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Short communication

Synthesis and antibacterial activity of naphthyridone derivatives containing mono/difluoro-methyloxime pyrrolidine scaffolds

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ABSTRACT

A series of novel naphthyridone derivatives containing mono/difluoro-methyloxime pyrrolidine scaffolds were designed and synthesized. These derivatives were initially evaluated for their in vitro antibacterial activity and compounds **13a1, b1** were chosen for further evaluation their in vivo activity against systemic infections in mice. The results indicate that all of the target compounds have considerable in vitro antibacterial activity. In the in vivo experiments, **13b1** was found to be more effective than the parent drug gemifloxacin against the tested five strains, and especially its activity (ED_{50}:21.27 mg/kg) is 5.2–6.1 times more potent than gemifloxacin and ciprofloxacin against clinically important Gram-negative pathogen *Pseudomonas aeruginosa*.

1. Introduction

Quinolones have become a major class of antibacterial agents mainly used to fight both community-acquired and serious hospital-acquired infections. These antibiotics act by binding two type II bacterial topoisomerase enzymes, DNA gyrase and topoisomerase IV [1,2].

Since the discovery of norfloxacin by Koga et al. in 1980 [3], most of the research concerning the quinolone antibacterials has been focused on the basic group at the C-7 position which greatly influences their potency, spectrum and safety [4]. As a result, ciprofloxacin (CPFX), lomefloxacin, fleroxacin, ofloxacin (OFLX), sparfloxacin and so on containing a piperazinyl group at the C-7 position were successfully introduced into the market. These early fluoroquinolones are currently used for the treatment of infections mainly caused by most Gram-negative organisms. Recently, the introduction of the noted pyrrolidine derivatives to the quinolones resulted in a dramatic improvement of Gram-positive activity compared to piperazinyl analogs [5]. The pyrrolidinyl-based fluoroquinolones, such as trovafloxacin, tosufloxacin, sitafloxacin, moxifloxacin (MXFX) and gemifloxacin (GMFX, Fig. 1), are generally characterized by a broad antimicrobial spectrum, but their activity against clinically important Gram-positive cocci including *Staphylococci, Streptococci* and *Enterococci* is relatively moderate. This has not only limited their use in infections caused by these organisms, but has also contributed to rapidly developing quinolone resistance [6]. Thus, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of early fluoroquinolones, such as CPFX and OFLX [7,8].

Previous work on pyrrolidinyl quinolones suggested the importance of the methyloxime functional group with respect to biological activity. For example, GMFX displays much more antibacterial activity than its desmethyloximino analog [5]. DW286 (Fig. 1), a methylation analog of GMFX, shows improved in vitro and in vivo activity than GMFX against important Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and quinolone-resistant *S. aureus*, while maintaining an excellent pharmacokinetic profile [9,10]. It was reported that some cephalosporins possessing a monofluoro-methyloxime moiety, such as CP6679 [11] and cefluprenam (E-1077) [12] have superior antibacterial activity to the corresponding methyloxime analogs against MRSA and *Pseudomonas aeruginosa*.

Inspired by these research results, we considered replacement of the methyloxime moiety in GMFX and DW286 with the bioisosteric fluoro-methyloximes which can be readily obtained from the corresponding oximes in a single operation. A series of novel naphthyridone compounds containing fluoro-methyloxime functionalized pyrrolidine side chains at the C-7 position were designed and synthesized. These derivatives are structurally unique, having an aminomethyl (and a methyl) and a mono/difluoro-methoxyimino group at the 3- and 4-positions of the

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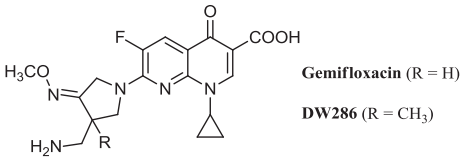


Fig. 1. Structures of Gemifloxacin and DW286.

pyrrolidine ring, respectively. Our primary objective was to optimize the potency of these naphthyridones against Gram-positive and Gram-negative organisms.

2. Results and discussion

2.1. Chemistry

Detailed synthetic pathways to new pyrrolidine derivatives **8a**–**c** and novel naphthyridones **13a**–**c** are depicted in Schemes 1 and 2, respectively. Catalytic hydrogenation of readily available *tert*-butyl 3-cyano-4-oxopyrrolidine-1-carboxylate (**1**) [13,14] yielded the primary amine with in situ *N*-*tert*-butoxycarbonyl (Boc) protection in a poor yield (43%). The resulting ketone **2** was reacted with hydroxyamine hydrochloric acid in the presence of pyridine to give oxime **3** ($R^1 = H$).

Nucleophilic substitution of **1** with iodomethane in the presence of anhydrous K_2CO_3 gave cyano ketone **4**. Selective hydrogenation of the cyano group of **4** by 5% Pd/C to the primary amine with in situ Boc protection yielded bis-Boc protected compound **5** in a good yield (78%) by conventional treatments. The methylated oxime **6** ($R^1 = CH_3$) was then obtained by introduction of an oxime group to **5** in a similar manner as for the preparation of **3**.

The oximes **3**, **6** were condensed with bromofluoromethane or chlorodifluoromethane in the presence of tetra-n-butylammonium bromide (TBAB) as the phase transfer catalyst to give fluoro-methyloximes **7a**–**c**, which upon removal of bis-Boc protected groups afforded the desired pyrrolidine derivatives **8a**–**c** by methanesulfonic acid in ethanol or hydrogen chloride gas in methanol. Surprisingly, condensation of **3** with trifluoromethyliodide yielded the bis-Boc protected compound containing a trifluoro-methoxyimino group successfully [19], but the latter was not converted to the desired product in a similar manner as for preparation of **8a, b** even though various attempts were made.

