European Journal of Medicinal Chemistry 104 (2015) 73–85

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis, antimycobacterial and antibacterial activity of fluoroquinolone derivatives containing an 3-alkoxyimino-4-(cyclopropylanimo)methylpyrrolidine moiety

Tingting Zhang [a, b, 1], Weiyi Shen [b, 1], Mingliang Liu [a, *], Rui Zhang [a], Minghua Wang [a], Linhu Li [a], Bin Wang [c], Huiyuan Guo [a], Yu Lu [c, **]

[a] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[b] Zhejiang Starry Pharmaceutical Co. Ltd., Xianju 317300, China
[c] Beijing Key Laboratory of Drug Resistance Tuberculosis Research, Department of Pharmacology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Capital Medical University, Beijing 101149, China

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
15 September 2015
Accepted 24 September 2015
Available online 30 September 2015

Keywords:
Fluoroquinolone derivatives
Synthesis
Antimycobacterial activity
Antibacterial activity

ABSTRACT

A series of novel fluoroquinolone derivatives containing an 3-alkoxyimino-4-(cyclopropylanimo)methylpyrrolidine moiety were designed, synthesized and evaluated for their biological activity. Our results revealed that **19b2** shows good activity against MTB H37Rv ATCC 27294 (MIC: <0.25 μg/mL) and MDR-MTB 6133 clinical isolate (MIC: 0.11 μg/mL). Most of them have potent potency against Gram-positive strains, although they are generally poor active against Gram-negative strains. Especially, compounds **22b1** and **23a3** (MICs: <0.008–8 μg/mL) were found to 2–128 times more potent than ciprofloxacin and levofloxacin against all of the tested Gram-positive strains including quinolone-resistant MRSA, MRSE, *Enterococcus faecium* and *Enterococcus faecalis*.

1. Introduction

Fluoroquinolones (FQs) represent an extremely successful family of antibiotics that have a broad spectrum of antibacterial activity and relatively few side effects [1]. Targeting two type II bacterial topoisomerase enzymes, DNA gyrase and topoisomerase IV, FQs are used mainly to fight community-acquired and serious hospital-acquired infections [2]. Among of them, 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), currently in Phase III clinical trials [4], show particularly strong in vitro and in vivo activity against *Mycobacterium tuberculosis* (MTB) and MDR-MTB [5,6].

However, FQ resistance increases in almost all Gram-negative and Gram-positive species as well as MTB, due mainly to the high level of use and to some degree of abuse [7,8]. The ideal strategy to such challenges is to find novel agents that inhibit new targets in pathogens, but it now remains extremely difficult. A more practical approach is to modify the structures of existing antibacterial agents to increase potency and to overcome resistance [9].

From the chemical structural point of view, FQs consist of a 4-quinolone/naphthyridone-3-carboxylic acid core and a secondary amino group attached to the C-7 position of the heterocyclic core (Fig. 1). CPFX, GTFX, LVFX and gemifloxacin (GMFX) represent the most common cores of important FQs on the market. Some new FQs, such as sitafloxacin (STFX), delafloxacin (DLFX) and AM-1954 have novel cores which are different from the traditional ones. On the other hand, the basic substituent at C-7 position, playing an important role in the antibacterial potency, spectrum and safety of FQs [10], 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 FQs [11].

Recently, methyloxime-functionalized pyrrolidines as novel C-7 substituents have attracted great attention and led to the discovery

* Corresponding author.
** Corresponding author.
E-mail addresses: lmllyx@126.com (M. Liu), luyu4876@hotmail.com (Y. Lu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2015.09.030
0223-5234/

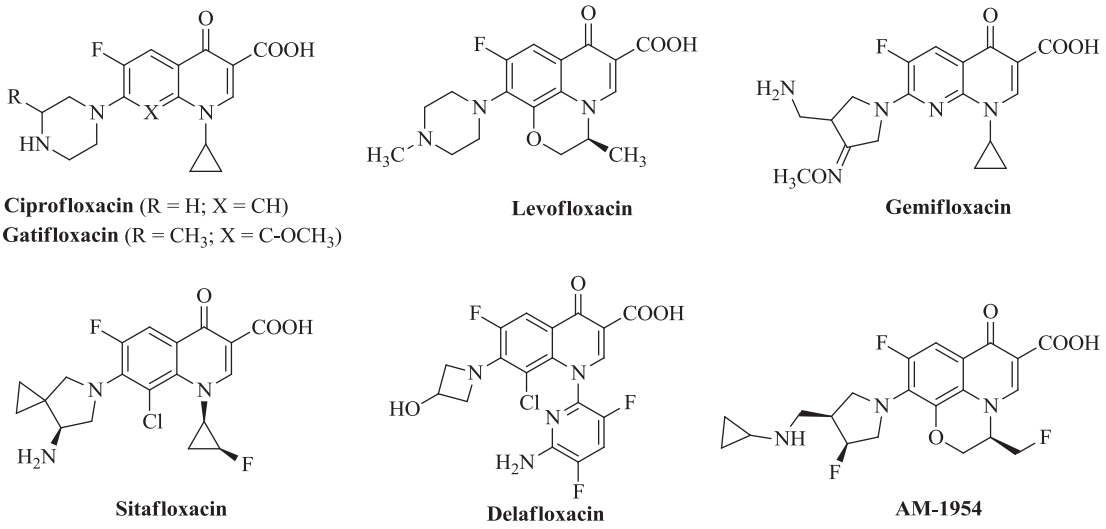


Fig. 1. Structures of some fluoroquinolones.

of new FQs (GMFX, zabofloxacin and DW286) [12–14]. In our previous works, some FQs containing oxime-functionalized azetidines, pyrrolidines or piperidines were found to have considerable biological activity [1,15–18]. These studies suggest the importance of the oxime functional group with respect to biological activity and pharmacokinetic profiles of FQs. Therefore, we intended to apply this modification strategy to the C-7 substituent of AM-1954 (Fig. 1) which has good in vitro activity against MDR-Gram-positive organisms [19]. More specifically, introduction of an oxime group of GMFX (and an alkyl group) instead of the fluorine atom on the pyrrolidine ring of AM-1954 develops 3-alkoxyimino-4-(cyclopropylanimo)methylpyrrolidine and 3-alkoxyimino-4-(cyclopropylanimo)methylpyrrolidine (Fig. 2) as new side chains at the 7-position of FQs. A series of novel FQ derivatives were designed and synthesized by condensation of the side chains with the traditional and new FQ cores. 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 structure-activity relationship (SAR) study was also explored to facilitate the further development of FQs.

