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Original article

Synthesis, antimycobacterial and antibacterial evaluation of l-[(1R, 2S)-2-fluorocyclopropyl]fluoroquinolone derivatives containing an oxime functional moiety

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ABSTRACT

A series of novel 1-[(1R, 2S)-2-fluorocyclopropyl]fluoroquinolone derivatives **9a**–**d** containing an oxime functional moiety were synthesized and evaluated for their biological activity. Our results reveal that **9a1** and **9b3** have good *in vitro* activity against MTB H37Rv ATCC 27294 (MIC: 0.25 μg/mL) and two MDR-MTB clinical isolates (MICs: 0.065–0.125 μg/mL). Most of **9a**–**d** show potent activity against *Escherichia coli* and *Klebsiella pneumoniae* (MICs: <0.008–4 μg/mL) except extended-spectrum β-lactamase-producing strains. Especially, **9a1** and **9d4** possessing excellent *in vitro* activity against all of the fourteen Gram-positive strains including MRSA and MRSE (MICs: <0.008–2 μg/mL) comparable to or better than the four reference drugs, show considerable *in vivo* efficacy against five Gram-negative and Gram-positive isolate strains (ED_{50}s: 11.43–26.04 mg/kg).

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1. Introduction

Fluoroquinolones (FQs), one of the important classes of weapons in our antibacterial arsenal, target two type II bacterial topoisomerase enzymes, DNA gyrase and/or topoisomerase IV and are used mainly to fight both community-acquired and serious hospital-acquired infections [1,2]. On the other hand, some FQs, such as ciprofloxacin (CPFX), ofloxacin and levofloxacin (LVFX) are frequently used for the treatment of tuberculosis (TB) including multi-drug resistant TB (MDR-TB) as components of second-line regiments [3]. Two C-8 methoxy FQs gatifloxacin (GTFX) and moxifloxacin (MXFX) possessing particularly strong *in vitro* and *in vivo* activity against *Mycobacterium tuberculosis* (MTB) [4,5], are currently in Phase III clinical trials to establish whether drug-susceptible TB can be effectively treated in four months by substituting GTFX for ethambutol, or MXFX for ethambutol or isoniazid (INH) [6].

Unfortunately, FQs resistance increases in almost all Gram-negative and Gram-positive species as well as MTB [7,8]. The continued increase in resistance has put enormous pressure on public health systems worldwide, due mainly to the high level of use and to some degree of abuse [9]. Therefore, there is an urgent need for the discovery and development of effective novel FQs to confer desirable biological and pharmacological properties [10].

Structure activity relationship (SAR) studies of FQs show that the C-4 carbonyl and C-3 carboxylic groups are known to be essential for antibacterial activity. Cyclopropyl and methoxyl (hydrogen or nitrogen) groups are generally accepted as the optimal substituents at N-1 and C-8 positions of FQs, respectively [11]. The basic substituent at C-7 position, playing an important role in the antibacterial potency, spectrum and safety of FQs [12], is recognized as the most adaptable site for chemical change, and the presence of five- or six-membered nitrogen heterocycle including pyrrolidine, piperazine and piperidine at this position is particularly structural feature of important FQs on the market [13], such as CPFX, LVFX, MXFX, balofloxacin and gemifloxacin (GMFX, Fig. 1), and so on.

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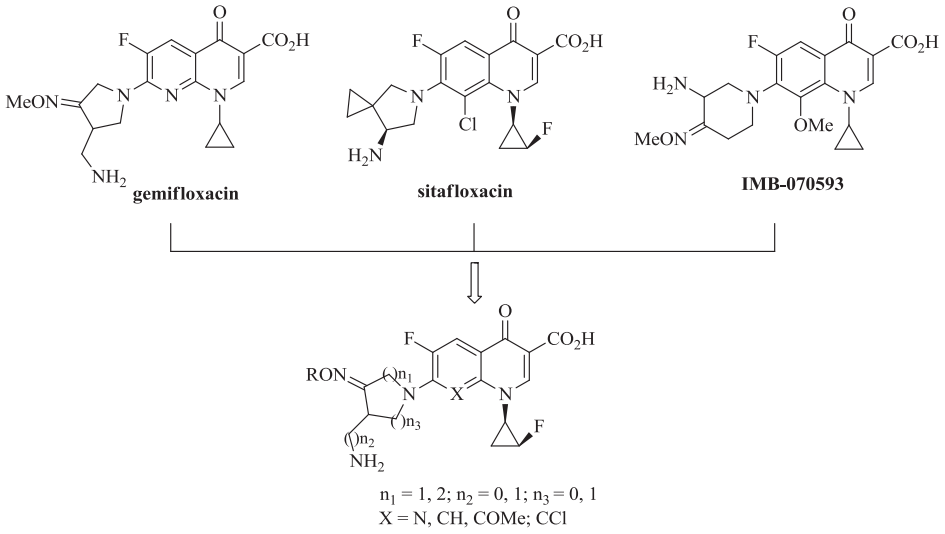


Fig. 1. Design of the novel fluoroquinolone derivatives.

It is of concern that the central nervous system (CNS) toxicity of N1-cyclopropyl FQs with outstanding antibacterial activity has been pointed in the clinical field [14], the corresponding 2-fluorocyclopropyl counterparts could modulate the lipophilicity and reduce the CNS toxicity [15]. Sitafloxacin (STFX, Fig. 1), a known FQ antibacterial agent, containing a (1R, 2S)-2-fluorocyclopropyl group at the N1-position, shows a broad spectrum of antibacterial and anti-MTB activity [16,17] and was approved in Japan for the treatment of a number of bacterial infections [18].

In recent years, methyloxime-functionalized pyrrolidines as novel C-7 substituents have attracted great attention and led to the discovery of some new FQ agents, such as GMFX, zabofloxacin (DW224a) and DW286 which show excellent antibacterial activity and pharmacokinetic profiles [19–21]. Similarly, many series of FQ derivatives containing an azetidine, a pyrrolidine or piperidine moiety with an oxime group at C-7 position were synthesized by our team and others, and some of them were found to have considerable biological activity [22–24]. For example, IMB-070593 (Fig. 1), a piperidinyl-based FQ candidate discovered in our lab and in late pre-clinical stage of development currently, possesses potent *in vitro* and *in vivo* antibacterial activity [25] and *in vitro* anti-MTB activity [26] as well as extremely low phototoxicity, hepatotoxicity and cardiac toxicity (unpublished data). All of the works emphasize the importance of the oxime functional group with respect to biological activity.

Inspired by the above research results, it was decided to introduce a four-, five- or six-membered nitrogen heterocyclic amine moiety with various alkyloxime groups at the C-7 position of STFX, and meanwhile do structural modifications to the C-8 position (Fig. 1). Thus, a series of novel 1-[(1R, 2S)-2-fluorocyclopropyl] FQ derivatives with an (R)/(S)-3-alkoxyimino-2-aminomethylazetidyl, 3-alkoxyimino-4-aminomethylpyrrolidyl or 3-alkoxyimino-4-aminopiperidyl group at the C-7 position were designed, synthesized and evaluated for their biological activity in this study. Our primary objective was to optimize the potency of these compounds against clinically important pathogens (especially Gram-positive ones) and MTB including MDR-MTB. A preliminary SAR study is also explored to facilitate the further development of FQs.

2. Results and discussion

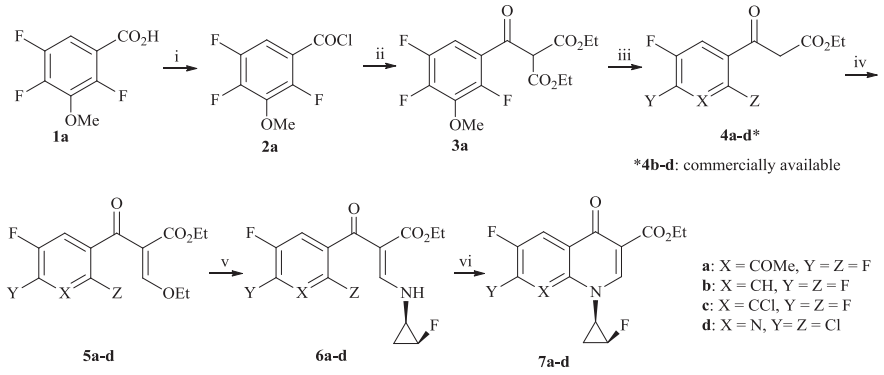
2.1. Chemistry

Four quinolone/naphthyridone core esters **7a**–**d** were first prepared according to Scheme 1. Commercially unavailable ethyl (3-methyoxyl-2,4,5-trifluorobenzoyl) acetate (**4a**) was conveniently obtained from 2,4,5-trifluoro-3-methoxybenzoic acid (**1a**) by chloroformylation, condensation with diethyl malonate and partial hydrolysis followed by decarboxylation successively according to well established procedures [27]. Condensation of **4a** and commercially available keto esters **4b**–**d** with triethyl orthoformate in acetic anhydride gave the enol ethers (**5a**–**d**), which upon evaporation of the solvent was allowed to react with a slight excess of (1R, 2S) -2-fluorocyclopropanamine tosylate in the presence of triethylamine to yield the enamino ethers (**6a**–**d**). Base-assisted cyclization of **6a**–**d** in N,N-dimethylformamide (DMF) afforded core esters (**7a**–**d**) (Scheme 1).

