}N_3NaO_4$ $(M+Na)^+$: 408.1894, found: 408.1897.

*4.1.13.27. (S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(4-fluorophenyl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1m′**).* Prepared from **A_2**, **B_1**, and 4-fluoroaniline (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1m′** (13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.87 (bs, 1H), 9.48 (s, 1H), 7.59 (s, 1H), 7.37 (m, 2H), 6.78 (m, 2H), 6.04 (m, 1H), 5.88 (m, 1H), 5.43 (s, 1H), 4.74 (d, J = 14 Hz, 1H), 4.42 (d, J = 15 Hz, 1H), 3.75 (t, J = 12 Hz, 1H), 3.49 (dd, J = 12 Hz, 2.5 Hz, 1H), 3.24 (m, 1H), 2.05–1.40 (m, 9H), 1.20 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 167.8, 157.6, 134.0, 128.1, 125.6, 120.9, 115.2, 115.0, 68.4, 54.3, 51.3, 40.2, 37.3, 36.4, 33.1, 32.8, 25.0; HR-MS (ESI) calc. for $C_{21}H_{26}FN_3NaO_4$ $(M+Na)^+$: 426.1800, found: 426.1793.

4.1.13.28. (S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(5-methylthiazol-2-yl)-2,5-dihydro-1H-

*pyrrole-2-carboxamide (**1o′**).* Prepared from **A_2**, **B_1**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1o′** (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.50 (bs, 1H), 7.63 (s, 1H), 5.95 (m, 2H), 5.72 (m, 1H), 4.94 (m, 1H), 4.33 (m, 1H), 3.97 (m, 1H), 3.44 (m, 1H), 3.14 (m, 1H), 2.26 (s, 3H), 1.97 (m, 2H), 1.82 (m, 2H), 1.65–1.52 (m, 5H), 1.26 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 166.6, 159.0, 158.4, 133.8, 128.6, 127.0, 125.0, 66.6, 53.0, 51.1, 39.9, 37.6, 35.5, 33.3, 32.4, 29.5, 25.0, 11.2; HR-MS (ESI) calc. for $C_{19}H_{26}N_4NaO_4S$ $(M+Na)^+$: 429.1567, found: 429.1582.

*4.1.13.29. (S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(5-fluoropyridin-2-yl)-2,5-dihydro-1H-pyrrole-2-carboxamide (**1p′**).* Prepared from **A_2**, **B_1**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1p′** (14% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.22 (bs, 1H), 9.59 (s, 1H), 8.18 (s, H), 7.98 (s, 1H), 7.67 (s, 1H), 7.26 (s, 1H), 5.98 (s, 1H), 5.92 (s, 1H), 5.50 (s, 1H), 4.74 (d, J = 15 Hz, 1H), 4.43 (d, J = 15 Hz, 1H), 3.85 (m, 1H), 3.47 (m, 1H), 3.21 (m, 1H), 1.88–1.70 (m, 4H), 1.65–1.50 (m, 5H), 1.13 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 168.0, 157.7, 154.9, 147.5, 134.8, 128.0, 125.5, 125.1, 124.9, 115.0, 68.2, 54.0, 51.3, 40.2, 37.4, 36.3, 33.0, 32.8, 29.5, 25.0; HR-MS(ESI) calc. for $C_{20}H_{25}FN_4NaO_4$ $(M+Na)^+$: 427.1752, found: 427.1766.

*4.1.13.30. 2-((S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-2,5-dihydro-1H-pyrrole-2-carboxamido)-5-fluoropyridine 1-oxide (**1q′**).* Prepared from **A_2**, **B_1**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **1p′** (12% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.59 (s, 1H), 8.42 (m, 1H), 8.14 (s, 1H), 7.64 (s, 1H), 7.11 (m, 1H), 6.04 (s, 1H), 5.95 (s, 1H), 5.65 (s, 1H), 4.82 (d, J = 10 Hz, 1H), 4.39 (d, J = 10 Hz, 1H), 3.89 (m, 1H), 3.41 (d, J = 10 Hz, 1H), 3.15 (m, 1H), 1.87 (m, 2H), 1.76 (m, 2H), 1.56 (m, 5H), 1.11 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 168.4, 158.3, 155.8, 141.4, 128.5, 127.3, 127.0, 125.1, 114.9, 68.3, 53.7, 51.1, 39.9, 37.3, 35.9, 33.0, 32.5, 29.5, 24.9; HR-MS (ESI) calc. for $C_{20}H_{25}FN_4NaO_5$ $(M+Na)^+$: 443.1701, found: 443.1700.