2. Results and discussion

2.1. Chemistry

Detailed synthetic pathways to pyrrolidine derivatives **11a**,**b** and target compounds **19**–**25** are depicted in Schemes 1 and 2, respectively. Reduction of readily available pyrrolidones (**1a**,**b**) [20,21] with $NaBH_4$ in methanol gave alcohols (**2a**,**b**), which upon hydroxyl protection by treatment with 3,4-dihydro-2H-pyran (DHP) in the presence of 4-methylbenzenesulfonic acid (p-TsOH) yielded esters (**3a**,**b**). Aldehydes (**5a**,**b**) were prepared via reduction of the esters **3a**,**b** with $LiAlH_4$ in tetrahydrofuran (THF) and then oxidation of the resulting alcohols (**4a**,**b**) with Dess-Martin Periodinane (DMP). Condensation of **5a**,**b** and cyclopropanamine produced secondary amines (**6a**,**b**), which were protected by treatment with di-tert-butyl dicarbonate (Boc_2O) and then deprotection of the hydroxyl groups gave the desired bis-Boc-protected amino alcohols (**8a**,**b**). Oxidation of compounds **8a**,**b** by DMP afforded the corresponding ketones (**9a**,**b**), on which the oxime function groups were introduced via condensation with alkoxylamines to yield amines (**10a**,**b**). The bis-Boc-protecting groups of **10a**,**b** were removed by pumping hydrogen chloride gas in methylene chloride to afford the pyrrolidine derivative dihydrochlorides (**11a**,**b**) (Scheme 1).

Finally, the target compounds **19**–**25** were obtained by coupling the new pyrrolidine derivatives **11a**,**b** with various compounds containing quinolone and naphthyridone cores according to well-established literature procedures (Scheme 2) [22]. In the case of naphthyridones **19**–**21**, direct condensation of **11a**,**b** with **12**–**14** was performed in the presence of triethylamine. However for quinolones **22**–**25**, boric chelates **15**–**18** were required to increase reactivity. All of the synthetic compounds were well characterized through the spectral characteristics.

2.2. Anti-MTB activity

Fifteen of the target compounds were selected initially for evaluation their in vitro activity against MTB H37Rv ATCC 27294 (MTB-1) using the Microplate Alamar Blue Assay (MABA) [23,24]. 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 these compounds along with isoniazid (INH) and rifampicin (RFP) for comparison are presented in Table 1. The data reveal that

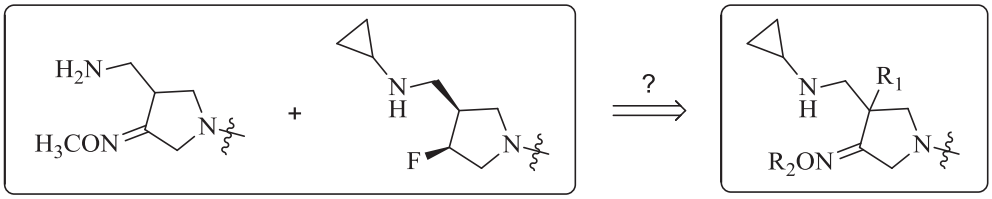
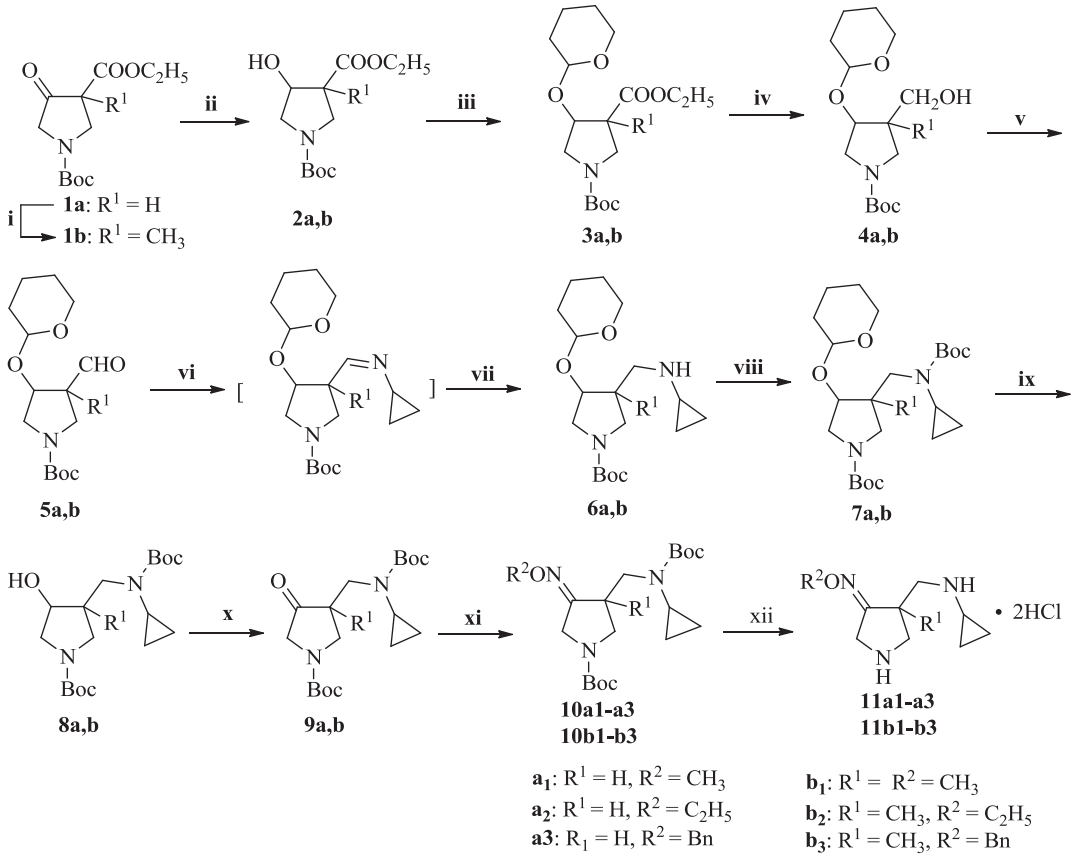
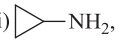
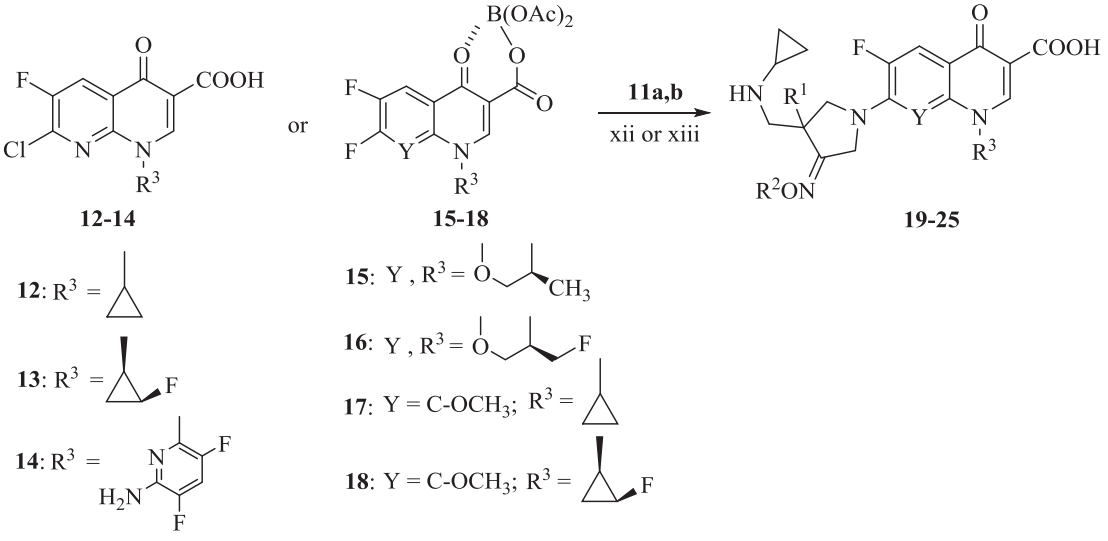


Fig. 2. Design of the new pyrrolidyl side chains.