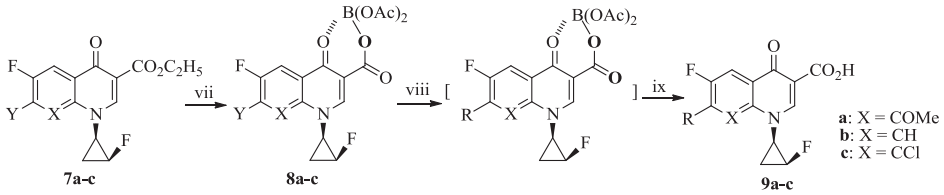
Synthetic pathways to novel quinolone derivatives **9a**–**c** and naphthyridinone derivatives **9d** are depicted in Schemes 2 and 3, respectively. Quinolone core esters (**7a**–**c**) were first converted to the corresponding boric chelates (**8a**–**c**) to increase reactivity. Condensation of **8a**–**c** with various side chain compounds (RH) in the presence of triethylamine followed by hydrolysis gave the desired derivatives **9a**–**c** (Scheme 2). Similarly, naphthyridone derivatives **9d** were synthesized through direct condensation of the carboxylic acid **8d** obtained by hydrolysis of the core ester **7d** with RH (Scheme 3) according to the routine synthetic procedures of naphthyridone agents [28].

2.2. Anti-MTB activity

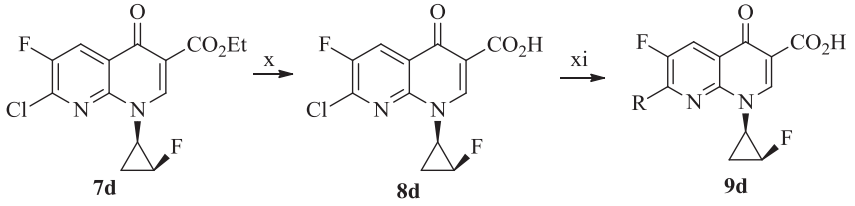
The target compounds **9a**–**d** were initially evaluated for their *in vitro* activity against MTB H37Rv ATCC 27294 (MTB-1) using the Microplate Alamar Blue Assay (MABA) [29,30]. The minimum inhibitory concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacterium-only controls and MICs of **9a**–**d** along with CPFX, LVFX, INH and rifampicin (RFP) for comparison are presented in Table 1. The data reveal that most of **9a**–**d** have



Scheme 1. Synthesis of quinolone/naphthyridione core esters **7a–d.**



Scheme 2. Synthesis of quinolone derivatives **9a–c.**



Scheme 3. Synthesis of naphthyridinone derivatives **9d**.

considerable activity against this strain (MICs: 0.25–4 μg/mL). The most active compounds **9a1**, **9b3** and **9b4** (MICs: 0.25 μg/mL) were found to be comparable to CPFX and LVFX, but less than INH and RFP (MICs: 0.05 μg/mL). Although it is generally believed that simply increasing the lipophilicity could improve the anti-MTB and antibacterial activity of FQs [31], our results suggest that the lipophilicity of **9a–d** which is expressed in the term of their Clog P values (Table 1) seems not to be an important parameter affecting the anti-MTB activity.

Compounds **9a1**, **9a3**, **9b3–5**, **9b7** and **9d2–4**, characterized by their better activity, were chosen for further evaluation their *in vitro* activity against MDR-MTB 6133 (MTB-2) and MDR-MTB 11277 (MTB-3) clinical isolates. All of them show good activity against the two strains (MICs: 0.0625–0.5 μg/mL). Especially, the most active compound **9a1** (MICs: 0.0625–0.125 μg/mL) was found to be 2-fold more potent than that of CPFX and LVFX against MTB-2, which is comparable to that of CPFX and LVFX against MTB-3. The two MDR-MTB isolates (MTB-2, MTB-3) are resistant to both of INH and RFP (Table 1).

2.3. Antibacterial activity

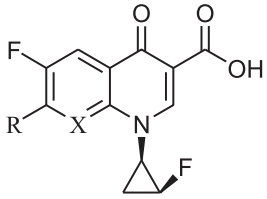
2.3.1. In vitro activity

The target compounds **9a–d** were evaluated for their *in vitro* antibacterial activity against representative strains using standard techniques [32]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth, and MIC values of **9a–d** against Gram-negative and Gram-positive strains along with IMB-070593, MXFX, CPFX and LVFX for comparison, are listed in Tables 2 and 3, respectively.

Generally, the derivatives **9a–d**, similar to the four reference drugs, have potent activity against *Escherichia coli* and *Klebsiella pneumoniae* (MICs: <0.008–4 μg/mL) with a few exceptions, but they have virtually no activity against the extended-spectrum β-lactamase-producing ($ESBL^+$) strains, due partly to resistance of these $ESBL^+$ strains inherent to FQs. Moreover, compounds **9b2–4** and **9d3** possess good potency against clinically important pathogens *Pseudomonas aeruginosa* including CPFX-/LVFX-/MXFX-resistant *P. aeruginosa* 12–14 (P.a.2) (MICs: 0.125–2 μg/mL).

On the other hand, the target compounds **9a–d** have potent *in vitro* antibacterial activity against the tested Gram-positive strains. For example, they show good potency in inhibiting the growth of methicillin-susceptible *Staphylococcus aureus* (MSSA, four strains), methicillin-susceptible *S. epidermidis* (MSSE, four strains) and *Streptococcus pneumoniae* (two strains) (MICs: <0.008–4 μg/mL) with a few exceptions, although their activity is similar to the four reference drugs, generally poor against methicillin-resistant *S. aureus* (MRSA, three strains) and

Table 1
Structures, physical data and antimycobacterial activity of compounds **9a**–**d**.



Compd.	R	X	mp [°C][a]	Clog P[b]	MIC (μg/mL)		
					MTB-1	MTB-2	MTB-3
9a1		C–OMe	145–147	−0.49	0.25	0.0625	0.125
9a2		C–OMe	94–96	0.26	32		
9a3		C–OMe	>250	−0.96	0.5	0.125	0.25
9b1		CH	176–179	−0.43	1		
9b2		CH	213–215	−0.20	1		
9b3		CH	>250	0.32	0.25	0.125	0.125
9b4		CH	148–150	1.42	0.25	0.125	0.25
9b5		CH	189–192	−0.67	0.5	0.125	0.25
9b6		CH	181–184	−0.16	2		
9b7		CH	158–160	0.99	0.5	0.25	0.5
9b8		CH	194–196	−0.67	1		
9b9		CH	>250	−0.16	2		
9b10		CH	>250	0.99	8		
9c1		C–Cl	126–128	0.28	16		
9c2		C–Cl	99–101	0.51	4		
9c3		C–Cl	116–118	1.04	4		
9c4		C–Cl	180–182	2.14	16		
9d1		N	155–157	−1.25	4		
9d2		N	178–180	−1.02	0.5	0.125	0.25
9d3		N	178–181	−0.49	0.5	0.125	0.125

Table 1 (*continued*)

Compd.	R	X	mp [°C][a]	Clog P[b]	MIC (μg/mL)		
					MTB-1	MTB-2	MTB-3
9d4		N	152–155	0.60	0.5	0.125	0.5
9d5		N	125–128	−1.50	1		
9d6		N	126–129	−0.97	1		
9d7		N	180–183	0.18	2		
9d8		N	146–148	−1.50	4		
9d9		N	115–117	−0.97	4		
9d10		N	176–178	0.18	2		
IMB				−0.72	0.25		
CPFX				1.32	0.25	0.125	0.125
LVFX				1.35	0.25	0.125	0.125
INH					0.05	4	1.0
RFP					0.05	>40	>40

[a] Melting points are uncorrected.
[b] The Clog P is calculated by Chemoffice 2010 software. The properties of all the compounds are maintained as described by Lipinski's rule of five; IMB: IMB-070593; CPFX: Ciprofloxacin; LVFX: Levofloxacin; INH: Isoniazid; RFP: Rifampicin; MTB-1: MTB H37Rv ATCC 27294; MTB-2: MDR-MTB 6133 resistant to INH and RFP; MTB-3: MDR-MTB 11277 resistant to INH and RFP.

methicillin-resistant *S. epidermidis* (MRSE, two strains). Among of them, the most active compounds **9a1** and **9d4** have excellent activity against all of the sixteen Gram-positive strains including MRSA and MRSE (MICs: <0.008–2 μg/mL), which is comparable to or better than IMB-070593 and MXFX, 2–64 fold more than CPFX and LVFX with a few exceptions. Notably, both of them show also useful activity against CPFX-/LVFX-/MXFX-resistant MRSA and MRSE (MICs: 0.5–2 μg/mL).