*4.1.13.31. (2S,3aR,7aS)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(5-methylthiazol-2-yl)octahydro-1H-indole-2-carboxamide (**2a**).* Prepared from **A_1**, **B_3**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2a** (21% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (bs, 1H), 6.92 (s, 1H), 4.59 (m, 1H), 3.97 (m, 1H), 3.73 (m, 1H), 3.33 (m, 1H), 3.06 (m, 1H), 2.34 (m, 1H), 2.28 (s, 3H), 2.08 (m, 2H), 1.62 (m, 5H), 1.25–1.18 (m, 9H), 0.82 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.3, 158.3, 157.8, 132.7, 127.0, 60.1, 58.1, 53.4, 41.7, 37.3, 30.6, 30.3, 29.6, 29.4, 29.3, 28.8, 25.6, 23.8, 22.8, 19.8, 14.1, 11.5; HR-MS (ESI) calc. for $C_{21}H_{32}N_4NaO_4S$ $(M+Na)^+$: 459.2036, found: 459.2040.

*4.1.13.32. (2S,3aR,7aS)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(5-methylpyridin-2-yl)octahydro-1H-indole-2-carboxamide (**2b**).* Prepared from **A_1**, **B_3**, and 5-methylpyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2b** (16% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.01 (s, 1H), 7.82 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 4.64 (m, 1H), 4.02 (m, 1H), 3.83 (m, 1H), 3.43 (m, 1H), 3.16 (m, 1H), 2.30 (m, 1H), 2.26 (s, 3H), 2.10 (m, 1H), 1.86 (m, 1H), 1.68 (m, 4H) 1.43–1.01 (m, 10H), 0.84 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 170.3, 156.6, 149.3, 147.0, 139.1, 128.8, 113.9, 60.6, 58.6, 52.4, 41.8, 37.1, 30.5, 29.6, 29.4, 28.7, 25.6, 23.9, 22.8, 19.8, 17.8, 13.7; HR-MS (ESI) calc. for $C_{23}H_{34}N_4NaO_4$ $(M+Na)^+$: 453.2472, found: 453.2491.

*4.1.13.33. (2S,4S)-4-fluoro-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-N-(5-methylthiazol-2-yl)pyrrolidine-2-carboxamide (**2c**).* Prepared from **A_1**, **B_4**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2c** (17% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.58 (s, 1H), 7.66 (s, 2H), 5.46 (d, J = 52.5 Hz, 1H), 4.43 (m, 1H), 4.05 (m, 1H), 3.94 (dd, J = 12.5 Hz, 1H), 3.48–3.40 (m, 2H), 3.11 (s, 1H), 2.46 (m, 2H), 2.25 (s, 3H), 1.59 (m, 2H), 1.43 (m, 4H), 0.98 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 167.5, 158.7, 133.5, 127.3, 93.3, 91.5, 58.2, 53.2, 51.4, 40.2, 29.3, 28.6, 22.6, 14.0, 11.4; HR-MS (ESI) calc. for $C_{17}H_{25}FN_4SNaO_4$ $(M+Na)^+$: 423.1473, found: 423.1457.

*4.1.13.34. 5-Fluoro-2-((2S,4S)-4-fluoro-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)pyrrolidine-2-carboxamido)pyridine 1-oxide (**2d**).* Prepared from **A_1**, **B_4**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2d** (13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.47 (s, 1H), 8.18 (s, 1H), 7.71 (s, 1H), 7.12 (s, 1H), 5.38 (d, J = 52.5 Hz, 1H), 4.99 (d, J = 9.0 Hz, 1H), 4.19 (m, 1H), 3.95 (m, 2H), 3.42 (d, J = 13 Hz, 1H), 3.14 (bs, 1H), 2.72 (m, 1H), 2.45 (m, 1H), 1.76 (bs, 1H), 1.56 (s, 1H), 1.35 (m, 4H), 0.90 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 169.4, 157.8, 156.0, 141.5, 127.3, 114.7, 93.3, 91.5, 60.0, 54.1, 51.3, 40.8, 34.9, 31.9, 29.6, 22.6, 13.8; HR-MS(ESI) calc. for $C_{18}H_{24}F_2N_4NaO_5$ $(M+Na)^+$: 437.1607, found: 437.1635.