Reagents and conditions: (i) MeI, K_2CO_3, Me_2O; (ii) $NaBH_4$, MeOH; (iii) DHP, p-TsOH, CH_2Cl_2; (iv) $LiAlH_4$, THF; (v) DMP, CH_2Cl_2; (vi)  , CH_2ClCH_2Cl; (vii) $NaBH(OAc)_3$, CH_2ClCH_2Cl; (viii) Boc_2O, MeOH; (ix) $MeSO_3H$, MeOH; (x) DMP, CH_2Cl_2; (xi) $RONH_2$·HCl, NaOH, MeOH; (xii) HCl (gas), CH_2Cl_2

Scheme 1. Synthesis of pyrrolidine derivatives **11a,b**.



Reagents and conditions: (xii) Et_3N, CH_3CN, 50 °C for **12-14**; (xiii) 1) Et_3N, CH_3CN, 50 °C, 2) 5% HOAc, 3) $NH_3 \cdot H_2O$ for **15-18**

Scheme 2. Synthesis of target compounds **19**–**25**.

six compounds (**19a1**, **19b2**, **20a2**, **20b1**, **20b2**, **22b1**) have considerable activity against MTB-1 (MICs: <0.25–0.72 μg/mL). The most active **19b2** was chosen for further evaluation its activity against MDR-MTB 6133 (MTB-2) clinical isolate which is resistant to both of INH and RFP, and it was found to have good activity against MTB-2 (MIC: 0.11 μg/mL). Although it is generally believed that simply increasing the lipophilicity could improve the anti-MTB and antibacterial activity of FQs [25], our results suggest that the lipophilicity of these compounds 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 (**20a1** *vs* **20a2** *vs* **20a3**, **21b1** *vs* **21b2** *vs* **21b3**).

2.3. Antibacterial activity

The target compounds **19**–**25** were evaluated for their in vitro antibacterial activity against representative strains using standard techniques [26]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth, and MIC values of **19**–**25** against Gram-negative and Gram-positive strains along with CPFX and LVFX for comparison, are listed in Tables 2 and 3, respectively. These data suggest that most of the target compounds **19**–**25** have considerable potency against Gram-positive strains, although they are generally less active than CPFX and LVFX against the Gram-negative strains. For example, these target compounds except for **19a**, **21a**,**b**, **22a1** and **24a1**, show good activity (MICs: <0.008–32 μg/mL), against all of the tested seventeen Gram-positive strains including methicillin-resistant *Staphylococcus aureus* (MRSA, two strains) and methicillin-resistant *Staphylococcus epidermidis* (MRSE, four strains). Among of them, compounds **22b1** and **23a3** (MICs: <0.008–8 μg/mL) were found to 2–128 times more potent than the two reference drugs CPFX and LVFX (MIC: 0.125 → 128 μg/mL) against all of the tested Gram-positive strains including both of CPFX- and LVFX-resistant MRSA, MRSE, *Enterococcus faecium* and *Enterococcus faecalis*.

In the case of Gram-positive strains, the activity of some groups at the N-1 position of naphthyridone derivatives (**19**–**21**) is in the order (1*R*, 2*S*)-2-fluorocyclopropyl ≥ cyclopropyl >> 6-amino-3,5-difluoro-2-pyridinyl against S.a. and MSSA1-3. In the series of FQ derivatives (**22**–**25**) with same group at the C-7 position, the contribution of the FQ nucleus to the activity is as follows: AM-1594 nucleus ≥ LVFX nucleus (**22a3** *vs* **23a3**) ≥ GTFX nucleus (**22a1** *vs* **24a1**), but LVFX nucleus ≥ AM-1594 nucleus (**22b1** *vs* **23b1**) when R_2 = Bn. On the other hand, introduction of a methyl group at the 3-position of the pyrrolidine ring can increase Gram-positive antibacterial activity (**19a** *vs* **19b**, **20a** *vs* **20b**, **21a** *vs* **21b**, **22a1** *vs* **22b1**), which is consistent with the SAR in Yun's study [27]. In addition, naphthyridone derivatives (**19**–**21**) featuring a methyloxime-incorporated pyrrolidino-substitution at C-7 position are comparable to corresponding analogs containing an ethyloxime or a benzyloxime with a few exceptions.

3. Conclusion

In summary, a series of novel FQ derivatives containing an 3-alkoxyimino-4-(cyclopropylanimo)methylpyrrolidine moiety were designed, synthesized and evaluated for their biological activity. Our results revealed that compound **19b2** shows good activity against MTB H37Rv ATCC 27294 (MIC: <0.25 μg/mL) and MDR-MTB 6133 (MIC: 0.11 μg/mL). On the other hand, most of the target compounds **19**–**25** have potent potency against Gram-positive strains (MICs: <0.008–32 μg/mL) with a few exceptions, although they are generally poor active against Gram-negative strains. Notedly, **22b1** and **23a3** shows useful activity (MICs: <0.008–8 μg/mL) against all of the tested Gram-positive strains including both of CPFX- and LVFX-resistant MRSA, MRSE, *E. faecium* and *E. faecalis*. However, our results suggested 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. ^{1}H 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) 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. *tert*-Butyl 3-ethyl 3-methyl-4-oxopyrrolidine-1,3-dicarboxylate ***1b***

To a stirring solution of **1a** (43.53 g, 169.2 mmol) dissolved in anhydrous acetone (846 mL) was added potassium carbonate (70.14 g, 507.6 mmol). Iodomethane (21.08 mL, 338.4 mmol) was added dropwise at 0 °C for about 28 min. After the addition was complete, the reaction mixture was stirred at the same temperature for 0.5 h and then allowed to stir for another 3 h at room temperature, and then filtered. The filtrate was concentrated under reduce pressure at below 35 °C to give a yellow oil. The residue was dissolved with dichloromethane (600 mL), and washed with water (100 mL × 3), brine (80 mL × 2), dried over anhydrous sodium sulfate, and evaporated under vacuo. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 5: 1) to afford the title compound **1b** (26.67 g, 58.1%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.28 (d, J = 11.8 Hz, 1H), 4.19 (qd, J = 7.1, 1.5 Hz, 2H), 4.05 (t, J = 19.7 Hz, 1H), 3.81 (d, J = 19.4 Hz, 1H), 3.47 (d, J = 11.8 Hz, 1H), 1.49 (s, 9H), 1.41 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H). MS-ESI (*m/z*): 289.13 $(M + NH_4)^+$.