2.3.2. *In vivo activity*

Mice protection tests were used to evaluate *in vivo* efficacy of compounds **9a1** and **9d4** having better *in vitro* activity. The efficacy of them was initially tested against two clinical isolate strains (MSSA 12-1, *E. coli* 12-1), and then **9a1** with better *in vivo* activity against the two strains was chosen for further evaluation its *in vivo* activity against other four clinical isolates (MRSE 12-1, MRSA 12-5, *S. pneumoniae* 12-10, *K. pneumoniae* 12-1), and IMB-070593 was used as the control drug (Table 4).

The data suggest that compound **9a1** (2-fluorocyclopropyl counterpart of IMB-070593) exhibits better *in vivo* efficacy than **9d4** (GMFX derivative) against Gram-positive and Gram-negative strains. Furthermore, **9a1** possesses strong efficacy against MSSA 12-1, MRSA 12-5, *S. pneumoniae* 12-10 and *K. pneumoniae* 12-1 (ED_{50}s: 11.43, 26.04, 25.28 and 21.11 mg/kg, respectively) roughly comparable to IMB-070593, but less than IMB-070593 against the other two strains (*E. coli* 12-1, MRSE 12-1). The inconsistent of the above results with the corresponding *in vitro* activity data may be partially due to the poorer solubility of **9a1** and **9d4** (3.59 and 0.24 mg/mL, respectively) than IMB-070593 (22.50 mg/mL) [33] and also due to the different structural modification. Because of

Table 2
In vitro antibacterial activity of compounds **9a**–**d** against Gram-negative strains.

Compd	Strains MIC (μg/mL)														
	E.coli	E.co.1	E.co.2	E.co.3	E.co.4	E.co.5	K.p.1	K.p.2	K.p.3	K.p.4	K.p.5	K.p.6	K.p.7	P.a.1	P.a.2
9a1	0.06	0.5	32	32	16	32	0.25	0.5	0.5	16	16	2	64	4	4
9a2	4	16	>128	>128	>128	>128	4	4	4	>128	128	32	>128	8	128
9a3	0.25	2	64	32	128	64	0.5	0.5	0.5	64	16	2	64	2	16
9b1	0.25	2	>128	16	128	>128	2	1	1	>128	16	4	>128	4	32
9b2	0.03	0.25	128	32	32	128	0.25	0.25	0.25	32	16	1	64	2	2
9b3	0.06	0.5	32	16	16	128	0.25	0.25	0.25	16	8	1	32	0.25	1
9b4	0.06	0.06	64	32	16	64	0.06	0.25	0.25	16	16	16	64	1	1
9b5	<0.008	1	128	16	64	>128	0.125	0.125	0.015	64	8	0.5	64	0.25	32
9b6	0.03	0.125	32	8	16	128	0.06	0.06	0.06	32	8	0.5	32	0.5	32
9b7	0.125	2	128	16	64	>128	0.125	0.06	0.25	32	8	1	64	2	6
9b8	0.06	0.5	>128	128	128	>128	1	0.5	0.25	128	16	4	128	1	64
9b9	0.06	0.25	128	16	64	>128	0.25	0.25	0.25	64	16	1	64	1	32
9b10	0.25	8	>128	16	128	>128	0.5	0.5	0.5	128	16	2	128	2	32
9c1	1	2	>128	32	>128	>128	2	2	1	>128	32	16	>128	16	>128
9c2	0.125	1	64	32	64	128	0.25	0.25	0.25	32	16	4	32	1	16
9c3	0.5	2	128	32	128	>128	0.5	1	1	32	32	4	64	4	32
9c4	0.5	32	>128	32	>128	>128	2	2	2	>128	32	16	>128	8	64
9d1	0.25	8	>128	16	>128	>128	2	1	1	>128	64	32	>128	16	>128
9d2	0.06	0.5	128	32	64	>128	0.25	0.25	0.25	32	0.5	2	64	0.5	8
9d3	<0.008	0.06	64	16	16	128	0.03	0.06	0.125	16	8	0.125	32	0.125	2
9d4	<0.008	0.125	64	16	16	128	0.03	0.125	0.06	16	16	1	32	1	1
9d5	0.06	1	128	16	128	>128	0.25	0.25	0.25	64	8	2	128	2	32
9d6	0.125	2	>128	16	128	>128	0.5	0.5	0.25	128	8	4	64	1	32
9d7	0.125	0.25	>128	32	>128	>128	0.5	1	1	>128	16	4	>128	4	64
9d8	0.25	2	>128	16	>128	>128	1	0.5	0.5	>128	16	4	128	2	>128
9d9	0.5	2	>128	32	>128	>128	1	1	1	>128	32	8	>128	4	>128
9d10	0.5	4	>128	16	>128	>128	1	1	1	>128	32	8	128	8	128
IMB	0.06	0.5	32	32	16	32	0.06	0.125	0.06	32	16	0.5	64	4	2
MXFX	<0.008	0.5	32	64	16	32	0.015	0.06	0.03	32	0.5	0.5	64	2	16
CPFX	<0.008	0.125	32	32	32	64	0.125	0.03	<0.008	32	4	0.5	64	0.125	32
LVFX	<0.008	0.25	16	32	16	32	0.06	<0.008	<0.008	16	8	0.5	16	0.5	16

E.coli: *E. coli* ATCC 25922. E.co.1: *E. coli* 12-1. E.co.2: Extended-spectrum β-lactamase-producing ($ESBL^+$) *E. coli* 12-2. E.co.3: $ESBL^+$ *E. coli* 12-3. E.co.4: $ESBL^+$ *E. coli* 12-14. E.co.5: $ESBL^+$ *E. coli* 12-15. K.p.1: *K. pneumoniae* 12-1. K.p.2: *K. pneumoniae* 12-2. K.p.3: *K. pneumoniae* 12-4. K.p.4: $ESBL^+$ *K. pneumoniae* 12-3. K.p.5: $ESBL^+$ *K. pneumoniae* 12-4. K.p.6: $ESBL^+$ *K. pneumoniae* 12-7. K.p.7: $ESBL^+$ *K. pneumoniae* 12-14. P.a.1: *P. aeruginosa* 12-12. P.a.2: *P. aeruginosa* 12-14. IMB: IMB-070593. MXFX: Moxifloxacin. CPFX: Ciprofloxacin. LVFX: Levofloxacin.

this, structural modifications on **9a1** and **9d4** are in progress currently to improve their solubility.

The antibacterial activity of the 1-[(1R, 2S)-2-fluorocyclopropyl] FQs in this study is closely related to both of the groups at the 7- and 8-positions. Generally, the activity of the group at the 8-position seems to be in the order: N > CH > $COCH_3$ > CCl when an azetidine or a pyrrolidine served as the C-7 side chain, and but the order is $COCH_3$ > CH > N ≥ CCl for the piperidinyl-based FQs (**9a1** vs **9b1** vs **9d1** vs **9c1**).

On the other hand, the sizes of the heterocycle and R group at the 7-position are especially important for the activity. In the series of naphthyridinones and 8-hydrogen derivatives, pyrrolidyl-based FQs generally show the best antibacterial activity, followed by azetidyl- and piperidinyl-based ones with the same R group successively, and but piperidinyl-based 8-OCH3 FQs are more active than the corresponding azetidyl analogs (**9a1** vs **9a3**). Similarly, the contribution of the alkyl groups of the oxime moiety to the activity is also relevant to the ones at the 8-position. For example, the activity of the R groups as follows: benzyl > ethyl > methyl for naphthyridinones and 8-hydrogen FQs, which suggest that simply increasing the lipophilicity could improve the antibacterial activity. Conversely, the lipophilicity of the R groups goes against the activity for 8-Cl FQs. Moreover, the chirality at C2 of the azetidinyl group of the target compounds influences the activity, and the S enantiomers are more active than the corresponding the R ones (**9b5** vs **9b8**, **9d5** vs **9d8**, etc.).