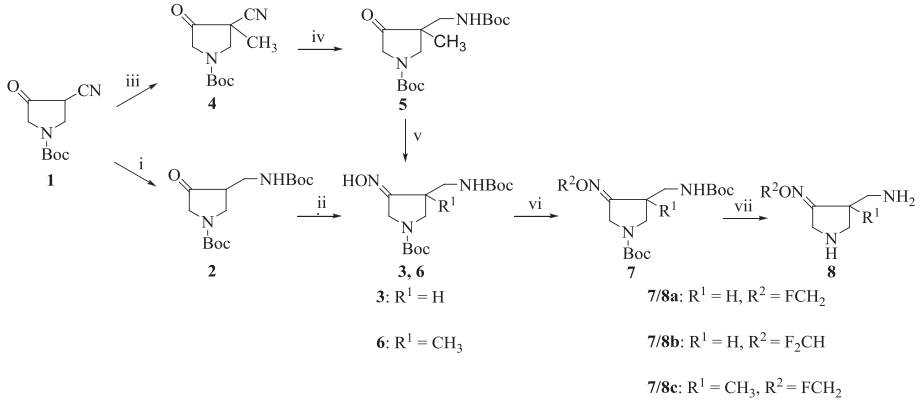
Although quinolone agents are usually synthesized by direct condensation of quinolone nuclei and the side chain compounds [20–22], preparation of naphthyridones **13a**–**c** met with no success through this strategy. In this case, the reaction was complex and it was very difficult to obtain the desired compounds with enough purity even though column chromatography separation technique was used. This was due partly to the fact that both the primary and second amino groups of pyrrolidine derivatives **8a**–**c** could attack 6-des/fluoronaphthyridone cores **9**–**11** [15–17] simultaneously. Therefore, the primary amino group of **8a**–**c** was first protected by iminization with benzaldehyde, and subsequently condensation with **9**–**11** in the presence of triethylamine gave compounds **12a**–**c**. Finally, the resulting condensates **12a**–**c** were treated with methanesulfonic acid or through silica gel column with weak acidity to give the target naphthyridones **13a**–**c** (Scheme 2).

Since the oxime group can exist in the E or Z configuration, it was necessary to determine the geometries of all the oxime target compounds **13a**–**c**. It was a pity that we were not successful in preparing X-ray quality single crystals of any oxime intermediate or product. We have done some NOE experiments with final compounds **13a1**, **b1**, but we did not observe any positive correlation between the crucial protons. Anyway, the oxime geometry would be expected to have the Z configuration, like GMFX [9]. It is also obvious that the target compounds **13a**–**c** and intermediates **8a**–**c** are all racemes.

2.2. Antibacterial activity

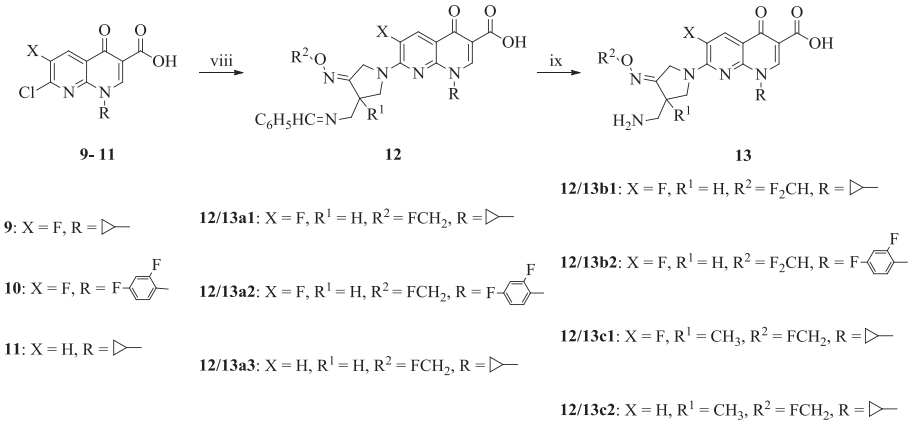
2.2.1. In vitro activity

The target compounds **13a**–**c** were screened for their in vitro antibacterial activity against representative Gram-positive and Gram-negative organisms including standard strains and clinical isolates by means of standard two fold serial dilution method using agar media [18]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the reference drugs CPFX, GMFX and MXFX for comparison are reported in Table 1.



Reagents and conditions: (i) 70 psi H_2, 5% Pd/C, $(Boc)_2O$, CH_3OH, 12 h, 43.1%; (ii) $NH_2OH{\cdot}HCl$, Pridine, CH_3OH, 5 h, 80.1%; (iii) CH_3I, K_2CO_3, $(CH_3)_2CO$, 3 h, 84.0%; (iv) 75 psi H_2, 5% Pd/C, $(Boc)_2O$, CH_3OH, 8 h, 78.0%; (v) $NH_2OH{\cdot}HCl$, Pridine, CH_3OH, 5 h, 93.1%; (vi) CH_2FBr/ CHF_2Cl, NaOH, TBAB, toluene,10 min-48 h, 28.4-96.1%; (vii) **8a, b:** CH_3SO_3H, CH_3CH_2OH, 16 h, 72.6-76.4%; **8c**: HCl gas, CH_3OH, 0.5 h, 90.0%.

Scheme 1. Synthesis of pyrrolidine derivatives **8a**–**c**.