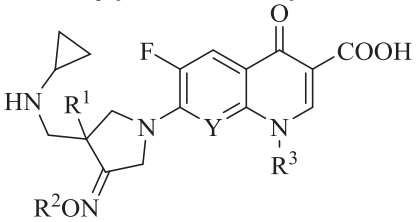
4.2.2. *tert*-Butyl 3-ethyl 4-hydroxypyrrolidine-1,3-dicarboxylate ***2a***

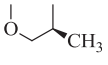
To a suspension of $NaBH_4$ (2.74 g, 72.3 mmol) in anhydrous methanol (290 mL) was added **1a** (37.19 g, 144.6 mmol) at below 10 °C. The reaction mixture was stirred at the same temperature for 50 min, and then 20% aqueous acetic acid was added dropwise to adjust pH 7.0 at 0 °C, and then concentrated under reduced pressure. The residue was dissolved with ethyl acetate (400 mL), and washed with water (50 mL × 3), brine (40 mL × 2), dried over anhydrous sodium sulfate, and evaporated under vacuo to afford the title compound **2a** (37.11 g, 98.9%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.53–4.42 (m, 1H), 4.16–4.08 (m, 2H), 3.36–3.60 (m, 2H), 3.43 (brs, 1H), 3.28–3.25 (brs, 2H), 3.01–2.97 (brs, 1H), 1.42 (s, 9H), 1.28–1.21 (m, 3H). MS-ESI (*m/z*): 282.75 $(M + Na)^+$.

4.2.3. *tert*-Butyl 3-ethyl 4-hydroxy-3-methylpyrrolidine-1,3-dicarboxylate ***2b***

The title compound **2b** (60.2%) was obtained from **1b** in a similar manner as for the preparation of **2a** as a white solid, mp: 68.8–70.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.50 (t, J = 6.3 Hz, 1H), 4.22–4.16 (m, 3H), 3.74–3.64 (m, 1H), 3.59 (s, 1H), 3.40–3.22 (m, 2H), 1.45 (d, J = 3.8 Hz, 9H), 1.31–1.28 (m, 3H), 1.25 (d, J = 7.3 Hz, 3H). MS-ESI (*m/z*): 296.16 $(M + Na)^+$.

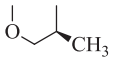
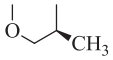
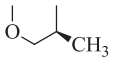
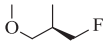
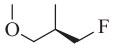
Table 1
Structures, physical data and antimycobacterial activity of compounds **19**–**25**.



Compd.	R^1	R^2	R^3	Y	Mp [°C][a]	$[\alpha]_D^{20}$ (c, CH_3OH)[b]	Clog P[c]	MIC (μg/mL)	
								MTB-1	MTB-2
19a1	H	CH_3		N	190–192		−0.38	0.44	
19a2	H	C_2H_5		N	156–157		0.15		
19a3	H	Bn		N	160–161		1.25		
19b1	CH_3	CH_3		N	193–195		0.14		
19b2	CH_3	C_2H_5		N	182–184		0.67	<0.25	0.11
19b3	CH_3	Bn		N	127–128		1.77		
20a1	H	CH_3		N	136–139	NT	−0.15	1.25	
20a2	H	C_2H_5		N	151–153	41.176° (0.034)	0.38	0.72	
20a3	H	Bn		N	112–114	NT	1.48	2.10	
20b1	CH_3	CH_3		N	150-152	40.698° (0.086)	0.37	0.45	
20b2	CH_3	C_2H_5		N	165–166	NT	0.90	0.60	
20b3	CH_3	Bn		N	>300	NT	1.99		
21a1	H	CH_3		N	132–133		−0.45	24.26	
21a2	H	C_2H_5		N	141–142		0.07	22.00	
21a3	H	Bn		N	161–162		1.17	>32	
21b1	CH_3	CH_3		N	155–157		0.06	15.63	
21b2	CH_3	C_2H_5		N	120–121		0.59	27.74	
21b3	CH_3	Bn		N	125–126		1.69	15.98	
22a1	H	CH_3			287–288	NT	0.20		

(*continued on next page*)

Table 1 (*continued*)

Compd.	R^1	R^2	R^3	Y	Mp [°C][a]	$[\alpha]_D^{20}$ (c, CH_3OH)[b]	Clog P[c]	MIC (μg/mL)	
								MTB-1	MTB-2
22a3	H	Bn			149–150	NT	1.83		
22b1	CH_3	CH_3			185–186	NT	0.72	0.64	
22b2	CH_3	C_2H_5			186-187	−66.667° (0.054)	1.25	2.76	
23a3	H	Bn			142–143	NT	1.70		
23b1	CH_3	CH_3			189–190	NT	0.59		
24a1	H	CH_3		C–OCH_3	116–118		0.38	>32	
25a2	H	C_2H_5		C–OCH_3	168–170	NT	1.13		
INH								0.05	4
RFP								0.05	>40

[a] Melting points are uncorrected.
[b] NT: not tested due to their limited solubilities or/and poor optical activities.
[c] The Clog P is calculated by Chemoffice 2010 software; INH: Isoniazid; RFP: Rifampicin; MTB-1: MTB H37Rv ATCC 27294; MTB-2: MDR-MTB 6133 resistant to INH and RFP.

4.2.4. *tert*-Butyl 3-ethyl 4-((tetrahydro-2H-pyran-2-yl)oxy) pyrrolidine-1,3-dicarboxylate ***3a***

To a solution of **2a** (37.11 g, 143.1 mmol) in dichloromethane (150 mL) was added p-toluenesulfonic acid monohydrate (1.36 g, 7.1 mmol) at 0 °C. 3,4-2*H*-dihydropyran (26.1 mL, 286.1 mmol) was added dropwise at the same temperature for about 40 min. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 10% aqueous sodium carbonate solution (30 mL). The organic phase was washed with water (50 mL × 3) and brine (40 mL × 2), dried over anhydrous sodium

Table 2
In vitro antibacterial activity of compounds **19**–**25** against Gram-negative strains.

Compd	Strains MIC (μg/mL)															
	E. coli	E.co.1	E.co.2	E.co.3	E.co.4	K.p.1	K.p.2	K.p.3	K.p.4	K.p.5	K.p.6	K.p.7	P.a.	P.a.1	P.a.2	P.a.3	P.a.4
19a1	0.5	16	>128	>128	>128	16	16	32	128	128	128	>128	16	8	32	32	32
19a2	2	8	128	128	128	8	8	8	32	32	128	128	8	8	32	32	16
19a3	4	32	>128	>128	>128	128	128	128	64	64	128	>128	64	64	64	128	64
19b1	0.5	32	>128	>128	>128	64	64	64	64	128	>128	>128	32	16	64	64	64
19b2	1	32	>128	>128	>128	64	64	64	64	64	128	>128	32	32	128	64	32
19b3	1	32	>128	>128	>128	128	128	128	64	64	>128	>128	64	64	128	128	128
20a1	4	16	>128	>128	>128	32	32	64	64	64	>128	>128	8	8	32	64	32
20a2	0.03	16	128	128	>128	32	32	64	32	32	128	128	16	8	32	32	32
20a3	0.25	16	>128	>128	>128	64	64	128	128	64	128	>128	32	8	64	64	64
20b1	1	16	>128	>128	>128	64	64	32	32	64	128	>128	16	16	64	64	32
20b2	2	32	>128	>128	>128	64	128	64	64	128	128	>128	32	32	128	64	64
20b3	4	32	>64	>64	>64	64	64	>64	64	64	64	>64	64	32	64	64	64
21a1	4	16	>128	>128	>128	64	64	64	32	32	>128	>128	16	32	128	128	64
21a2	4	32	>128	>128	>128	128	128	128	64	64	128	>128	32	32	128	128	128
21a3	8	64	>128	>128	>128	128	>128	128	64	128	>128	>128	128	128	128	128	128
21b1	16	128	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	128	128	>128	128	>128
21b2	32	64	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	128	64	128	128	>128
21b3	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	128	128	128	128	>128
22a1	0.5	>64	>64	>64	>64	4	4	8	32	32	>64	>64	64	64	>64	>64	64
22a3	1	2	>64	>64	>64	32	32	64	32	64	64	64	32	16	64	64	32
22b1	2	4	>128	64	64	16	16	32	16	16	128	>128	16	8	32	32	32
22b2	2	8	>128	>128	>128	32	32	32	64	64	>128	>128	64	64	128	64	64
23a3	1	4	>64	>64	>64	64	32	32	64	32	64	>64	32	32	64	64	32
23b1	2	8	>128	>128	>128	32	32	>128	>128	>128	128	128	32	32	128	128	64
24a1	32	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128
25a2	2	4	>32	>32	>32	32	32	>32	>32	>32	>32	>32	16	32	32	32	32
LVFX	0.125	4	8	8	8	0.5	0.5	64	16	8	32	16	1	0.5	2	2	2
CPFX	<0.008	8	32	8	8	0.5	0.5	64	32	16	>128	32	0.5	0.5	1	1	1