3. Conclusions

In summary, a series of novel 1-[(1R, 2S)-2-fluorocyclopropyl]FQ derivatives containing a four-, five- or six-membered nitrogen heterocyclic amine moiety with various alkyloxime groups at the C-7 position were designed, synthesized and evaluated for their biological activity. Our results reveal that compounds **9a1** and **9b3** have good *in vitro* activity against MTB H37Rv ATCC 27294 (MICs: 0.25 μg/mL), MDR-MTB 6133 and MDR-MTB11277 clinical isolates (MICs: 0.065–0.125 μg/mL) which is comparable to or better than CPFX and LVFX. On the other hand, most of compounds **9a**–**d** show potent activity against *E. coli* and *K. pneumoniae* (MICs: <0.008–4 μg/mL) except $ESBL^+$ strains. Especially, **9a1** and **9d4** possess excellent *in vitro* activity against all of the sixteen Gram-positive strains including MRSA and MRSE (MICs: <0.008–2 μg/mL), and considerable *in vivo* efficacy against five clinical isolate strains (ED_{50}s: 11.43–26.04 mg/kg). However, our results suggest that the lipophilicity seems not to be an important parameter affecting both the anti-MTB and antibacterial activity.

4. Experimental protocol

4.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. ^1H NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6, D_2O or $CDCl_3$ using tetramethylsilane as an internal standard. Electrospray ionization (ESI)

Table 3
In vitro antibacterial activity of compounds **9a**–**d** against Gram-positive strains.

Compd	Strains MIC (μg/mL)														
	S.a.	MSSA1	MSSA2	MSSA3	MSSA4	MRSA1	MRSA2	MRSA3	MSSE1	MSSE2	MSSE3	MSSE4	MRSE1	MRSE2	S.p.1	S.p.2
9a1	0.015	<0.008	<0.008	<0.008	<0.008	2	2	2	<0.008	<0.008	<0.008	0.25	0.5	1	0.125	1
9a2	0.015	0.25	0.25	0.25	0.25	128	128	64	0.25	2	0.25	16	32	>128	4	4
9a3	<0.008	0.125	0.125	0.06	0.125	8	8	4	0.125	0.125	0.125	2	2	2	0.25	2
9b1	0.5	0.5	0.5	0.5	0.5	64	128	64	0.5	1	0.5	2	8	2	1	2
9b2	<0.008	0.125	0.125	0.06	0.125	16	4	8	0.06	0.06	0.125	0.25	2	0.125	0.03	0.25
9b3	<0.008	0.06	0.015	0.06	0.06	8	8	8	0.06	0.25	0.06	0.5	1	1	0.03	0.25
9b4	<0.008	0.06	<0.008	0.06	0.06	4	8	4	0.125	0.125	0.06	0.125	1	0.5	0.06	0.25
9b5	0.06	0.5	0.5	0.25	0.5	32	64	32	0.25	0.25	0.25	0.125	8	4	0.125	0.5
9b6	0.03	0.125	0.06	0.06	0.125	16	32	16	0.125	0.5	0.125	2	4	8	0.06	1
9b7	0.015	0.125	0.03	0.03	0.125	16	16	8	0.125	0.25	0.125	2	4	16	0.25	1
9b8	0.5	0.5	0.5	0.25	0.5	64	128	64	0.5	1	0.5	8	16	16	0.5	2
9b9	<0.008	0.06	0.06	0.03	0.03	32	32	32	0.06	0.125	0.06	4	8	64	0.25	1
9b10	0.25	1	1	1	1	>128	>128	128	1	2	1	2	32	8	0.5	8
9c1	1	0.25	0.25	0.25	0.25	32	64	32	0.5	1	0.5	4	16	32	1	8
9c2	<0.008	0.125	0.125	0.06	0.125	16	16	16	0.125	0.5	0.125	2	4	4	0.25	1
9c3	0.5	0.25	0.125	0.25	0.25	16	16	16	0.5	1	0.25	2	4	16	1	4
9c4	0.25	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	1	0.5	64	0.5	64	1	8
9d1	1	2	2	1	2	>128	>128	>128	1	<0.008	1	<0.008	>128	1	1	4
9d2	0.015	0.125	0.125	0.06	0.125	16	32	16	0.125	0.5	0.125	1	4	4	0.125	0.25
9d3	<0.008	0.015	0.015	0.015	0.015	4	4	4	<0.008	0.03	0.015	0.25	1	4	0.03	0.06
9d4	<0.008	<0.008	<0.008	<0.008	<0.008	1	2	1	<0.008	<0.008	<0.008	0.125	0.25	2	0.03	0.25
9d5	0.125	0.06	0.06	0.03	0.06	32	32	32	0.06	0.5	0.06	2	4	4	0.5	2
9d6	0.5	0.5	0.25	0.25	0.5	64	64	64	0.5	1	0.25	16	16	16	0.5	4
9d7	0.125	0.25	0.25	0.125	0.25	64	32	32	0.25	0.5	0.25	8	16	8	0.5	4
9d8	0.25	1	0.5	0.5	0.5	>128	>128	>128	0.5	0.5	0.5	32	64	64	1	4
9d9	0.25	0.5	0.5	0.25	0.5	>128	>128	>128	0.5	2	0.5	32	64	32	2	8
9d10	0.5	0.5	0.25	0.25	0.25	128	128	128	0.5	1	0.5	32	32	32	1	4
IMB	<0.008	<0.008	<0.008	<0.008	<0.008	2	2	2	0.03	0.06	0.03	0.125	0.125	2	0.03	0.25
MXFX	<0.008	0.015	<0.008	<0.008	0.015	8	8	8	0.03	0.125	0.03	1	0.25	16	0.06	0.5
CPFX	0.25	0.25	0.25	0.25	0.25	64	64	32	0.25	2	0.25	4	8	16	0.25	1
LVFX	<0.008	0.125	0.125	0.125	0.125	32	32	32	0.125	0.5	0.125	4	4	32	0.125	2

S.a.: *S. aureus* ATCC25923. MSSA1: Methicillin-sensitive *S. aureus* 12-1. MSSA2: Methicillin-sensitive *S. aureus* 12-2. MSSA3: Methicillin-sensitive *S. aureus* 12-4. MSSA4: Methicillin-sensitive *S. aureus* 12-5. MRSA1: Methicillin-resistant *S. aureus* 12-2. MRSA2: Methicillin-resistant *S. aureus* 12-4. MRSA3: Methicillin-resistant *S. aureus* 12-5. MSSE1: Methicillin-sensitive *S. epidermidis* 12-1.MSSE2: Methicillin-sensitive *S. epidermidis* 12-3. MSSE3: Methicillin-sensitive *S. epidermidis* 12-6. MSSE4: Methicillin-sensitive *S. epidermidis* 12-8. MRSE1: Methicillin-resistant *S. epidermidis* 12-1.MRSE2: Methicillin-resistant *S. epidermidis* 12-6. S.p.1: *S. pneumoniae* ATCC 49619. S.p.2: *S. pneumoniae* 12-18. IMB: IMB-070593. MXFX: Moxifloxacin. CPFX: Ciprofloxacin. LVFX: Levofloxacin.

mass spectra and high resolution mass spectra (HRMS) were obtained on an MDSSCIEX Q-Tap 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. Ethyl 3-(2,4,5-trifluoro-3-methoxyphenyl)-3-oxopropanoate **4a**

A solution of 2,4,5-trifluoro-3-methoxybenzoic acid **1a** (20.60 g, 0.10 mol) and DMF (0.25 mL) in thionyl chloride (100 mL) was stirred for 10 h at reflux and concentrated under reduced pressure. The residue was diluted with dry toluene (30 mL) and then concentrated under reduced pressure to give crude product **2a** as a yellow oil.

To a stirred mixture of magnesium powder (2.88 g, 0.12 mol), anhydrous alcohol (100 mL) and carbon tetrachloride (1 mL) was added dropwise a solution of diethyl malonate (18.60 mL, 0.12 mol) in anhydrous alcohol (50 mL) over a period of 30 min at reflux and then stirred for 2 h at the same temperature. The reaction mixture was cooled to −10 °C and added dropwise a solution of the above **2a** in dry toluene (50 mL) over a period of 20 min, and then stirred 2 h at the same temperature. The mixture was adjusted to pH 5 with 6 N HCl and extracted with toluene (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to provide crude product **3a** as a yellow oil.

To a solution of the above **3a** dissolved in water (100 mL) was added p-toluenesulfonic acid (0.10 g, 0.6 mmol). The reaction mixture was stirred for 2 h at reflux and then extracted with dichloromethane (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give crude product **4a** (17.30 g, 62.6%, from **1a**) as a yellow oil, which was used directly without further purification.

Table 4
In vivo efficacy of compounds **9a1** and **9d4** against systemic infections in mice.