*4.1.13.35. 5-Fluoro-2-((2S,4S)-1-((R)-2-((N-hydroxyformamido)methyl)hexanoyl)-4-methylpyrrolidine-2-carboxamido)pyridine 1-oxide (**2e**).* Prepared from **A_1**, **B_5**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2e** (13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.46 (s, 1H), 8.50 (m, 1H), 8.29 (s, 1H), 7.74 (s, 1H), 7.12 (m, 1H), 4.70 (t, J = 8.0 Hz, 1H), 4.10 (t, J = 8.0 Hz, 1H), 3.88 (m, J = 13.0 Hz, 1H), 3.36 (d, J = 13.1 Hz, 1H), 3.20 (m, 1H), 3.14 (m, 1H), 2.54 (m, 1H), 2.39 (m, 1H), 1.71 (m, 2H), 1.37 (m, 4H), 1.11 (d, J = 13.8 Hz, 3H), 0.92 (t, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 171.0, 158.0, 141.6, 127.3, 127.0, 115.9, 115.0, 62.1, 54.7, 51.4, 40.5, 37.5, 33.8, 29.8, 28.7, 22.6, 16.6, 13.8; HR-MS(ESI) calc. for $C_{19}H_{27}FN_4NaO_5$ $(M+Na)^+$: 433.1858, found: 433.1863.

*4.1.13.36. (2S,3aR,7aS)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(5-methylthiazol-2-yl)octahydro-1H-indole-2-carboxamide (**2f**).* Prepared from **A_2**, **B_3**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2f** (14% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (bs, 1H), 6.99 (s, 1H), 4.63 (m, 1H), 4.06 (m, 1H), 3.75 (m, 1H), 3.43 (m, 1H), 3.17 (m, 1H), 2.35 (s, 4H), 2.17 (m, 2H), 1.91–1.38 (m, 15H), 1.29 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 170.1, 158.1, 157.8, 133.0, 127.0, 60.0, 58.2, 53.3, 41.0, 37.7, 37.3, 36.7, 33.2, 32.8, 30.2, 28.6, 25.6, 25.1, 24.9, 23.8, 19.8, 11.5; HR-MS (ESI) calc. for $C_{23}H_{34}N_4NaO_4S(M+Na)^+$: 485.2193, found: 485.2171.

*4.1.13.37. (2S,3aR,7aS)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(5-fluoropyridin-2-yl)octahydro-1H-indole-2-carboxamide (**2g**).* Prepared from **A_2**, **B_3**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel

(MeOH/DCM = 1:10) to obtain **2g** (13% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 8.19–8.09 (m, 2H), 7.78 (s, 1H), 7.38 (m, 1H), 4.67 (m, 1H), 4.02 (bs, 1H), 3.79 (m, 1H), 3.51 (m, 1H), 3.21 (m, 1H), 2.44–2.28 (m, 2H), 2.03 (m, 1H), 1.74 (m, 8H), 1.47 (m, 7H), 1.07 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 169.6, 161.9, 147.7, 135.3, 135.1, 124.9, 114.8, 60.3, 59.0, 50.8, 41.1, 37.9, 37.0, 33.1, 32.7, 28.6, 28.5, 25.6, 25.1, 24.9, 23.9, 19.7; HR-MS (ESI) calc. for $C_{24}H_{33}N_4FO_4$ $(M+Na)^+$: 483.2378, found: 483.2344.

*4.1.13.38. (2S,3aR,7aS)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-N-(5-methylpyridin-2-yl)octahydro-1H-indole-2-carboxamide (**2h**).* Prepared from **A_2**, **B_3**, and 5-methylpyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2h** (13% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 8.08 (m, 2H), 7.80 (s, 1H), 7.49 (m, 1H), 4.66 (t, J = 8.4 Hz, 1H), 4.03 (dd, J = 6.0 Hz, 4.8 Hz, 1H), 3.81 (m, 1H), 3.51 (m, 1H), 3.21 (m, 1H), 2.48(m, 1H), 2.31 (m, 1H), 2.27 (s, 3H), 2.07 (m, 1H), 1.76 (m, 7H), 1.53 (m, 7H), 1.25 (m, 3H) 1.06 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 168.8, 156.5, 154.1, 146.3, 134.5, 124.2, 113.4, 63.3, 57.3, 52.7, 41.7, 36.7, 35.6, 32.1, 31.5, 30.9, 28.7, 28.3, 27.3, 24.5, 24.0, 23.5, 21.6, 18.7; HR-MS(ESI) calc. for $C_{25}H_{37}N_4O_4S$ $(M+H)^+$: 457.2809, found: 457.2807.