E. coli: *E. coli* ATCC 25922. E.co.1: Extended-spectrum β-lactamase-producing ($ESBL^+$) *E. coli* 14-1. E.co.2: $ESBL^+$ *E. coli* 14-2. E.co.3: *E. coli* 14-1. E.co.4: *E. coli* 14-2. K.p.1: $ESBL^+$ *K. pneumoniae* 14–17. K.p.2: $ESBL^+$ *K. pneumoniae* 14–18. K.p.3: $ESBL^+$ *K. pneumoniae* 14–19. K.p.4: *K. pneumoniae* 14-1. K.p.5: *K. pneumoniae* 14-2. K.p.6: *K. pneumoniae* 14-3. K.p.7: *K. pneumoniae* 14-4. P.a.: *P. aeruginosa* ATCC 27853. P.a.1: *P. aeruginosa* 14-9. P.a.2: *P. aeruginosa* 14-14. P.a.3: *P. aeruginosa* 14–15. P.a.4: *P. aeruginosa* 14–16. LVFX: Levofloxacin. CPFX: Ciprofloxacin.

Table 3
In vitro antibacterial activity of compounds **19**–**25** against Gram-positive strains.

Compd	Strains MIC (μg/mL)															
	S.a.	MSSA1	MSSA2	MSSA3	MRSA1	MRSA2	MSSE1	MRSE1	MRSE 2	MRSE3	MRSE4	S.p.	E.fm. 1	E.fm. 2	E.fm. 3	E. fs.1	E. fs.2
19a1	<0.008	8	4	8	>128	8	>128	16	16	32	16	4	32	16	32	64	0.5
19a2	<0.008	8	8	8	128	8	128	16	16	32	16	2	32	16	8	64	0.125
19a3	<0.008	8	4	8	>128	16	64	16	16	16	32	8	16	32	8	64	1
19b1	<0.008	0.5	0.25	0.5	4	4	16	4	2	4	2	0.25	4	4	2	16	0.5
19b2	<0.008	0.25	0.5	0.25	4	4	8	4	4	2	4	0.25	4	8	4	16	0.125
19b3	<0.008	2	0.5	0.5	2	2	16	8	4	2	2	0.5	4	2	4	64	0.25
20a1	<0.008	8	8	16	>128	8	>128	32	16	64	32	4	16	32	64	128	0.5
20a2	<0.008	4	4	8	16	8	64	16	8	16	32	1	4	16	32	32	0.125
20a3	<0.008	4	4	16	32	8	32	16	8	16	64	1	8	16	32	64	1
20b1	<0.008	1	1	2	4	2	16	4	4	2	4	0.5	4	4	2	16	1
20b2	0.015	1	2	1	4	2	16	4	4	2	4	0.25	4	2	4	16	1
20b3	0.125	8	2	8	16	16	64	16	16	16	32	1	16	32	32	64	2
21a1	0.25	16	16	16	64	16	128	64	32	64	16	32	32	16	32	16	4
21a2	0.125	16	32	32	64	8	>128	128	32	64	64	16	64	32	128	128	4
21a3	0.015	16	32	32	64	8	128	64	128	128	64	16	64	64	128	64	8
21b1	0.25	16	16	8	32	32	128	128	32	64	128	4	64	128	64	128	8
21b2	0.5	32	16	4	64	64	128	64	128	128	64	16	128	128	64	64	8
21b3	1	16	8	4	16	32	64	32	128	64	128	8	32	>128	128	32	16
22a1	4	64	16	16	>64	64	>64	>64	>64	64	>64	8	>64	64	>128	>64	16
22a3	<0.008	2	0.125	0.06	4	4	8	8	4	2	4	0.125	4	8	16	16	0.5
22b1	<0.008	2	0.015	0.015	4	4	8	4	2	2	2	0.5	4	4	8	8	0.5
22b2	<0.008	4	0.03	0.06	64	16	32	8	8	4	8	0.25	16	4	16	32	1
23a3	<0.008	1	0.015	0.03	2	4	8	2	2	2	2	0.125	1	8	8	1	0.25
23b1	<0.008	4	0.03	0.03	8	4	16	16	4	16	2	0.25	8	4	16	16	0.5
24a1	8	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	>128	>128	>128	64
25a2	0.06	8	0.03	0.06	8	16	16	8	8	8	4	0.25	8	4	16	32	2
LVFX	0.25	8	0.125	0.5	64	8	>128	64	32	64	64	0.5	32	64	64	128	1
CPFX	0.5	32	0.125	0.5	>128	16	>128	64	128	128	128	2	32	128	>128	64	1

S.a.: *S. aureus* CMCC 26003. MSSA1: Methicillin-sensitive *S. aureus* 14-1. MSSA2: Methicillin-sensitive *S. aureus* 14-3. MSSA3: Methicillin-sensitive *S. aureus* 14-4. MRSA1: Methicillin-resistant *S. aureus* 14-4. MRSA2: Methicillin-resistant *S. aureus* 14-5. MSSE1: Methicillin-sensitive *S. epidermidis* 14-2. MRSE1: Methicillin-resistant *S. epidermidis* 14-21. MRSE2: Methicillin-resistant *S. epidermidis* 14-22. MRSE3: Methicillin-resistant *S. epidermidis* 14-37. MRSE4: Methicillin-resistant *S. epidermidis* 14-39. S.p.: *S. pneumoniae* ATCC 19615. E. fm. 1: *E. faecium* 14-2. E. fm. 2: *E. faecium* 14-5. E. fm. 3: *E. faecium* 14-6. E. fs. 1: *E. faecalis* 14-1. E. fs. 2: *E. faecalis* 14-2. LVFX: Levofloxacin. CPFX: Ciprofloxacin.

sulfate, and filtered. The filtrate was concentrated under reduce pressure. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 6: 1) to give the crude product which was recrystallized from petroleum ether (about 180 mL) to give the title compound **3a** (26.85 g, 54.6%) as a white solid, mp: 89.4–90.4 °C. The mother liquor was then concentrated to afford an additional 21.20 g (43.2%) of the material. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.73–4.47 (m, 1H), 4.24–4.15 (m, 2H), 3.85–3.52 (m, 4H), 3.15–2.99 (m, 1H), 1.66–1.50 (m, 9H), 1.47 (s, 9H), 1.31–1.25 (m, 3H). MS-ESI (*m/z*): 366.24 $(M + Na)^+$.