Infected bacteria [challenge dose (cfu/mL)]	Compd[a]	MIC (mg/mL)	ED_{50} (mg/kg)[b]	95% confidence limit (mg/kg)
MSSA 12-1 (3.0×10^4)	**9a1**	<0.008	11.43	8.22–15.94
	9d4	<0.008	16.94	11.30–28.53
	IMB	<0.008	10.0	7.03–14.23
Escherichia coli 12-1 (6.0×10^5)	**9a1**	0.5	12.14	8.46–17.68
	9d4	0.125	18.04	12.18–30.30
	IMB	0.5	8.23	5.91–11.40
MRSE 12-1 (4.5×10^6)	**9a1**	0.5	25.36	20.10–32.68
	IMB	0.125	15.33	11.47–20.06
MRSA 12-5 (4.5×10^6)	**9a1**	2	26.04	18.73–40.67
	IMB	2	25.36	20.10–32.68
Streptococcus pneumoniae 12-10 (5.2×10^8)	**9a1**	1	25.28	19.78–33.25
	IMB	0.06	27.57	21.85–36.07
Klebsiella pneumoniae 12-1 (4.5×10^6)	**9a1**	0.25	21.11	16.81–27.16
	IMB	0.06	18.56	14.55–23.97

MRSA: Methicillin-resistant *S. aureus*; MRSE: Methicillin-resistant *S. epidermidis*; IMB: IMB-070593.

[a] Antimicrobial agents were orally administrated twice at 0 and 6 h after infection.

[b] ED_{50}: 50% effective dose.

*4.2.2. General procedure for the synthesis of compounds **7a–d***

A solution of keto esters **4a–d** (1 mmol) and triethyl orthoformate (2.50 mL, 1.5 mmol) in acetic anhydride (5.5 mL, 6 mmol) was stirred for 8 h at reflux and concentrated under reduced pressure to give crude products **5a–d** as yellow oils. To a solution of **5a–d** and triethylamine (2.80 mL, 2 mmol) dissolved in dichloromethane (10 mL) was added (1R, 2S)-fluorocyclopropanamine tosylate (3.71 g, 1.5 mmol) in portions and stirred for 2 h at room temperature, and then concentrated under reduced pressure to give crude products **6a–d** as yellow oils. A mixture of **6a–d**, DMF (10 mL) and K_2CO_3 (2.80 g, 2 mmol) was stirred for 1 h at 90 °C and then poured into water (100 mL). The precipitate was collected by filtration and purified by silica gel column chromatography to give the title compounds**7a–d** (42.5–61.3%, from **4a–d**) as white solids.

*4.2.2.1. Ethyl 6,7-difluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4- dihydroquinoline-3-carboxylate **7a**.* The title compound **7a** was obtained from **4a** (46.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (1H, s, C_2–H), 8.05 (1H, d, J = 12.0 Hz, C_5–H), 5.02–4.80 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.39 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.88–3.83 (1H, m, fluorocyclopropyl CH), 1.68–1.56 (m, 2H, fluorocyclopropyl CH), 1.39 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 342.16 $(M+H)^+$.

*4.2.2.2. Ethyl 6,7-difluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4- dihydroquinoline-3-carboxylate **7b**.* The title compound **7b** was obtained from **4b** (42.5%). δ 8.59 (1H, s, C_2–H), 8.18–8.05 (2H, m, C_5–H, C_8–H), 5.40–5.20 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.23 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.64–3.69 (1H, m, fluorocyclopropyl CH), 1.70–1.61 (m, 2H, fluorocyclopropyl CH), 1.28 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 312.53 $(M+H)^+$.

*4.2.2.3. Ethyl 8-chloro-6,7-difluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4- dihydroquinoline-3-carboxylate **7c**.* The title compound **7c** was obtained from **4c** (54.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (1H, s, C_2–H), 8.10 (1H, d, J = 12.0 Hz, C_5–H), 5.20–5.01 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.16–4.27 (3H, m, O–CH_2CH_3, fluorocyclopropyl CH), 1.70–1.61 (m, 2H, fluorocyclopropyl CH), 1.28 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 346.07 $(M+H)^+$.

*4.2.2.4. Ethyl 6,7-difluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8- naphthyridine-3-carboxylate **7d**.* The title compound **7d** was obtained from **4d** (61.3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (1H, s, C_2–H), 8.46 (1H, d, J = 12.0 Hz, C_5–H), 5.26–5.06 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.26 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.65–3.71 (1H, m, fluorocyclopropyl CH), 1.70–1.61 (m, 2H, fluorocyclopropyl CH), 1.28 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 329.25 $(M+H)^+$.

*4.2.3. General procedure for the synthesis of core chelates **8a–c***

A mixture of boric acid (0.10 g, 1.5 mmol) and acetic anhydride (4.60 mL, 5 mmol) was stirred for 1.5 h at 110 °C and acetic acid (5.20 mL, 9 mmol) was added, and then stirred for 1 h at the same temperature. To the reaction mixture was added **7a–c** (1 mmol) at 95 °C and stirred for 2–3.5 h at the same temperature. After cooling to room temperature, the mixture was poured into ice water (20 mL) slowly and stirred for 0.5 h. The resulting solid was collected by suction and washed successively with water (20 mL), chilled ethanol (10 mL) and then dried under vacuum to afford the title compounds **8a–c** as white solids.

*4.2.4. General procedure for the synthesis of 8-methoxyFQ derivatives **9a1–3***

A mixture of **8a** (0.44 g, 1 mmol), RH (1.1 mmol) and triethylamine (0.42 mL, 3 mmol) in dry acetonitrile (10 mL) was stirred for 5–8 h at 50 °C under an atmosphere of nitrogen and then concentrated under reduced pressure. The residue was dissolved in 1% NaOH (10 mL) and stirred for 1 h at room temperature, adjusted to pH 6.5–7.0 with 20% HCl, and extract with chloroform (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluting with dichloromethane and methanol (30:1, v/v) to get the title compounds **9a1–3** (31.2–37.6%) as off-white solids.

*4.2.4.1. 7-[3-Amino-4-(methoxyimino)piperidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9a1**.* The title compound **9a1** was obtained from **8a** and 3-aminopiperidin-4-one O-methyl oxime dihydrochloride [1] as an off-white solid (37.6%).$[\alpha]_D^{20}$ = +18.75 (c 0.064, CH_3OH), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (1H, s, C_2–H), 7.80 (1H, d, J = 11.9 Hz, C_5–H), 4.98 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.14 (1H, s, fluorocyclopropyl CH), 3.85–3.73 (6H, s, O–CH_3), 3.70 (1H, m, H), 3.64 (1H, m, H), 3.48 (1H, m, H), 3.20 (1H, m, H), 3.01 (1H, m, H), 2.75 (1H, m, H), 2.38 (1H, m, H), 1.61–1.48 (m, 2H, fluorocyclopropyl CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.83, 165.86, 157.57 (J = 86, d) 154.75, 152.63, 146.32, 134.63, 121.34, 106.88, 73.99, 71.79, 63.65, 61.56, 58.33, 51.63, 50.03, 45.91, 41.46, 24.65, 15.63. MS-ESI (m/z): 437.29 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{20}H_{23}O_5N_4F_2$ $(M+H)^+$: 437.161761; Found 437.16096.

*4.2.4.2. 7-[3-(Aminomethyl)-4-(ethoxyimino)pyrrolidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9a2**.* The title compound **9a2** was obtained from **8a** and 4-aminopyrrolidin-3-one O-ethyl oxime dimesylate [34] as an off-white solid (31.2%). $[\alpha]_D^{20}$ = −23.68 (c 0.076, CH_3OH), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (1H, s, C_2–H), 7.86 (1H, d, J = 12.4 Hz, C_5–H), 4.99–4.67 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.14 (2H, q, J = 7.0 Hz, O–CH_2CH_3), 3.89 (2H, m), 3.78–3.62 (5H, m), 3.53 (1H, m, H), 3.40 (1H, m, H), 3.01 (2H, m), 1.70–1.47 (2H, m, fluorocyclopropyl CH), 1.26 (3H, t, J = 7.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 451.25 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{21}H_{25}O_5N_4F_2$ $(M+H)^+$: 451.17741; Found 451.17694.

*4.2.4.3. 7-[(S)-2-(Aminomethyl)-3-(methoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)- 2-fluorocyclopropyl]-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9a3**.* The title compound **9a3** was obtained from **8a** and (S)-2-(aminomethyl)azetidin-3-one O-methyl oxime dihydrochloride [23] as an off-white solid (35.3%). $[\alpha]_D^{20}$ = −14.00 (c 0.050, CH_3OH), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60,8.59 (1H, 2 × s, C_2–H), 7.73 (1H, d, J = 12.0 Hz, C_5–H), 5.26 (1H, m), 5.08 (2H, m), 4.81 (1H, m, J = 64.0 Hz, fluorocyclopropyl CH), 4.04 (1H, m), 3.83 (3H, s, O–CH_3), 3.64 (3H, s, O–CH_3), 3.08 (2H, m), 1.46–1.38 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 423.19 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_5N_4F_2$ $(M+H)^+$: 423.14745; Found 423.14749.