*4.1.13.39. (2S,4S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-4-fluoro-N-(5-methylthiazol-2-yl)pyrrolidine-2-carboxamide (**2i**).* Prepared from **A_2**, **B_4**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2i** (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.48 (s, 1H), 7.67 (s, 1H), 7.61 (s, 1H), 5.38–5.18 (m, 2H), 4.41 (m, 1H), 3.97 (m, 2H), 3.54 (m, 1H), 3.07 (m, 1H), 2.48 (m, 2H), 2.37 (m, 3H), 1.97 (m, 3H), 1.69 (m, 2H), 1.59 (m, 4H), 1.26 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 167.5, 158.9, 133.6, 127.2, 93.3, 91.5, 58.3, 53.3, 51.3, 40.2, 37.8, 35.5, 33.3, 32.4, 25.1, 25.0, 11.3; HR-MS(ESI) calc. for $C_{19}H_{27}FN_4NaO_4S$ $(M+Na)^+$: 449.1629, found: 449.1622.

*4.1.13.40. 2-((2S,4S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-4-fluoropyrrolidine-2-carboxamido)-5-fluoropyridine 1-oxide (**2j**).* Prepared from **A_2**, **B_4**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2j** (15% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.57 (s, 1H), 8.50 (m, 1H), 8.21 (s, 1H), 7.74 (s, 1H), 7.14 (s, 1H), 5.40 (d, J = 52.5 Hz, 1H), 5.00 (d, J = 9.3 Hz, 1H), 4.21 (m, 1H), 3.93 (m, 2H), 3.50 (d, J = 13.4 Hz, 1H), 3.19 (bs, 1H), 2.77 (t, J = 16.5 Hz 1H), 2.45 (m, 1H), 1.94–1.79 (m, 4H), 1.65 (m, 4H), 1.20 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 169.3, 158.0, 153.5, 141.6, 127.4, 114.8, 93.3, 91.5, 60.0, 54.1, 51.3, 40.4, 37.5, 36.4, 33.1, 32.7, 29.6, 25.0; HR-MS (ESI) calc. for $C_{20}H_{26}F_2N_4NaO_5$ $(M+Na)^+$: 463.1763, found: 463.1759.

*4.1.13.41. (2S,4S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-4-methyl-N-(5-methylthiazol-2-yl)pyrrolidine-2-carboxamide (**2k**).* Prepared from **A_2**, **B_5**, and 5-methylthiazol-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2k** (22% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (m, 1H), 4.60 (m, 1H), 4.27 (m, 1H), 4.02 (m, 1H), 3.43 (m, 1H), 3.15 (m, 1H), 2.52 (m, 1H), 2.39 (m, 3H), 2.20 (m, 1H), 1.97 (m, 1H), 1.86 (m, 1H), 1.58 (m, 6H), 1.00 (m, 5H), 0.87 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 170.7, 157.6, 133.9, 125.3, 113.0, 59.3, 53.6, 52.8, 36.4, 31.8, 30.9, 28.6, 24.1, 21.6, 15.6, 13.1, 10.5; HR-MS(ESI) calc. for $C_{20}H_{30}N_4NaO_4S$ $(M+Na)^+$: 445.1880, found: 445.1873.

*4.1.13.42. 2-((2S,4S)-1-((R)-3-cyclopentyl-2-((N-hydroxyformamido)methyl)propanoyl)-4-methylpyrrolidine-2-carboxamido)-5-fluoropyridine 1-oxide (**2l**).* Prepared from **A_2**, **B_5**, and 5-fluoropyridin-2-amine (R_3NH_2) according to the general procedure and purified by flash column chromatography on silica gel (MeOH/DCM = 1:10) to obtain **2l** (16% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (s, 1H), 8.50 (m, 1H), 8.21 (s, 1H), 7.74 (s, 1H), 7.14 (m, 1H), 4.67 (t, J = 8.0 Hz, 1H), 4.12 (m, J = 8.0 Hz, 1H), 3.86 (m, 1H), 3.44 (m, J = 13.7 Hz, 1H), 3.24 (m, 1H), 3.13 (m, 1H), 2.50 (m, 1H), 2.38 (bs, 1H), 1.90 (m, 3H), 1.70–1.54 (m, 7H), 1.10 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 170.9, 157.7, 155.9, 141.7, 127.4, 115.7, 114.9, 62.1, 54.7, 51.3, 40.2, 37.5, 37.4, 36.3, 33.8, 33.0, 32.8, 29.6, 25.1, 16.7; HR-MS (ESI) calc. for $C_{21}H_{29}FN_4NaO_5$ $(M+Na)^+$: 459.2014, found: 459.2019.