Reagents and conditions: (viii) **8a-c**, C_6H_5CHO, triethylamine, CH_3CN/DMSO, 12 h, 69.3-85.4% (**12a1, a2, b1**); (ix) CH_3SO_3H, H_2O, $C_2H_5OH/CHCl_3$, 10-16 h, 65.4- 68.9% (**13a1, a2, b1**), 42.1% (**13a3**, from **11**), 60.1% (**13b2,** from **10**); or silica gel column chromatography, 23.5-25.0% (**13c1, c2,** from **9, 11**)

Scheme 2. Synthesis of naphthyridone derivatives **13a**–**c**.

Table 1
In vitro antibacterial activity of naphthyridone derivatives **13a**–**c** against selected strains.

Strains	MIC (μg/mL)									
	13a1	**13a2**	**13a3**	**13b1**	**13b2**	**13c1**	**13c2**	CPFX	GMFX	MXFX
S.a. ATCC25923	0.015	0.25	0.25	0.015	0.008	0.008	0.03	0.25	0.008	0.008
MRSA 10-1	32	64	>128	16	16	4	128	>128	16	8
MRSA 10-2	2	16	64	1	4	0.25	8	16	0.5	8
MRSA 10-3	32	64	>128	16	16	4	128	>128	16	8
MRSA 10-4	64	64	>128	32	16	4	128	>128	16	8
MSSA 10-1	0.06	0.06	0.03	0.015	0.03	0.06	0.5	0.015	0.008	0.008
MSSA 10-2	8	4	128	4	1	1	32	64	2	4
MSSA 10-3	0.03	0.125	0.25	0.06	0.015	0.25	0.015	0.06	0.06	0.008
MSSA 10-4	0.25	0.125	0.25	0.5	0.008	0.06	0.015	0.06	0.015	0.008
MSSA 10-5	0.125	0.125	0.125	0.008	0.015	0.03	0.03	0.03	0.5	0.03
MSSA 10-6	0.5	0.125	0.125	0.06	0.015	0.125	0.03	0.125	0.008	0.008
MRSE 10-1	2	4	32	4	2	1	4	16	0.25	0.5
MRSE 10-2	1	8	64	0.25	1	0.5	8	8	0.5	1
MSSE 10-1	32	32	16	64	32	8	4	8	2	2
MSSE 10-2	64	32	32	32	64	64	64	64	16	16
*S.p.*10–1	0.06	0.125	0.25	0.03	0.06	0.5	0.015	0.03	0.015	0.008
E.fm. 09-1	32	64	64	32	32	16	32	>128	16	16
E.fm. 09-2	32	64	64	32	32	16	32	>128	16	16
E.fm. 09-3	16	32	64	16	16	8	2	128	4	2
E.fm. 09-4	0.06	0.5	2	0.125	0.125	0.06	0.5	0.5	0.03	0.008
E.fm. 09-5	32	64	16	16	32	16	32	64	16	16
E.fm. 09-6	0.03	0.5	1	0.06	0.125	0.06	0.5	0.5	0.03	0.125
E.fs. 09-1	8	32	64	4	8	4	32	64	2	8
E.fs. 09-2	32	32	64	8	16	1	0.015	64	4	16
E.co. ATCC25922	0.008	0.125	0.06	0.008	0.008	0.008	0.015	0.008	0.008	0.008
E.co. 09-1	32	128	>128	16	32	64	>128	32	16	16
E.co. 09-2	32	>128	>128	32	>128	>128	>128	32	32	32
E.co. 09-3	32	>128	>128	16	64	32	>128	64	16	16
E.co. 10-1	32	128	>128	16	>128	64	>128	32	16	16
E.co. 10-2	0.5	4	4	0.5	4	0.125	1	0.03	0.25	0.5
E.co. 10-3	16	32	>128	8	8	32	128	4	4	4
K.p. 09-2	32	>128	>128	32	64	128	>128	32	16	16
K.p. 10-1.	0.03	0.5	0.5	0.015	0.25	0.015	0.25	0.06	0.015	0.03
K.p. 10–3	0.5	4	4	0.5	2	0.125	2	0.25	0.5	0.5
P.a. ATCC27853	0.5	16	4	0.5	4	0.125	1	0.25	0.5	1
P.a. 10-1	0.5	4	2	0.5	1	0.008	0.5	0.06	0.25	0.5
P.a. 10-2	32	>128	>128	32	128	16	64	>128	16	16
P.a. 10-3	4	32	32	4	8	4	8	0.5	2	4

CPFX: ciprofloxacin; GMFX: gemifloxacin; MXFX: moxifloxacin. *S.a., Staphylococcus aureus*; MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-sensitive *Staphylococcus aureus*; MRSE, Methicillin-resistant *Staphylococcus epidermidis*; MSSE, Methicillin-sensitive *Staphylococcus epidermidis*; *S.p., Streptococcus pneumonia*; *E.fm., Enterococcus faecium*; *E.fs., Enterococcus faecalis*; *E.co., Escherichia coli*; *K.p., Klebsiella pneumoniae*; *P.a., Pseudomonas aeruginosa.*

Table 2
In vivo efficacy of compounds **13a1, b1** against systemic infections in mice.