*4.2.5. General procedure for the synthesis of 8-hydrogenFQ derivatives **9b1–10***

A mixture of **8b** (0.42 g, 1 mmol), RH (1.1 mmol) and triethylamine (0.42 mL, 3 mmol) in dry acetonitrile (10 mL) was stirred for 25–20 h at room temperature under an atmosphere of nitrogen and concentrated under reduced pressure. The residue was dissolved in 0.5% NaOH (15 mL) and stirred for 1 h at room temperature, adjusted to pH 6.5–7.0 with 20% HCl, and extract with chloroform (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluting

with dichloromethane and methanol (35:1, v/v) to get the title compounds **9b1**–**10** (33.5–46.8%) as off-white and yellow solids.

*4.2.5.1. 7-[3-Amino-4-(methoxyimino)piperidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b1**.* The title compound **9b1** was obtained from **8b** and 3-aminopiperidin-4-one O-methyl oxime dihydrochloride as a yellow solid (42.7%). $[\alpha]_D^{20}$ = +14.63 (c 0.082, CH_3OH), ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 (1H, s, C_2–H), 8.04 (1H, d, J = 13.2 Hz, C_5–H), 7.21 (1H, s, C_8–H), 5.12 (1H, d, J = 65.8 Hz, fluorocyclopropyl CH), 3.91 (3H, s, O–CH_3), 3.82 (1H, s), 3.73 (1H, m), 3.57–3.49 (2H, m), 3.47–3.26 (2H, m), 3.25–3.07 (1H, m), 2.66–2.58 (1H, m), 1.81–1.74 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 407.31 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 407.15254; Found 407.15103.

*4.2.5.2. 7-[3-(Aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl)-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b2**.* The title compound **9b2** was obtained from **8b** and 4-aminopyrrolidin-3-one O-methyl oxime dimesylate [34] as an off-white solid (39.6%). $[\alpha]_D^{20}$ = +4.76 (c 0.042, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, s, C_2–H), 7.89 (1H, d, J = 14.2 Hz, C_5–H), 7.15 (1H, d, J = 7.3 Hz, C_8–H), 5.37 (1H, d, J = 64.8 Hz, fluorocyclopropyl CH), 4.40 (2H, s), 4.03–3.91 (1H, m), 3.86 (4H, m), 3.76–3.60 (1H, m), 3.15 (1H, s), 2.94–2.76 (2H, m), 1.96–1.83 (1H, m, fluorocyclopropyl CH), 1.85–1.70 (1H, m, fluorocyclopropyl CH). MS-ESI (m/z): 407.50 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 407.15254; Found 407.15106.

*4.2.5.3. 7-[3-(Aminomethyl)-4-(ethoxyimino)pyrrolidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b3**.* The title compound **9b3** was obtained from **8b** and 4-aminopyrrolidin-3-one O-ethyl oxime dimesylate as an off-white solid (34.6%). $[\alpha]_D^{20}$ = +11.11 (c 0.056, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (1H, s, C_2–H), 7.88 (1H, d, J = 14.2 Hz, C_5–H), 7.15 (1H, d, J = 7.3 Hz, C_8–H), 5.36 (1H, d, J = 64.2 Hz, fluorocyclopropyl CH), 4.41 (2H, s), 4.11 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.91 (1H, m), 3.78 (1H, m), 3.66 (1H, m), 3.11 (1H, s), 2.89–2.78 (2H, m), 1.95–1.81 (1H, m, fluorocyclopropyl CH), 1.78–1.70 (1H, m, fluorocyclopropyl CH), 1.23 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 421.15 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{20}H_{23}O_4N_4F_2$ $(M+H)^+$: 421.16853; Found 421.16819.

*4.2.5.4. 7-[3-(Aminomethyl)-4-(benzyloxyimino)pyrrolidin-1-yl]-6-fluoro-1-[(1R, 2S)- 2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b4**.* The title compound **9b4** was obtained from **8b** and 4-aminopyrrolidin-3-one O-benzyl oxime dimesylate [34] as an off-white solid (46.7%). $[\alpha]_D^{20}$ = +6.25 (c 0.048, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (1H, s, C_2–H), 7.89 (1H, d, J = 14.2 Hz, C_5–H), 7.38–7.31 (5H, m, Ar–H), 7.15 (1H, d, J = 7.8 Hz, C_8–H), 5.36 (1H, d, J = 64.2 Hz, fluorocyclopropyl CH), 5.13 (2H, s, O–CH_2Ar), 3.87 (2H, m), 3.87 (2H, m), 3.69 (1H, m), 3.07 (1H, s), 2.85–2.72 (2H, m), 1.97–1.70 (2H, m, fluorocyclopropyl CH), 1.78–1.70 (1H, m, fluorocyclopropyl CH). MS-ESI (m/z): 483.32 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{25}H_{25}O_4N_4F_2$ $(M+H)^+$: 483.18384; Found: 483.18179.

*4.2.5.5. 7-[(S)-2-(aminomethyl)-3-(methoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b5**.* The title compound **9b5** was obtained from **8b** and (S)-2-(aminomethyl)azetidin-3-one O-methyl oxime dihydrochloride as an off-white solid (33.5%). $[\alpha]_D^{20}$ = +27.94 (c 0.068, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.67 (1H, s, C_2–H), 7.87 (1H, d, J = 12.0 Hz, C_5–H), 7.34 (1H, d, J = 7.8 Hz, C_8–H), 5.38 (1H, d, J = 64.2 Hz, fluorocyclopropyl CH), 5.22–5.15 (1H, m), 4.90 (2H, m), 3.83 (3H, s, O–CH_3) 3.75 (1H, m), 3.08 (2H, m), 1.96–1.78 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 393.69 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{19}O_4N_4F_2$ $(M+H)^+$: 393.13689; Found: 393.13584.

*4.2.5.6. 7-[(S)-2-(aminomethyl)-3-(ethoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b6**.* The title compound **9b6** was obtained from **8b** and (S)-2-(aminomethyl)azetidin-3-one O-ethyl oxime dihydrochloride [23] as an off-white solid (37.9%). $[\alpha]_D^{20}$ = +52.78 (c 0.036, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.74 (1H, s, C_2–H), 7.94 (1H, d, J = 12.0 Hz, C_5–H), 7.24 (1H, m, C_8–H), 5.51–5.40 (2H, m), 4.94 (2H, m), 4.13 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.37 (1H, s), 3.33–3.24 (2H, m), 1.94–1.80 (2H, m, fluorocyclopropyl CH), 1.23 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 407.82 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 407.15254; Found: 407.15128.

*4.2.5.7. 7-[(S)-2-(aminomethyl)-3-(benzyloxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b7**.* The title compound **9b7** was obtained from **8b** and (S)-2-(aminomethyl)azetidin-3-one O-benzyl oxime dihydrochloride [23] as an off-white solid (43.5%). $[\alpha]_D^{20}$ = +46.55 (c 0.058, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.69 (1H, s, C_2–H), 7.89 (1H, d, J = 12.0 Hz, C_5–H), 7.40–7.27 (6H, m, C_8–H, Ar–H), 5.48–5.25 (2H, m), 5.10 (2H, s, O–CH_2Ar), 4.97–4.89 (2H, m), 3.78 (1H, m), 3.31–3.10 (2H, m), 1.90–1.74 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 469.35 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{23}O_4N_4F_2$ $(M+H)^+$: 469.16819; Found: 419.16635.

*4.2.5.8. 7-[(R)-2-(aminomethyl)-3-(methoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b8**.* The title compound **9b8** was obtained from **8b** and (R)-2-(aminomethyl)azetidin-3-one O-methyl oxime dihydrochloride [23] as an off-white solid (46.2%). $[\alpha]_D^{20}$ = +8.82 (c 0.068, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (1H, s, C_2–H), 7.89 (1H, d, J = 12.0 Hz, C_5–H), 7.38 (1H, m, C_8–H), 5.48–5.24 (2H, m), 5.22–5.15 (1H, m), 4.95–4.84 (2H, m), 3.83 (3H, s, O–CH_3) 3.78 (1H, m), 3.13 (2H, m), 1.91–1.76 (2H, m). MS-ESI (m/z): 393.33 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{18}H_{19}O_4N_4F_2$ $(M+H)^+$: 393.13689; Found: 393.13592.