4.2. Biology

4.2.1. Antibiotic susceptibility tests

Whole-cell antimicrobial activity was determined by broth microdilution using the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS; Document M7-A4). All organisms were brought from The People's Hospital of Jiangsu Province and consisted of ATCC strains and clinical isolates of relevant pathogens, including methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), enterococci, methicillin-susceptible *S. aureus* (MSSA), methicillin-susceptible *Staphylococcus haemolyticus* (MSSE), penicillin-resistant *S. pneumonia* (PRSP), *Enterococcus faecalis*, *Moraxella catarrhalis*, and *E. coli*. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of compound that inhibited visible growth after 24 h at 35 °C.

4.2.2. Pharmacokinetics study

Briefly, outbred male Sprague–Dawley rats (Shanghai Slaccas Laboratory Animal Breeding Co., Ltd) were used for pharmacokinetic analysis of compounds **1o**, **1q**, **1o′**, **1q′**, **1x**, and LBM415. The compounds were formulated in PBS (pH 6.5, 20% DMSO) and filter sterilized. Rats (weight, 274–375 g each) were administered 5 mg of the chosen compound per kilogram of body weight in a volume of 2 mL/kg by intravenous (i.v.) route, or 25 mg of the chosen compound per kilogram of body weight in a volume of 10 mL/kg by oral (p.o.) route. Blood samples were collected from anesthetized rats via cardiac puncture 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h after dosing. Groups of four rats were used for each time point. The blood was allowed to clot, and the serum samples collected and stored immediately at −80 °C. For analysis, standards were prepared by spiking PDF inhibitor candidates in control serum and extracting the samples with an equal volume of acetonitrile; the supernatant was analyzed by UFLC (3.0 × 50 mm pro-C18 column from ACE on HP1100 ChemStation from Agilent with UV detection at 240 nm). The runs were performed with a linear gradient of A (10 mM ammonium formate aqueous solution) and B (acetonitrile:methanol = 7:3): t = 0.1 min, 36% B; t = 2 min, 95% B; t = 2.3 min, 95% B; t = 2.4 min, 36% B; t = 4 min, stop). The pharmacokinetics parameters, including time to maximum concentration (T_{max}), maximum concentration measured (C_{max}), terminal half-life ($t_{1/2}$), and area under the curve (AUC) were calculated using WinNorlin (Version 5.3, Pharsight). The oral bioavailability was calculated as the ratio of the AUC for p.o. administration and the AUC for i.v. administration.

4.2.3. hERG inhibition

All compounds to be tested were dissolved in dimethyl sulphoxide at a concentration of 100 mM and then diluted to produce stock concentrations. Fresh solutions were made each

experimental day, and the final experimental concentrations for testing were 1 μM and 10 μM. An Axopatch 200A (Axon Instruments, CA) amplifier was used, controlled by a PC connected via a Digidata 1322A A/D converter (Axon Instruments, CA), which was also used for data acquisition and analysis. Fractional block of I_{hERG} was calculated using the following equation:

$$\text{Fractional block}\,\% = \left[1 - \left(I_{hERG-Drug} \Big/ I_{hERG-Control}\right)\right]\%$$

Where $I_{hERG\text{-}control}$ is the whole-cell peak tail current of hERG under the conditions listed, and $I_{hERG\text{-}drug}$ is the peak tail current of that cell under the same conditions after superfusion of the drug. Tail current was used to quantify the current mediated by the mutant channels as well as wild-type.

4.2.4. Protection from infection in mouse septicemia model (MRSA)

A standard peritonitis model (Shanghai Slaccas Laboratory Animal Breeding Co., Ltd) of infection was used to evaluate the efficacy of **1q**. Briefly, outbred mice (KM, weight, 20–25 g each) were inoculated intraperitoneally with a 90-to-100%-lethal dose (5×10^7 CFU/mL) of MRSA08-12 in 0.5 mL of brain heart infusion broth containing 5% mucin. Compound **1q** was dissolved in 20% DMSO and administered at doses of 1.25, 2.5, 5, 10, and 20 mg/kg in a dosing volume of 0.1 mL via i.v. injection. LBM415 was used as a control antibiotic, and was dosed with the same formulation as the drug treated group. Groups of 10 mice were used for each dosage. Mice were monitored daily for 14 days and cumulative mortality used to determine the 50% effective dose (ED_{50}), which was calculated using Bliss's method.