Infected bacteria [challenge dose (cfu/mL)]	Compd[a]	MIC (μg/mL)	ED_{50} (mg/kg) [b]	95% confidence limit (mg/kg)
MRSA10-2 (5.2×10^5)	**13a1**	2	16.91	28.53–11.30
	13b1	1	14.30	21.46–9.91
	GMFX	1	14.86	26.46–9.49
	CPFX	16	15.92	28.55–10.27
MRSE10-4 (5.0×10^5)	**13a1**	2	8.00	11.95–5.15
	13b1	2	16.91	28.53–11.30
	GMFX	4	24.00	55.92–15.30
	CPFX	8	24.54	46.91–16.64
*E. coli*10-2 (3.0×10^5)	**13a1**	0.5	8.79	16.92–5.62
	13b1	0.5	5.83	8.78–3.94
	GMFX	0.25	6.86	11.59–4.43
	CPFX	0.06	4.00	5.97–2.58
K. pneumoniae10-3 (3.0×10^5)	**13a1**	0.5	>160	–[c]
	13b1	0.5	100.26	119.79–84.66
	GMFX	0.25	108.46	130.43–91.42
	CPFX	0.25	87.45	101.30–74.08
P.aeruginosa10-1 (5.2×10^5)	**13a1**	1	39.28	93.64–23.96
	13b1	1	21.27	30.30–14.97
	GMFX	0.25	129.40	188.85–105.99
	CPFX	0.25	110.89	139.41–92.62

CPFX: ciprofloxacin; GMFX: gemifloxacin.
MRSA, Methicillin-resistant *Staphylococcus aureus*; MRSE, Methicillin-resistant *Staphylococcus epidermidis*.
[a] Antimicrobial agents were orally administrated twice at 1 and 4 h after infection.
[b] ED_{50}:50% effective dose.
[c] –, confidence limits could not be calculated.

The data shows that all of the target compounds **13a–c** have generally potent in vitro antibacterial activity against the tested strains. The activity of compounds **13a1, b1, b2, c1** against Gram-positive organisms is better than CPFX, and comparable to GMFX and MXFX. For example, compound **13c1** (MICs: 0.25–1 μg/mL) is 2- to 64-fold more potent than the three reference drugs against MRSA 10-2 and *Enterococcus faecalis* 09-2. For Gram-negative organisms, compounds **13a1, b1** (MICs: 0.015–32 μg/mL) are comparable to the reference drugs.

Generally, the activity of the naphthyridone nuclei in this study is in the order: 1-cyclopropyl-6-fluoro-1,8-naphthyridone > 1-(2,4-difluorophenyl) -6-fluoro –1,8- naphthyridone > 1-cyclopropyl-1,8-naphthyridone. In addition, naphthyridones featuring difluoro-methyloxime-incorporated pyrrolidino substitution at C-7 position are more potent than the analogs containing a monofluoro-methyloxime.

2.2.2. *In vivo activity*

Mice protection tests were used to evaluate further in vivo efficacy of compounds **13a1, b1** having better in vitro activity, and CPFX and GMFX were used as control drugs (Table 2). The efficacy of these compounds was tested against five clinical isolate strains: MRSA 10-2 and methicillin-resistant *Staphylococcus epidermidis* (MRSE) 10-4 were selected for Gram-positive bacteria, and *Escherichia coli*10-2, *Klebsiella pneumoniae*10-3 and *P. aeruginosa* 10-1 were chosen for Gram-negative bacteria.

The data suggests that monofluoro-methyloxime derivative **13a1** exhibits lower in vivo efficacy than the corresponding difluoro-methyloxime analog **13b1** against the tested strains except for MRSE10-4, which is approximately consistent with the in vitro activity results. The ED_{50}s of **13a1, b1** are comparable to those of GMFX and CPFX against the Gram-positive strain MRSA 10-2, but both of them (ED_{50}s of 8 and 16.91 mg/kg, respectively) are 1.4–3.1 times stronger than the two reference drugs (ED_{50}s of 24 and 24.54 mg/kg, respectively) against MRSE10-4. For Gram-negative strain *P. aeruginosa* 10-1, compounds **13a1, b1** (ED_{50}s of 39.28 and 21.27 mg/kg, respectively) are both more effective than GMFX and CPFX (ED_{50}s of 129.4 and 110.89 mg/kg, respectively). In addition, compound **13b1** is marginally more effective than GMFX against *E. coli*10-2 and *K. pneumoniae*10-3.

3. Conclusion

In conclusion, a series of novel naphthyridone derivatives containing mono/difluoro-methyloxime pyrrolidine scaffolds were designed, synthesized and characterized by ^{1}H NMR, MS, HRMS and ^{13}C NMR. These derivatives were initially evaluated for their in vitro activity against representative Gram-positive strains including MRSA and MRSE, and Gram-negative strains including *P. aeruginosa*. Compounds **13a1, b1** were chosen for further evaluation using their in vivo activity against systemic infections in mice. Our results demonstrate that all of the synthesized compounds have considerable in vitro antibacterial activity. For example, the activity of compounds **13a1, b1, b2, c1** is better than CPFX, and comparable to GMFX and MXFX against Gram-positive organisms. Compounds **13a1, b1** are comparable to the three reference drugs against Gram-negative organisms. It is important to note that **13b1** is more effective than the parent drug GMFX against the tested five strains, and especially its activity (ED_{50}:21.27 mg/kg) is 5.2–6.1 times more potent than the references GMFX and CPFX against clinically important Gram-negative pathogen *P. aeruginosa* in the in vivo experiments.

4. Experimental protocol

4.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra and high resolution mass spectra (HRMS) were obtained on an MDSSCIEX Q-Tap mass spectrometer. Fast Atom Bombardment (FAB) mass spectra and high resolution mass spectra (HRMS) were obtained on a MICROMASS AutoSpec Ultima-TOF mass spectrometer. The reagents were all of analytical grade or

chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254).