4.2.5. *tert*-Butyl 3-ethyl 3-methyl-4-((tetrahydro-2H-pyran-2-yl) oxy)pyrrolidine-1,3-dicarboxylate ***3b***

The title compound **3b** (60.4%) was obtained from **2b** in a similar manner as for the preparation of **3a** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.60 (d, J = 18.3 Hz, 1H), 4.44 (dd, J = 48.5, 4.9 Hz, 1H), 4.07 (q, J = 6.9 Hz, 2H), 3.73 (dd, J = 18.7, 9.2 Hz, 2H), 3.64–3.54 (m, 1H), 3.49–3.41 (m, 1H), 3.32–3.13 (m, 2H), 1.71 (d, J = 5 Hz, 1H), 1.62 (d, J = 10 Hz, 1H), 1.49–1.44 (m, 4H), 1.36 (s, 9H), 1.27–1.17 (m, 6H). MS-ESI (*m/z*): 380.24 $(M + Na)^+$.

4.2.6. *tert*-Butyl 3-(hydroxymethyl)-4-((tetrahydro-2H-pyran-2-yl) oxy)pyrrolidine-1-carboxylate ***4a***

To a solution of tetrahydrofuran (500 mL) was added Lithium aluminum hydride (3.49 g, 92.0 mmol) in portions at 0 °C, and **3a** (26.34 g, 76.7 mmol) in tetrahydrofuran (100 mL) was added dropwise within 20 min. The reaction mixture was stirred at room temperature for 2 h. Water (3.5 mL), 7% aqueous sodium hydroxide solution (3.5 mL), and water (10.5 mL) was added successively until the mixture became too thick to stir at 0 °C. The mixture was filtered and the filter cake was dissolved ethyl acetate (200 mL), washed successively with water (50 mL × 2), brine (30 mL). The organic layer was dried over anhydrous sodium sulfate, and filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 2: 1) to give the title compound **4a** as a colorless oil (16.66 g, 72.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.69–4.50 (m, 1H), 4.23–4.09 (m, 1H), 4.00–3.75 (m, 2H), 3.68–3.61 (m, 2H), 3.53–3.42 (m, 3H), 3.16–3.09 (m, 1H), 2.45–2.40 (brs, 1H), 1.81–1.69 (m, 2H), 1.62–1.54 (m, 4H), 1.45 (s, 9H). MS-ESI (*m/z*): 324.26 $(M + Na)^+$.

4.2.7. *tert*-Butyl 3-(hydroxymethyl)-3-methyl-4-((tetrahydro-2H-pyran-2-yl)oxy)pyrrolidine-1-carboxylate ***4b***

The title compound **4b** (80.6%) was obtained from **3b** in a similar manner as for the preparation of **4a** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.47 (d, J = 8 Hz, 1H), 3.96 (t, J = 10.4 Hz, 2H), 3.86–3.80 (m, 1H), 3.59–3.27 (m, 6H), 3.14–3.03 (m, 1H), 1.78–1.73 (m, 2H), 1.51 (d, J = 4.6 Hz, 4H), 1.44 (s, 9H), 1.07 (s, 3H). MS-ESI (*m/z*): 316.39 $(M + H)^+$.

4.2.8. *tert*-Butyl 3-formyl-4-((tetrahydro-2H-pyran-2-yl)oxy) pyrrolidine-1-carboxylate ***5a***

To a solution of **4a** (16.66 g, 55.3 mmol) and in dichloromethane (150 mL) was added Dess-Martin periodinane (30.47 g, 71.8 mmol) in portions at room temperature, and then added slowly dichloromethane (1300 mL) containing water (1.3 mL). The clear solution grew cloudy toward the end of wet dichloromethane addition, which required 30 min. The reaction mixture was stirred

at room temperature for 3.5 h and then filtered. The filtrate was washed with 10% $Na_2S_2O_3$: saturated $NaHCO_3$ (300 mL, v: v = 1: l), water (200 mL × 2) and brine (100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the title compound **5a** as a colorless oil (15.08 g, 91.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.86 (s, 1H), 4.84–4.64 (m, 3H), 3.82–3.75 (m, 3H), 3.62–3.51 (m, 2H), 1.77–1.62 (m, 7H), 1.53 (s, 9H). MS-ESI (*m/z*): 322.26 $(M + Na)^+$.

4.2.9. tert-Butyl 3-formyl-3-methyl-4-((tetrahydro-2H-pyran-2-yl) oxy)pyrrolidine-1-carboxylate **5b**

The title compound **5b** (74.9%) was obtained from **4b** in a similar manner as for the preparation of **5a** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.77 (t, *J* = 17.0 Hz, 1H), 4.65 (d, *J* = 5.0 Hz, 1H), 4.30 (d, *J* = 17.8 Hz, 1H), 3.93–3.86 (m, 1H), 3.72 (t, *J* = 9.9 Hz, 1H), 3.59–3.40 (m, 3H), 3.27–3.17 (m, 1H), 1.69 (d, *J* = 11.4 Hz, 2H), 1.54 (d, *J* = 18.4 Hz, 4H), 1.47 (s, 9H), 1.22 (s, 3H). MS-ESI (*m/z*): 314.00 $(M + H)^+$.

4.2.10. tert-Butyl 3-((cyclopropylamino)methyl)-4-((tetrahydro-2H-pyran-2-yl)oxy)pyrrolidine-1-carboxylate **6a**

To a solution of **5a** (15.08 g, 50.4 mmol) and in 1,2-dichloroethane (150 mL) was added cyclopropanamine (3.8 mL, 54.8 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 1–5 h, and then added sodium triacetoxyborohydride solid (12.80 g, 60.4 mmol) in portions at the same temperature. The reaction mixture was stirred at the same temperature for 4 h and then added saturated aqueous sodium bicarbonate solution (160 mL) cautiously. The organic phase was separated and the aqueous phase was extracted with dichloromethane (50 mL × 2). The combined organic phases were washed with brine (40 mL × 3), dried over sodium sulfate, filtered and evaporated, the residue was purified by column chromatography (silica gel) eluted with methanol and dichloromethane (v: v = 1: 5) to give the title compound **6a** as a light yellow oil (15.71 g, 91.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.70 (s, 1H), 4.20 (s, 1H), 3.88–3.85 (brs, 1H), 3.53–3.48 (m, 4H), 3.16–3.12 (m, 1H), 2.91–2.89 (m, 1H), 2.75–2.72 (m, 1H), 2.33 (brs, 1H), 2.13–2.10 (m, 1H), 1.83–1.70 (m, 3H), 1.71–1.58 (m, 4H), 1.53 (s, 9H), 0.45–0.33 (m, 4H). MS-ESI (*m/z*): 341.22 $(M + H)^+$.