*4.2.5.9. 7-[(R)-2-(aminomethyl)-3-(ethoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b9**.* The title compound **9b9** was obtained from **8b** and (R)-2-(aminomethyl)azetidin-3-one O-ethyl oxime dihydrochloride [23] as an off-white solid (35.2%). $[\alpha]_D^{20}$ = +7.84 (c 0.102, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.69 (1H, s, C_2–H), 7.88 (1H, d, J = 12.0 Hz, C_5–H), 7.25 (1H, m, C_8–H), 5.48–5.25 (2H, m), 4.95–4.75 (2H, m), 4.08 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.78 (1H, s), 3.30–3.12 (2H, m), 1.94–1.78 (2H, m, fluorocyclopropyl CH), 1.23 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 407.15 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 407.15254; Found: 407.15247.

*4.2.5.10. 7-[(R)-2-(aminomethyl)-3-(benzyloxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **9b10**.* The title compound **9b10** was obtained from **8b** and (R)-2-(aminomethyl)azetidin-3-one O-benzyl oxime dihydrochloride [23] as an off-white solid (46.8%). $[\alpha]_D^{20}$ = +6.12 (c 0.050, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, s, C_2–H), 7.90 (1H, d, J = 12.0 Hz, C_5–H), 7.39–7.31 (6H, m, C_8–H, Ar–H), 5.42–5.24 (2H, m), 5.11 (2H, s, O–CH_2Ar), 4.95–4.85 (2H, m), 3.79 (1H, m), 3.28–3.12 (2H, m), 1.91–1.76 (2H, m,

fluorocyclopropyl CH). MS-ESI (*m*/*z*): 469.28 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{24}H_{23}O_4N_4F_2$ $(M+H)^+$: 469.16819; Found: 469.16821.

4.2.6. General procedure for the synthesis of 8-chloroFQ derivatives ***9c1–4***

A mixture of **8c** (0.45 g, 1 mmol), RH (1.1 mmol) and triethylamine (0.42 mL, 3 mmol) in dry acetonitrile (10 mL) was stirred for 2–4 h at room temperature under an atmosphere of nitrogen, and concentrated under reduced pressure. The residue was dissolved in 1% NaOH (10 mL) and stirred for 1 h at room temperature, adjusted to pH 6.5–7.0 with 20% HCl, and extract with chloroform (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluting with dichloromethane and methanol (40:1, v/v) to get the title compounds **9c1**–**4** (39.6–46.3%) as off-white and yellow solids.

4.2.6.1. 7-[3-Amino-4-(methoxyimino)piperidin-1-yl]-8-chloro-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9c1****.* The title compound **9c1** was obtained from **8c** and 3-aminopiperidin-4-one O-methyl oxime dihydrochloride as an off-white solid (44.3%). $[\alpha]_D^{20} = -64.52$ (c 0.062, CH_3OH), ^1H NMR (400 MHz, $CDCl_3$) δ 8.74 (1H, s, C_2–H), 7.94 (1H, d, $J = 12.0$ Hz, C_5–H), 5.16 (1H, d, $J = 68.0$ Hz, fluorocyclopropyl CH), 4.48 (1H, s), 4.32 (1H, m), 3.71 (3H, s, O–CH_3), 3.12 (1H, m), 2.93–2.85 (1H, m), 2.77 (2H, m), 2.39 (2H, m), 1.66–1.53 (2H, m, fluorocyclopropyl CH). MS-ESI (*m*/*z*): 441.13 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{19}H_{20}O_4N_4F_2Cl$ $(M+H)^+$: 441.11174; Found 441.11222.

4.2.6.2. 7-[-3-(Aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl]-8-chloro-6-fluoro-1- [(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9c2****.* The title compound **9c2** was obtained from **8c** and 4-aminopyrrolidin-3-one O-methyl oxime dimesylate as an off-white solid (39.6%). $[\alpha]_D^{20} = -52.17$ (c 0.046, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.72 (1H, s, C_2–H), 7.90 (1H, d, $J = 12.0$ Hz, C_5–H), 5.10 (1H, d, $J = 64.0$ Hz, fluorocyclopropyl CH), 4.32 (1H, s), 3.74 (3H, s, O–CH_3), 3.68–3.55 (3H, m), 3.15 (1H, s), 2.94–2.76 (2H, m), 1.58–1.45 (2H, m, fluorocyclopropyl-H). MS-ESI (*m*/*z*): 441.50 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{19}H_{20}O_4N_4F_2Cl$ $(M+H)^+$: 441.11174; Found 441.11203.

4.2.6.3. 7-[3-(Aminomethyl)-4-(ethoxyimino)pyrrolidin-1-yl]-8-chloro-6-fluoro-1- [(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9c3****.* The title compound **9c3** was obtained from **8c** and 4-aminopyrrolidin-3-one O-ethyl oxime dimesylate as an off-white solid (42.5%). $[\alpha]_D^{20} = -34.85$ (c 0.066, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, s, C_2–H), 7.88 (1H, d, $J = 12.0$ Hz, C_5–H), 5.09 (1H, d, $J = 64.0$ Hz, fluorocyclopropyl CH), 4.31 (1H, s), 3.99 (2H, q, $J = 8.0$ Hz, O–CH_2CH_3), 3.70 (2H, m), 3.45 (2H, m), 3.04 (1H, m), 2.89–2.81 (2H, m), 1.68–1.51 (2H, m, fluorocyclopropyl CH), 1.15 (3H, t, $J = 8.0$ Hz, OCH_2–CH_3). MS-ESI (*m*/*z*): 455.59 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{20}H_{22}O_4N_4F_2Cl$ $(M+H)^+$: 455.12739; Found 455.12751.

4.2.6.4. 7-[3-(Aminomethyl)-4-(benzyloxyimino)pyrrolidin-1-yl]-8-chloro-6-fluoro-1- [(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9c4****.* The title compound **9c4** was obtained from **8c** and 4-aminopyrrolidin-3-one O-benzyl oxime dimesylate as an off-white solid (46.3%). $[\alpha]_D^{20} = -67.50$ (c 0.054, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, s, C_2–H), 7.88 (1H, d, $J = 14.0$ Hz, C_5–H), 7.33–7.26 (5H, m, Ar–H), 5.17-5.00 (3H, m), 4.30 (1H, m), 3.69–3.60 (2H, m), 3.51 (2H, m), 3.03 (1H, s), 1.66–1.50 (2H, m, fluorocyclopropyl CH). MS-ESI (*m*/*z*): 517.31 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{25}H_{24}O_4N_4F_2Cl(M+H)^+$: 517.14304; Found: 517.14251.

4.2.7. General procedure for the synthesis of naphthyridone derivatives ***9d1–10***

A mixture of **7d** (0.33 g, 1 mmol), acetic acid (10 mL) and concd HCl (40 mL) was stirred for 6 h at reflux. After cooling to room temperature, the mixture was poured into ice water (100 mL) slowly and stirred for 0.5 h. The resulting solid was collected by suction and washed with ethanol (20 mL) and ether successively, and then dried under vacuum to afford the title compound **8d** (0.22 g, 33%) as white and yellow solids.

A mixture of **8d** (0.30 g, 1 mmol), RH (1.1 mmol) and triethylamine (0.42 mL, 3 mmol) in dry acetonitrile (10 mL) was stirred for 2 h at room temperature under an atmosphere of nitrogen, and concentrated under reduced pressure. The residue was dissolved in 5% NaOH (5 mL) and stirred for 1 h, adjusted to pH 6.5–7.0 with 20% HCl, and extract with chloroform (50 mL × 3). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The solid was washed with ethanol and ether successively to get the title compounds **9d1**–**10** (52.6–72.1%) as off-white and yellow solids.

4.2.7.1. 7-[3-Amino-4-(methoxyimino)piperidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d1****.* The title compound **9d1** was obtained from **8d** and 3-aminopiperidin-4-one O-methyl oxime dihydrochloride as an off-white solid (66.5%). $[\alpha]_D^{20} = +35.00$ (c 0.060, CH_3OH), ^1H NMR (400 MHz, D_2O) δ 8.76 (1H, s, C_2–H), 7.94 (1H, s, C_5–H), 5.21–5.05 (d, $J = 64.9$ Hz, 1H, fluorocyclopropyl CH), 4.64 (1H, s), 4.64–4.20 (2H, m), 3.95 (3H, s, O–CH_3), 3.80–3.69 (3H, m), 3.20 (1H, m), 2.53 (1H, s), 1.92–1.71 (2H,m, fluorocyclopropyl CH). MS-ESI (*m*/*z*): 408.21 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{18}H_{20}O_4N_5F_2$ $(M+H)^+$: 408.14779; Found: 408.14638.