4.2. Synthesis

4.2.1. tert-Butyl 3-(tert-butoxycarbonyl)aminomethyl-4-oxopyrrolidine-1-carboxylate **2**

A mixture of *tert*-butyl 3-cyano-4-oxopyrrolidine-1-carboxylate **1** (21.02 g, 100 mmol), $(Boc)_2O$ (26.19 g, 120 mmol) and 5% Pd/C (6.00 g) in methanol (250 mL) was pressurized at 70 psi of hydrogen at room temperature for 12 h, and then filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (*v*: *v* = 5: 1) to give the title compound **2** (13.53 g, 43.1%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.97 (1H, brs), 3.79–3.63 (3H, m), 3.51–3.36 (1H, m), 3.27–3.18 (1H, m), 3.10–3.01 (1H, m), 2.85–2.73 (1H, m), 1.41 (9H, s), 1.39 (9H, s). MS-ESI (*m/z*): 315 $(M + H)^+$.

4.2.2. tert-Butyl 3-(tert-butoxycarbonyl)aminomethyl-4-(hydroxyimino)pyrrolidine-1-carboxylate **3**

To a solution of **2** (9.43 g, 30 mmol) in methanol (200 mL) was added hydroxylamine hydrochloride (2.50 g, 36 mmol) and pyridine (2.85 g, 36 mmol). The reaction mixture was stirred for 5 h at room temperature, and concentrated under reduced pressure. The residue was treated with ethyl acetate (50 mL), washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure, and the crude product was recrystallized from petroleum ether (400 ml) to afford the title compound **3** (7.91 g, 80.1%) as a white solid, mp: 138–140 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.91 (1H, s), 6.94 (1H, s), 3.92–3.85 (2H, m), 3.54–3.48 (1H, m), 3.32–3.12 (2H, m), 3.00–3.90 (2H, m), 1.44 (18H, s). MS-ESI (*m/z*): 330 $(M + H)^+$.

4.2.3. tert-Butyl 3-cyano-3-methyl-4-oxopyrrolidine-1-carboxylate **4**

To a mixture of **1** (21.02 g, 100 mmol) and anhydrous K_2CO_3 (41.46 g, 300 mmol) in dry acetone (800 mL) was added dropwise iodomethane (17.03 g, 120 mmol) over a period of 30 min at room temperature. The reaction mixture was stirred for 3 h at the same temperature, and then filtered. The filtrate was concentrated under reduced pressure. The residue was treated with petroleum ether (300 mL) and filtered, dried to afford the title compound **4** (18.83 g, 84.0%) as a white solid, mp: 71–73 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.15–3.69 (4H, m), 1.58 (3H, s), 1.49 (9H, s). MS-ESI (*m/z*): 225 $(M + H)^+$.

4.2.4. tert-Butyl 3-(tert-butoxycarbonyl)aminomethyl-3-methyl-4-(hydroxyimino) pyrrolidine-1-carboxylate **5**

A mixture of **4** (11.20 g, 50 mmol), $(Boc)_2O$ (13.09 g, 60 mmol) and 5% Pd/C (3.0 g) in methanol (200 mL) was pressurized at 75 psi of hydrogen at room temperature for 8 h and then filtered. The filtrate was concentrated under reduced pressure. The residue was treated with petroleum ether (200 mL), filtered and dried to afford the title compound **5** (12.80 g, 78.0%) as a white solid, mp: 106–108 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.77 (1H, brs), 3.83 (2H, s), 3.64–3.26 (4H, m), 1.50 (9H, s), 1.43 (9H, s), 1.15 (3H, s). MS-ESI (*m/z*): 329 $(M + H)^+$.

4.2.5. tert-Butyl 3-(tert-butoxycarbonyl)aminomethyl-3-methyl-4-(hydroxyimino) pyrrolidine-1-carboxylate **6**

The title compound **6** was obtained from **5** in a similar manner as for the preparation of **3** (93.1%) as a white solid, mp: 149–150 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.86 (1H, brs), 6.94 (1H, brs), 3.96 (2H, s), 3.50–2.98 (4H, m), 1.40 (9H, s), 1.36 (9H, s), 1.06 (3H, s). ESI-MS (*m/z*): 344 $(M + H)^+$.

4.2.6. tert-Butyl 3- (tert-butoxycarbonyl)aminomethyl-4-(fluoromethoxyimino) pyrrolidine-1-carboxylate **7a**

To a stirring suspension of **3** (6.59 g, 20 mmol) in toluene (400 mL) was added 12N NaOH solution (10 mL, 120 mmol) and bromofluoromethane (3.93 g, 30 mmol) at 0 °C. The reaction mixture was then stirred for 10 min at room temperature, and washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to afford the title compound **7a** (6.86 g, 95.0%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.07 (1H, brs), 5.68 (2H, d, J = 56 Hz), 4.05 (2H, brs), 3.57 (1H, brs), 3.31–3.27 (1H, m), 3.19 (1H, brs), 3.10–3.03 (2H, m), 1.45 (9H, s), 1.40 (9H, s). MS-ESI (*m/z*): 362 $(M + H)^+$.

4.2.7. tert-Butyl 3- (tert-butoxycarbonyl)aminomethyl-4-(difluoromethoxyimino) pyrrolidine-1-carboxylate **7b**

To a stirring suspension of **3** (3.29 g, 10 mmol) and TBAB (0.97 g, 3 mmol) in toluene (300 mL) was added 12N NaOH solution (5 mL, 60 mmol) at 0–5 °C, and pumped chlorodifluoromethane gas for 1 h at the same temperature. The reaction mixture was then stirred for 48 h at 70 °C, and washed successively with water and saturated saline solution, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (*v*: *v* = 6: 1) to afford the title compound **7b** (1.07 g, 28.4%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.07 (1H, brs), 7.06 (1H, t, J = 72 Hz), 4.09 (2H, brs), 3.58 (1H, brs), 3.32–3.28 (1H, m), 3.20 (1H, brs), 3.11–3.07 (2H, m), 1.41 (9H, s), 1.37 (9H, s). MS-ESI (*m/z*): 380 $(M + H)^+$.