4.2.11. tert-Butyl 3-((cyclopropylamino)methyl)-3-methyl-4-((tetrahydro-2H-pyran-2-yl)oxy)pyrrolidine-1-carboxylate **6b**

The title compound **6b** (82.2%) was obtained from **5b** in a similar manner as for the preparation of **6a** as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.66–4.52 (m, 1H), 3.90–3.75 (m, 2H), 3.66–3.55 (m, 1H), 3.51–3.46 (m, 2H), 3.30–3.25 (m, 1H), 3.10–3.02 (m, 1H), 2.85–2.82 (m, 1H), 2.66–2.59 (m, 1H), 2.12–2.07 (m, 1H), 1.78 (d, *J* = 8.9 Hz, 1H), 1.71–1.68 (m, 2H), 1.52 (d, *J* = 4.4 Hz, 3H), 1.44 (s, 9H), 1.24 (s, 1H), 1.03 (d, *J* = 3.6 Hz, 1H), 0.99 (d, *J* = 2.5 Hz, 2H), 0.39 (d, *J* = 5.9 Hz, 2H), 0.26 (d, *J* = 2.3 Hz, 2H). MS-ESI (*m/z*): 355.25 $(M + H)^+$.

4.2.12. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-4-((tetrahydro-2H-pyran-2-yl)oxy)pyrrolidine-1-carboxylate **7a**

To a solution of **6a** (15.71 g, 46.1 mmol) in methanol (150 mL) was added di-tert butyl dicarbonate (10.58 g, 48.5 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 3 h and then evaporated. The remaining yellow oil was dissolved in 100 mL of ethyl acetate, washed with water (30 mL × 2), brine (30 mL), dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 7: 1) to give the title compound **7a** as a light yellow oil (10.57 g, 52.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.68 (m, 1H), 4.10–3.97 (m, 1H), 3.87–3.77 (m, 1H), 3.65–3.38 (m, 2H), 3.34–3.27 (m, 2H), 3.28–3.03 (m, 2H), 2.64 (brs, 1H), 2.58 (brs, 1H), 1.80–1.67 (m, 2H), 1.57–1.53 (m, 5H), 1.45 (s, 18H), 0.87–0.85 (m, 2H), 0.59–0.55 (m, 2H). MS-ESI (*m/z*): 463.28 $(M + Na)^+$.

4.2.13. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-3-methyl-4-((tetrahydro-2H-pyran-2-yl)oxy)pyrrolidine-1-carboxylate **7b**

The title compound **7b** (62.1%) was obtained from **6b** in a similar manner as for the preparation of **7a** as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.63 (d, *J* = 13.9 Hz, 1H), 4.03–3.96 (m, 1H), 3.87–3.77 (m, 2H), 3.59–3.38 (m, 4H), 3.35–3.06 (m, 2H), 2.55–2.43 (m, 1H), 1.79 (d, *J* = 14.8 Hz, 1H), 1.75–1.68 (m, 1H), 1.58–1.53 (m, 4H), 1.47 (d, *J* = 5.0 Hz, 9H), 1.46 (s, 9H), 1.04–0.96 (m, 3H), 0.89–0.79 (m, 1H), 0.74 (s, 1H), 0.62–0.54 (m, 2H). MS-ESI (*m/z*): 477.34 $(M + Na)^+$.

4.2.14. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-4-hydroxypyrrolidine-1-carboxylate **8a**

To a solution of **7a** (1.14 g, 2.6 mmol) in the anhydrous methanol was added methanesulfonic acid (0.19 mL, 2.9 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 25 min and then saturated aqueous sodium bicarbonate solution (about 2 mL) was added to adjust pH to 7.0–8.0. The solvent was evaporated, and the residue was dissolved in 50 mL of ethyl acetate, washed with water (15 mL × 2), brine (20 mL), dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 5 → 3: 1) to give the title compound **8a** as a yellow oil (0.70 g, 75.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.06 (s, 1H), 3.81–3.75 (m, 1H), 3.51–3.44 (m, 3H), 3.07 (t, *J* = 8 Hz, 1H), 2.92–2.88 (m, 1H), 2.52–2.50 (m, 1H), 2.28–2.26 (brs, 1H), 1.56 (s, 1H), 1.48 (s, 9H), 1.45 (s, 9H), 0.86–0.82 (m, 1H), 0.79–0.73 (m, 2H), 0.70–0.54 (m, 1H). MS-ESI (*m/z*): 379.25 $(M + Na)^+$.

4.2.15. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-3-methyl-4-hydroxypyrrolidine-1-carboxylate **8b**

The title compound **8b** (72.6%) was obtained from **7b** in a similar manner as for the preparation of **8a** as a white solid, mp: 108.3–109.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.36 (s, 1H), 3.80 (t, *J* = 15.6 Hz, 1H), 3.71 (s, 1H), 3.57 (ddd, *J* = 28.6, 12.0, 4.1 Hz, 1H), 3.48–3.36 (m, 1H), 3.21–3.13 (m, 1H), 3.05 (d, *J* = 10 Hz, 1H), 2.76 (dd, *J* = 31.2, 14.7 Hz, 1H), 2.63–2.58 (m, 1H), 1.48 (s, 9H), 1.45 (s, 9H), 1.04 (d, *J* = 5.4 Hz, 3H), 0.91–0.85 (m, 1H), 0.79–0.70 (m, 1H), 0.66–0.57 (m, 2H). MS-ESI (*m/z*): 393.42 $(M + Na)^+$.

4.2.16. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-4-oxopyrrolidine-1-carboxylate **9a**

The title compound **9a** was obtained from **8a** and Dess-Martin periodinane in a similar manner as for the preparation of **5a** from **4a**, and then purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 4: 1) to give the title compound **9a** as a yellow oil (72.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.99 (brs, 1H), 3.79–3.74 (m, 2H), 3.63 (brs, 1H), 3.47–3.42 (m, 2H), 2.93–2.90 (m, 1H), 2.47 (s, 1H), 1.47 (s, 18H), 0.76 (brs, 2H), 0.61 (brs, 2H). MS-ESI (*m/z*): 377.36 $(M + Na)^+$.

4.2.17. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-3-methyl-4-oxopyrrolidine-1-carboxylate **9b**

The title compound **9b** (91.4%) was obtained from **8b** in a similar manner as for the preparation of **9a** as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.96–3.59 (m, 4H), 3.44 (d, J = 11.5 Hz, 1H), 3.27 (dd, J = 39.5, 14.1 Hz, 1H), 2.43 (d, J = 30.5 Hz, 1H), 1.48 (s,

9H), 1.46 (s, 9H), 1.18 (s, 3H), 0.89–0.81 (m, 1H), 0.74–0.66 (m, 1H), 0.64–0.59 (m, 1H), 0.55–0.53 (m, 1H). MS-ESI (*m/z*): 391.41 $(M + Na)^+$.

4.2.18. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-4-(methoxyimino)pyrrolidine-1-carboxylate ***10a1***

To a solution of methoxyamine hydrochloride (0.30 g, 3.6 mmol) in the methanol (20 mL) was added sodium hydroxide (0.15 g, 3.7 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 30 min. After the addition of **9a** (1.17 g, 3.3 mmol) was complete, the mixture was heated at reflux for 8 h. The solvent was evaporated, and the residue was dissolved in 20 mL of water. The mixture was extracted with ethyl acetate (40 mL × 3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and evaporated. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 6: 1) to give the title compound **10a1** as a light yellow oil (0.68 g, 53.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.12 (2H, s), 3.88 (3H, s), 3.65–3.53 (1H, m), 3.41 (1H, brs), 3.30–3.23 (1H, m), 2.60 (1H, s), 1.66–1.62 (2H, m), 1.51 (18H, s), 0.82–0.79 (2H, m), 0.71–0.62 (2H, m). MS-ESI (*m/z*): 383.88 $(M + H)^+$.