4.2.7.2. 7-[-3-(Aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl]-6-fluoro-1- [(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d2****.* The title compound **9d2** was obtained from **8d** and 4-aminopyrrolidin-3-one O-methyl oxime dimesylate as a yellow solid (54.6%). $[\alpha]_D^{20} = +25.00$ (c 0.060, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (1H, s, C_2–H), 8.04 (1H, d, $J = 12.8$ Hz, C_5–H), 5.18 (1H, d, $J = 65.2$ Hz, fluorocyclopropyl CH), 4.58 (2H, s), 4.24–4.13 (1H, m), 3.93–3.86 (4H, m), 3.71 (1H, m), 3.15 (1H, s), 2.96–2.82 (2H, m), 1.91–1.61 (2H, m, fluorocyclopropyl CH). MS-ESI (*m*/*z*): 408.13 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{18}H_{20}O_4N_5F_2$ $(M+H)^+$: 408.14779; Found: 408.14652.

4.2.7.3. 7-[3-(Aminomethyl)-4-(ethoxyimino)pyrrolidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d3****.* The title compound **9d3** was obtained from **8d** and 4-aminopyrrolidin-3-one O-ethyl oxime dimesylate as an off-white solid (52.6%). $[\alpha]_D^{20} = +27.30$ (c 0.044, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.70 (1H, s, C_2–H), 8.04 (1H, d, $J = 12.0$ Hz, C_5–H), 5.18 (1H, d, $J = 65.2$ Hz, fluorocyclopropyl CH), 4.57 (2H, s), 4.23 (1H, m), 4.10 (2H, q, $J = 8$ Hz, O–CH_2CH_3), 3.88–3.76 (2H, m), 3.08 (1H, s), 2.88–2.75 (2H, m), 1.79–1.64 (2H, m, fluorocyclopropyl CH), 1.21 (3H, t, $J = 8$ Hz, OCH_2–CH_3). MS-ESI (*m*/*z*): 422.18 $(M+H)^+$. HRMS-ESI (*m*/*z*): Calcd. for $C_{19}H_{22}O_4N_4F_2$ $(M+H)^+$: 422.16334; Found: 422.16171.

4.2.7.4. 7-[-3-(Aminomethyl)-4-(benzyloxyimino)pyrrolidin-1-yl]-6-fluoro-1-[(1R, 2S) -2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d4****.* The title compound **9d4** was obtained from **8d** and 4-aminopyrrolidin-3-one O-benzyl oxime dimesylate as a yellow solid (53.7%). $[\alpha]_D^{20} = +22.00$ (c 0.100,

CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, s, C_2–H), 8.05 (1H, d, J = 12.0 Hz, C_5–H), 7.39–7.31 (5H, m, Ar–H), 5.24–5.08 (3H, m), 4.65 (2H, s), 4.19 (1H, m), 3.92–3.75 (2H, m), 3.08 (1H, s), 2.85–2.76 (2H, m), 1.90–1.83 (1H, m, fluorocyclopropyl CH), 1.66–1.58 (1H, m, fluorocyclopropyl CH). ^{13}C NMR (150 MHz, TFA-d_1) δ 202.58, 174.17 (d, J = 83 Hz), 153.13, 140.76, 139.44, 138.82, 137.42, 136.66, 134.96, 133.92, 133.35, 133.26, 133.14, 132.61, 132.37, 113.81, 107.99, 75.01, 73.21, 71.72, 52.89, 42.42 (d, J = 90 Hz), 22.74, 16.67. MS-ESI (m/z): 484.19 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{24}O_4N_4F_2$ $(M+H)^+$: 484.17909; Found: 484.17712.

4.2.7.5. 7-[(S)-2-(Aminomethyl)-3-(methoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)- 2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d5****.* The title compound wasobtained from **8d** and (S)-2-(aminomethyl)azetidin-3-one O-methyl oxime dihydrochloride as an off-white solid (72.1%). $[\alpha]_D^{20}$ = +67.02 (c 0.094, CH_3OH), ^1H NMR (400 MHz, $CDCl_3$) δ 8.75 (1H, s, C_2–H), 8.10 (1H, d, J = 12.0 Hz, C_5–H), 5.43 (1H, m), 5.04–4.87 (3H, m), 3.96 (3H, s, O–CH_3) 3.57 (1H, m), 3.45–3.37 (2H, m), 1.73–1.64 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 394.21 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{17}H_{17}O_4N_5F_2$ $(M+H)^+$: 394.06339; Found: 394.06296.

4.2.7.6. 7-[(S)-2-(Aminomethyl)-3-(ethoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2- fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d6****.* The title compound **9d6** was obtained from **8d** and (S)-2-(aminomethyl)azetidin-3-one O-ethyl oxime dihydrochloride as an off-white solid (65.3%). $[\alpha]_D^{20}$ = +75.80 (c 0.066, CH_3OH), ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, s, C_2–H), 8.12 (1H, d, J = 12.0 Hz, C_5–H), 5.49–5.39 (1H, m), 5.05–4.90 (3H, m), 4.20 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.56 (1H, s), 3.42–3.37 (2H, m), 1.74–1.64 (2H, m, fluorocyclopropyl CH), 1.28 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 408.19 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 408.14779; Found: 408.14632.

4.2.7.7. 7-[(S)-2-(Aminomethyl)-3-(benzyloxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d7****.* The title compound **9d7** was obtained from **8d** and (S)-2-(aminomethyl)azetidin-3-one O-benzyl oxime dihydrochloride as an off-white solid (53.7%). $[\alpha]_D^{20}$ = +91.02 (c 0.078, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.72 (1H, s, C_2–H), 8.11 (1H, d, J = 12.0 Hz, C_5–H), 7.39–7.32 (5H, m, Ar–H), 5.42 (1H, m), 5.14–5.02 (5H, m), 3.71 (1H, m), 3.29–3.08 (2H, m), 1.91–1.59 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 470.23 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{23}O_4N_4F_2$ $(M+H)^+$: 470.16334; Found: 470.16328.

4.2.7.8. 7-[(R)-2-(Aminomethyl)-3-(methoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d8****.* The title compound **9d8** was obtained from **8d** and (R)-2-(aminomethyl)azetidin-3-one O-methyl oxime dihydrochloride as an off-white solid (63.2%). $[\alpha]_D^{20}$ = +20.00 (c 0.070, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.76 (1H, s, C_2–H), 8.17 (1H, d, J = 12.0 Hz, C_5–H), 5.58 (1H, m), 5.13–4.85 (3H, m), 3.87 (3H, s, O–CH_3) 3.76 (1H, m), 3.42–3.34 (2H, m), 1.93–1.62 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 394.16 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{17}H_{17}O_4N_5F_2$ $(M+H)^+$: 394.13214; Found: 394.13094.

4.2.7.9. 7-[(R)-2-(Aminomethyl)-3-(ethoxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d9****.* The title compound **9d9** was obtained from **8d** and (R)-2-(aminomethyl)azetidin-3-one O-ethyl oxime dihydrochloride as an off-white solid (66.8%). $[\alpha]_D^{20}$ = +3.66 (c 0.082, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.73 (1H, s, C_2–H), 8.11 (1H, d, J = 12.0 Hz, C_5–H), 5.48–5.39 (1H, m), 5.15–5.05 (3H, m), 4.10 (2H, q, J = 8.0 Hz, O–CH_2CH_3), 3.72 (1H, s), 3.32–3.16 (2H, m), 1.90–1.62 (2H, m, fluorocyclopropyl CH), 1.22 (3H, t, J = 8.0 Hz, OCH_2–CH_3). MS-ESI (m/z): 408.20 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{19}H_{21}O_4N_4F_2$ $(M+H)^+$: 408.14779; Found: 408.14960.

4.2.7.10. 7-[(R)-2-(Aminomethyl)-3-(benzyloxyimino)azetidin-1-yl]-6-fluoro-1-[(1R, 2S)-2-fluorocyclopropyl]-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***9d10****.* The title compound **9d10** was obtained from **8d** and (R)-2-(aminomethyl)azetidin-3-one O-benzyl oxime dihydrochloride as an off-white solid (52.6%). $[\alpha]_D^{20}$ = −5.41 (c 0.074, CH_3OH), ^1H NMR (400 MHz, DMSO-d_6) δ 8.75 (1H, s, C_2–H), 8.14 (1H, d, J = 12.0 Hz, C_5–H), 7.48–7.33 (5H, m, Ar–H), 5.51 (1H, m), 5.25–4.98 (5H, m), 3.74 (1H, m), 3.25–3.03 (2H), 1.91–1.55 (2H, m, fluorocyclopropyl CH). MS-ESI (m/z): 470.18 $(M+H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{23}O_4N_4F_2$ $(M+H)^+$: 470.16334; Found: 470.16145.

4.3. MIC determination

All compounds were screened for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains, by means of standard twofold serial dilution method using agar media [32]. Minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth after incubation at 35 °C for 18–24 h.

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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.2014.09.029.

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