4.2.8. tert-Butyl 3- (tert-butoxycarbonyl)aminomethyl-3-methyl-4-(fluoromethoxyimino)pyrrolidine-1-carboxylate **7c**

The title compound **7c** was obtained from **6** in a similar manner as for the preparation of **7a** (96.1%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.08 (1H, brs), 5.66 (2H, t, J = 56 Hz), 4.09 (2H, s), 3.53–2.97 (4H, m), 1.40 (9H, s), 1.36 (9H, s), 1.09 (3H, s). ESI-MS (*m/z*): 376 $(M + H)^+$.

4.2.9. 3-Aminomethyl-4-(fluoromethoxyimino)pyrrolidine dimethanesulfonate **8a**

To a solution of **7a** (3.61 g, 10 mmol) in ethanol (40 mL) was added methylsulfonic acid (2.88 g, 30 mmol). The reaction mixture was stirred for 16 h at room temperature, and then filtered. The precipitate was washed with ethanol, and dried in vacuo to yield the title compound **8a** (2.57 g, 72.6%) as a white solid, mp: 137–139 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.32 (2H, brs), 7.99 (3H, brs), 5.74 (2H, d, J = 56 Hz), 4.16–3.99 (2H, m), 3.75–3.71 (1H, m), 3.36–3.28 (2H, m), 3.19–3.07 (2H, m), 2.38 (6H, s). MS-ESI (*m/z*): 162 $(M + H)^+$.

4.2.10. 3-Aminomethyl-4-(difluoromethoxyimino)pyrrolidine dimethanesulfonate **8b**

The title compound **8b** was obtained from **7b** in a similar manner as for the preparation of **8a** (76.4%) as a white solid, mp: 168–170 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.35 (2H, brs), 7.97 (3H, brs), 7.16 (1H, t, J = 72 Hz), 4.22–4.05 (2H, m), 3.77–3.73 (2H, m), 3.45–3.40 (1H, m), 3.16–3.12 (2H, m), 2.37 (6H, s). MS-ESI (*m/z*): 180 $(M + H)^+$.

4.2.11. 3-Aminomethyl-3-methyl-4-(fluoromethoxyimino) pyrrolidine dihydrochloride **8c**

To a stirring solution of **7c** (7.50 g, 20 mmol) dissolved in methanol (50 mL) was pumped dried hydrochloride gas at 0–5 °C

for 30 min. The reaction mixture was allowed to stir for another 30 min at room temperature, and the precipitate was collected by suction, and dried in vacuo to give the title compound **8c** (4.47 g, 90.0%) as a off-white solid easily absorbing moisture.

*4.2.12. General procedure for the synthesis of condensates **12a1, a2, b1***

To a suspension of **8a, b** (1.1 mmol) in dry acetonitrile (15 mL) was added benzaldehyde (1.2 mmol) and triethylamine (3 mmol), and then stirred for 2 h at room temperature. To the reaction mixture was added **9, 10** (1 mmol), and stirred for another 12 h at the same temperature. The precipitate was filtered, washed with acetonitrile, and dried in vacuo to give the title compounds **12a1, a2, b1**.

*4.2.12.1. 7-(3-Benzylideneaminomethyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **12a1***. The title compound **12a1** was obtained from **8a** and **9** as an off-white solid (85.4%), mp: 176–178 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.22 (1H, s), 8.53 (1H, s), 8.38 (1H, s), 7.91 (1H, d, J = 13 Hz), 7.58 (2H, d, J = 8 Hz), 7.36–7.27 (3H, m), 5.77 (2H, d, J = 56 Hz), 4.71–4.62 (2H, m), 4.13–4.09 (2H, m), 3.93–3.85 (2H, m), 3.67–3.62 (1H, m), 3.52–3.49 (1H, m), 1.13–0.99 (4H, m). MS-FAB (m/z): 496 $(M + H)^+$.

*4.2.12.2. 7-(3-Benzylideneaminomethyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1- (2,4-difluorophenyl)-6-fluoro- 4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **12a2***. The title compound **12a2** was obtained from **8a** and **10** as a white solid (69.5%), mp: 248–250 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 14.74 (1H, s), 8.64 (1H, s), 8.27 (1H, s), 8.08 (1H, d, J = 13 Hz), 7.66–7.52 (2H, m), 7.43–7.32 (4H, m), 7.03–6.99 (2H, m), 5.61 (2H, d, J = 56 Hz), 4.46–3.36 (7H, m). MS-ESI (m/z): 568 $(M + H)^+$.

*4.2.12.3. 7-(3-Benzylideneaminomethyl-4-difluoromethoxyimino-1-pyrrolidinyl)-1- cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **12b1***. The title compound **12b1** was obtained from **8b** and **9** as an white solid (69.3%), mp: 205–207 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.19 (1H, brs), 8.55 (1H, s), 8.39 (1H, s), 7.94 (1H, d, J = 13 Hz), 7.58 (2H, d, J = 8 Hz), 7.36–7.27 (3H, m), 7.16 (1H, t, J = 72 Hz), 4.77–4.70 (2H, m), 4.20–4.13 (2H, m), 3.91–3.89 (2H, m), 3.68–3.62 (1H, m), 3.59–3.56 (1H, m), 1.14–1.00 (4H, m). MS-FAB (m/z): 514 $(M + H)^+$.