Similar procedures were employed to evaluate the in vivo efficacy of **1x**. Briefly, outbred mice were inoculated intraperitoneally with a 90-to-100%-lethal dose (5×10^6 CFU/mL) of MRSA (ATCC 33591) in 0.5 mL of brain heart infusion broth containing 5% mucin to establish the mouse septicemia model, and **1x** was dissolved in 20% DMSO and administered at doses of 5, 10, and 20 mg/kg in a dosing volume of 0.1 mL via the i.g. route after infection. Linezolid was included as a control antibiotic, and was dosed with the same formulation as the drug treated group. Groups of six mice were used for each dosage. Mice were monitored daily for 14 days and cumulative mortality used to determine the 50% effective dose (ED_{50}).

*4.2.5. Plasma protein binding test of **1q**, **1x**, and warfarin*

Pooled plasma (CD-1 Mouse Plasma purchased form Bioreclamation, frozen at <−70 °C) was thawed in a water bath at 37 °C immediately prior to the experiment and centrifuged at 3220 RCF for 5 min to remove the clots. A 200 μM working solution was made by diluting the appropriate volume of stock solution with 50% CH_3CN/H_2O. The 2 μM final solution was made by diluting 8 μL of the working solution (200 μM) with 792 μL of blank plasma. The dialysis instrument was assembled following the manufacturer's instructions. Each cell of the dialysis plate was loaded with 150 μL of plasma solution (in triplicate) and dialyzed against an equal volume of dialysis buffer (100 mM phosphate buffered saline, pH 7.4). Before dialysis, 50 μL aliquots of spiked plasma (in triplicate) were removed in a sample collection plate (T_0 sample) and stored at −40 °C. The dialysis plate was sealed and placed in an incubator at 37 °C for 4 h with rotation at 150 rpm. At the end of dialysis, aliquots of post-dialysis plasma (50 μL) and post-dialysis buffer (50 μL) were removed into a new 96-well polypropylene plate (sample collection plate). All samples in each well were matched with an equal volume of opposite blank matrix to obtain a final concentration ratio of 50/50 for the plasma and dialysis buffer in each well. Samples were diluted with 300 μL of 100% CH_3CN (containing 200 ng/mL tolbutamide plus 20 ng/mL buspirone) to precipitate the plasma proteins. The sample collection plate was shaken at 800 rpm for 5 min to mix the samples, and then centrifuged at 3220 RCF for 20 min. An aliquot of supernatant (100 μL) was transferred from each well and mixed with 200 μL ultra pure water before being subjected to LC-MS/MS analysis (30 × 2.00 mm Phenomenex Luna 5 μm C18; UV detection at 240 nm). The runs were performed with a linear gradient of A (0.1% FA in water) and B (0.1% FA in acetonitrile): t = 0.01 min, 10% B; t = 0.8 min, 95% B; t = 1.0 min, 95% B; t = 1.01 min, 10% B; t = 1.2 min, stop). The concentration was calculated using the peak area ratio of analyte and internal standard. The percent of unbound, bound and recovery were calculated using the following equations:

$$\%\,\text{Unbound} = 100{*}F_C/T_C;$$

$$\%\,\text{Bound} = 100 - \%\,\text{Unbound}$$

$$\%\,\text{Recovery} = 100{*}(F_C + T_C)/T_0$$

Where T_C is the total compound concentration as determined by the calculated concentration on the plasma side of the membrane, F_C is the free compound concentration as determined by the calculated concentration on the buffer side of the membrane, And T_0 is the total compound concentration as determined before dialysis.

4.2.6. Acute toxicity study in mice

Nine male and ten female outbred KM mice in SPF grade (weight, 20–25 g each, bought from Shanghai Slaccas Laboratory Animal Breeding Co., Ltd) were used for this study. Compound **1q** was formulated in PBS (pH 6.5, 20% DMSO) and given in a single-dose at 100 and 250 mg/kg, respectively, via intravenous injection of the tail vein over approximately 60s. The animals were monitored for 14 days and their behavior change, weight, coat color, anal temperature, daily food intake, daily water intake, and daily weight of urine and stool checked every day.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.106.

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