*4.2.13. General procedure for the synthesis of naphthyridine derivatives **13a1, a2, b1***

To a suspension of **12a1, a2, b1** (1 mmol) in ethanol (20 mL) was added methylsulfonic acid (3 mmol) and water (0.2 mL), and the reaction mixture was then stirred for 16 h at room temperature. The precipitate was filtered, washed with ethanol, and dried in vacuo to yield the title compounds **13a1, a2, b1**.

*4.2.13.1. 7-(3-Aminomethyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1-cyclopropyl- 6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate **13a1***. The title compound **13a1** was obtained from **12a1** as a white solid (68.9%), mp: 220–222 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.22 (1H, brs), 8.60 (1H, s), 8.08 (1H, d, J = 13 Hz), 7.95 (3H, brs), 5.79 (2H, d, J = 56 Hz), 4.71–4.64 (2H, m), 4.44–4.39 (1H, m), 3.88–3.83 (1H, m), 3.74–3.68 (1H, m), 3.52–3.49 (1H, m), 3.20–3.14 (2H, m), 2.31 (3H, s), 1.24–1.02 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 176.38, 165.67, 162.81, 148.33 (d, J = 15 Hz), 146.96, 146.90, 146.13 (d, J = 257 Hz), 118.15 (d, J = 21 Hz), 111.55, 107.60, 104.12 (d, J = 219 Hz), 50.57, 48.81, 48.54, 40.13, 34.95, 6.92, 6.76. MS-FAB (m/z): 408 $(M + H)^+$. HRMS-FAB (m/z): Calcd. for $C_{18}H_{20}N_5O_4F_2$ $(M + H)^+$: 408.1483; Found 408.1495.

*4.2.13.2. 7-(3-Aminomethyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1- (2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate **13a2***. The title compound **13a2** was obtained from **12a2** as a white solid (69.4%), mp: 168–170 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.84 (1H, s), 8.17 (1H, d, J = 13 Hz), 7.86–7.78 (4H, m), 7.59–7.56 (1H, m), 7.36–7.32 (1H, m), 5.72 (2H, d, J = 56 Hz), 4.46–3.90 (3H, m), 3.72–3.30 (2H, m), 3.20–3.00 (2H, m), 2.32 (3H, s). MS-FAB (m/z): 480 $(M + H)^+$. HRMS-FAB (m/z): Calcd. for $C_{21}H_{18}N_5O_4F_4$ $(M + H)^+$: 480.1295; Found 480.1279.

*4.2.13.3. 7-(3-Aminomethyl-4-difluoromethoxyimino-1-pyrrolidinyl)-1-cyclopropyl -6-fluoro-4-oxo-1,4-dihydro- 1,8-naphthyridine-3-carboxylic acid methanesulfonate **13b1***. The title compound **13b1** was obtained from **12b1** as a white solid (65.4%), mp: 235–237 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.19 (1H, brs), 8.59 (1H, s), 8.08 (1H, d, J = 13 Hz), 8.00 (3H, brs), 7.18 (1H, t, J = 72 Hz), 4.77–4.66 (2H, m), 4.46–4.41 (1H, m), 3.91–3.87 (1H, m), 3.74–3.68 (1H, m), 3.61–3.53 (1H, m), 3.45–3.41 (1H, m), 3.22–3.20 (1H, m), 2.32 (3H, s), 1.24–1.02 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 180.87, 170.20, 151.89 (d, J = 12 Hz), 151.53, 151.36, 150.60 (d, J = 257 Hz), 122.81 (t, J = 247 Hz), 122.56 (d, J = 12 Hz), 116.10, 112.07, 55.19, 53.55, 53.50, 44.38, 43.54, 11.45, 11.28. MS-FAB (m/z): 426 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{19}N_5O_4F_3$ $(M + H)^+$: 426.1404; Found 426.1415.

*4.2.13.4. 7-(3-Aminomethyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1-cyclopropyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate **13a3***. To a suspension of **8a** (0.39 g, 1.1 mmol) in dry acetonitrile (10 mL) was added benzaldehyde (0.13 g, 1.2 mmol) and triethylamine (0.3 g, 3 mmol) at room temperature and stirred for 30 min at the same temperature. To the reaction mixture was added **11** (0.26 g, 1.0 mmol) and stirred for another 12 h at room temperature, and then concentrated under reduced pressure. The residue was treated with ethanol (10 mL) and filtered. To the filtrate was added methylsulfonic acid (0.77 g, 8 mmol) and few drops of water. The reaction mixture was stirred for 10 h at room temperature. The precipitate was filtered and dried in vacuo to yield the title compound **13a3** [0.20 g, 42.1% (from **11**)] as a white solid, mp: >240 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 15.43 (1H, s), 8.59 (1H, s), 8.34 (1H, brs), 7.95 (3H, brs), 6.93 (1H, brs), 5.80 (2H, d, J = 56 Hz), 4.61–4.40 (2H, m), 4.29–4.00 (1H, m), 3.80–3.08 (5H, m), 2.31 (3H, s), 1.22–1.06 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ (ppm): 176.79, 165.98, 163.48, 157.25, 151.24, 147.22, 135.26, 110.93, 108.70, 107.61, 104.19 (d, J = 217 Hz), 49.13, 48.61, 47.54, 39.08, 34.63, 6.98, 6.83. MS-ESI (m/z): 390 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{21}N_5O_4F_1$ $(M + H)^+$: 390.1577; Found 390.1586.

*4.2.13.5. 7-(3-Aminomethyl-4-difluoromethoxyimino-1-pyrrolidinyl)-1- (2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate **13b2***. To a suspension of **8b** (0.39 g, 1.1 mmol) in DMSO (10 mL) was added benzaldehyde (0.13 g, 1.2 mmol) and triethylamine (0.30 g, 3.0 mmol) and stirred for 30 min at room temperature. **10** (0.35 g, 1.0 mmol) was added to the reaction mixture and stirred for another 12 h at room temperature. The reaction mixture was treated with distilled water (50 mL) and filtered. The obtained solid was diluted with chloroform (10 mL), and then methylsulfonic acid (0.38 g, 4 mmol) and few drops of water were added. The reaction mixture was stirred for 16 h at room temperature, and then treated with ether (40mL). The precipitate was filtered and dried in vacuo to yield the title compounds **13b2** [0.36 g, 60.1% (from **10**)] as a white solid, mp: 145–147 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 14.96 (1H, s), 8.88 (1H, s), 8.19 (1H, d, J = 13 Hz), 7.92–7.78 (4H, m), 7.58–7.51 (1H, m), 7.36–7.32 (1H, m), 7.10 (1H, t,

$J = 72$ Hz), 4.46–4.00 (3H, m), 3.92–3.60 (1H, m), 3.53–3.40 (1H, m), 3.18–3.03 (2H, m), 2.29 (3H, s). ^{13}C NMR (400 MHz, DMSO-d_6): 177.68, 165.78, 161.89, 159.09, 156.44, 148.83, 148.80, 146.71 (d, $J = 255$ Hz), 146.37, 131.36, 124.50, 118.95 (d, $J = 21$ Hz), 118.63 (t, $J = 243$ Hz), 112.85, 111.84, 109.55, 105.51, 50.64, 49.42, 49.40. MS-FAB (*m/z*): 498 $(M + H)^+$. HRMS-FAB (*m/z*): Calcd. for $C_{21}H_{17}N_5O_4F_5$ $(M + H)^+$: 498.1201; Found 498.1180.

4.2.14. 7-(3-Aminomethyl-3-methyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **13c1**

To a suspension of **8c** (2.5g, 10 mmol) in dry acetonitrile (20 mL) was added benzaldehyde (1.27 g, 12 mmol) and triethylamine (3.03 g, 30 mmol) at room temperature under an atmosphere of nitrogen and then stirred for 2 h at the same temperature. To the reaction mixture was added **9** (2.5g, 9mmol) and stirred for another 12 h at room temperature, and then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluted with methanol and methylene chloride (*v*: $v = 1$: 5) to afford the title compound **13c1** [0.95 g, 25.0% (from **9**)] as an off-white solid. mp: 195–196 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.58 (1H, s), 8.03 (1H, d, $J = 13$ Hz), 5.76 (2H, d, $J = 64$ Hz), 4.71 (2H, s), 4.28 (1H, d, $J = 11$ Hz), 3.75–3.71 (2H, m), 3.31 (2H, s), 2.73 (2H, q, $J = 12$ Hz), 1.25 (3H, s), 1.22–1.17 (2H, m), 1.12–1.09 (2H, m). ESI-MS (*m/z*): 422 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd for $C_{19}H_{22}F_2N_5O_4$ $(M + H)^+$: 422.1640; Found 422.1656.

4.2.15. 7-(3-Aminomethyl-3-methyl-4-fluoromethoxyimino-1-pyrrolidinyl)-1- cyclopropyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **13c2**

The title compound was obtained from **8c** and **11** in a similar manner as for the preparation of **13c2** (23.5%, from **11**) as a white solid, mp: 184–186 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.57 (1H, s), 8.29 (1H, s), 6.94 (1H, s), 5.76 (2H, d, $J = 65$ Hz), 4.53 (2H, s), 4.14–4.02 (1H, m), 3.77–3.71 (1H, m), 3.58–3.31 (1H, m), 2.70 (2H, q), 1.25 (3H, s), 1.20–1.12 (2H, m), 1.09–1.03 (2H, m). ESI-MS (*m/z*): 404 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{19}H_{23}FN_5O_4$ $(M + H)^+$: 404.1734; Found 404.1739.

4.3. MIC determination

Compounds **13a**–**c** were evaluated for their in vitro antibacterial activity using standard techniques in comparison to the reference drugs CPFX, GMFX and MXFX. Drugs (10.0 mg) were dissolved in 0.1 N NaOH solution and water (10 mL). Further progressive two fold serial dilution with melted Mueller-Hinton agar was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 and 0.008 μg/mL. Petri dishes were incubated with 10^4 colony-forming units (cfu) and incubated at 35 °C for 18 h. MIC was the lowest concentration of the test compound, which resulted in no visible growth on the plate.

4.4. ED_{50} determination

The in vivo activity of compounds **13a1, b1**, CPFX and GMFX were determined against systemic infection model in mice. Test organisms for infection were cultured on MH(Mueller-Hinton) Agar Medium (Difco) at 35 °C for 18 h and were suspended in gastric mucin (OXOID). Male KM mice (weight 19–21 g) in four groups of 10 mice each were infected intraperitoneally with 0.5 mL of a bacterial suspension corresponding to an inoculum range of 5–10 times the MLD (minimal lethal dose) of bacteria. Four dose levels were used for each antibiotic, depending on the in vitro antimicrobial activity of the compounds. Mice were orally administered twice at 1 and 4 h post infection with various dose regimens of antibiotics. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of mice (ED_{50}) was calculated by the method of Bliss. All untreated mice died within 2 days after infection.

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