4.2.19. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-3-methyl-4-(methoxyimino)pyrrolidine-1-carboxylate ***10b1***

The title compound **10b1** (81.5%) was obtained from **9b** and methoxyamine hydrochloride in a similar manner as for the preparation of **10a1** as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.16–4.05 (m, 2H), 3.86 (s, 3H), 3.71 (s, 1H), 3.60 (t, J = 16.1 Hz, 1H), 3.25–3.19 (m, 1H), 3.10 (d, J = 13.9 Hz, 1H), 2.57 (s, 1H), 1.46 (s, 9H), 1.46 (s, 9H), 1.29 (s, 3H), 0.85 (s, 1H), 0.74–0.69 (m, 1H), 0.63–0.58 (m, 1H), 0.55 (d, J = 4.3 Hz, 1H). MS-ESI (*m/z*): 398.11 $(M + H)^+$.

4.2.20. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-4-(ethoxyimino)pyrrolidine-1-carboxylate ***10a2***

The title compound **10a2** (88.7%) was obtained from **9a** and ethoxyamine hydrochloride in a similar manner as for the preparation of **10a1** as an off-white solid, mp: 158.7–160.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.17–4.06 (m, 4H), 3.66–3.19 (m, 4H), 2.55 (s, 1H), 1.69 (s, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.23 (t, J = 6.7 Hz, 3H), 0.87–0.58 (m, 4H). MS-ESI (*m/z*): 398.05 $(M + H)^+$.

4.2.21. tert-Butyl 3-(((tert-butoxycarbonyl) (cyclopropyl)amino) methyl)-3-methyl-4-(ethoxyimino)pyrrolidine-1-carboxylate ***10b2***

The title compound **10b2** (81.5%) was obtained from **9b** and ethoxyamine hydrochloride in a similar manner as for the preparation of **10a1** as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.07 (q, J = 17.7 Hz, 4H), 3.70 (s, 1H), 3.57 (t, J = 15.3 Hz, 1H), 3.20 (dd, J = 23.2, 12.3 Hz, 1H), 3.08 (d, J = 14.3 Hz, 1H), 2.53 (s, 1H), 1.44 (s, 9H), 1.43 (s, 9H), 1.26 (s, 3H), 1.23–1.20 (m, 3H), 0.84–0.76 (m, 1H), 0.70–0.66 (m, 1H), 0.59–0.52 (m, 2H). MS-ESI (*m/z*): 412.05 $(M + H)^+$.

4.2.22. tert-Butyl 3-((benzyloxy)imino)-4-(((tert-butoxycarbonyl) (cyclopropyl)amino)methyl)pyrrolidine-1-carboxylate ***10a3***

The title compound **10a3** (80.6%) was obtained from **9a** and *O*-benzylhydroxylamine hydrochloride in a similar manner as for the preparation of **10a1** as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.37–7.32 (m, 5H), 5.08 (s, 2H), 4.14–4.11 (m, 2H), 3.68–3.61 (m, 1H), 3.50–3.21 (m, 3H), 2.51 (s, 1H), 1.70–1.58 (m, 1H), 1.46 (s, 18H), 0.86–0.57 (m, 4H). MS-ESI (*m/z*): 460.16 $(M + H)^+$.

4.2.23. tert-Butyl 3-((benzyloxy)imino)-3-methyl-4-(((tert-butoxycarbonyl) (cyclopropyl)amino)methyl)pyrrolidine-1-carboxylate ***10b3***

The title compound **10b3** (59.8%) was obtained from **9b** and *O*-benzylhydroxylamine hydrochloride in a similar manner as for the preparation of **10a1** as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.34 (s, 2H), 7.31 (s, 3H), 5.13–5.05 (m, 2H), 4.18–4.08 (m, 2H), 3.70–3.57 (m, 2H), 3.30–3.11 (m, 2H), 2.49–2.43 (m, 1H), 1.46 (s, 18H), 1.26 (d, J = 4.5 Hz, 3H), 0.89–0.77 (m, 2H), 0.63–0.48 (m, 2H). MS-ESI (*m/z*): 474.14 $(M + H)^+$.

4.2.24. 4-((Cyclopropylamino)methyl)pyrrolidin-3-one O-methyl oxime dihydrochloride ***11a1***

To a stirring solution of **10a1** (3.06 g, 8.0 mmol) dissolved in anhydrous dichloromethane (200 mL) was pumped dried hydrochloride gas at room temperature for 2 h. The reaction mixture was stirred for 6 h at the same temperature, and then filtered. The precipitate was washed with anhydrous ethyl acetate (20 mL), and dried in vacuo at 45 °C for 10 h to yield the title compound **11a1** (1.70 g, 83.1%) as an off-white solid, mp: 157.4–158.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O exchanged) δ (ppm): 4.05–3.91 (m, 2H), 3.89 (s, 3H), 3.74–3.70 (m, 1H), 3.38–3.35 (m, 2H), 3.32–3.24 (m, 2H), 2.78–2.71 (m, 1H), 0.86–0.82 (m, 4H). MS-ESI (*m/z*): 184.11 $(M + H)^+$.

4.2.25. 4-((Cyclopropylamino)methyl)-4-methylpyrrolidin-3-one O-methyl oxime dihydrochloride ***11b1***

The title compound **11b1** (92.8%) was obtained from **10b1** in a similar manner as for the preparation of **11a1** as an off-white solid, mp: 117.6–118.9 °C. ^{1}H NMR (500 MHz, DMSO-*d6*) δ (ppm): 4.05–3.98 (m, 2H), 3.87 (s, 3H), 3.70–3.57 (m, 3H), 3.40–3.32 (m, 1H), 2.76 (s, 1H), 1.37 (s, 3H), 1.05–1.01 (m, 2H), 0.75 (d, J = 6.9 Hz, 2H). MS-ESI (*m/z*): 198.10 $(M + H)^+$.

4.2.26. 4-((Cyclopropylamino)methyl)pyrrolidin-3-one O-ethyl oxime dihydrochloride ***11a2***

The title compound **11a2** (83.1%) was obtained from **10a2** in a similar manner as for the preparation of **11a1** as an off-white solid, mp: 180.7–182.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.13 (q, J = 7.0 Hz, 2H), 3.92 (dd, J = 37.7, 17.1 Hz, 2H), 3.74 (dd, J = 10.7, 7.4 Hz, 1H), 3.42 (d, J = 8 Hz, 1H), 3.33–3.23 (m, 3H), 2.75 (s, 1H), 1.22 (t, J = 7.0 Hz, 3H), 0.99–0.68 (m, 4H). MS-ESI (*m/z*): 198.05 $(M + H)^+$.

4.2.27. 4-((Cyclopropylamino)methyl)-4-methylpyrrolidin-3-one O-ethyl oxime dihydrochloride ***11b2***

The title compound **11b2** (68.2%) was obtained from **10b2** in a similar manner as for the preparation of **11a1** as a yellow solid, mp: 146.5–147.3 °C. ^{1}H NMR (500 MHz, DMSO-*d6*) δ (ppm): 4.11 (t, J = 7.0 Hz, 2H), 4.00 (s, 2H), 3.69 (d, J = 11.9 Hz, 1H), 3.41 (s, 1H), 3.33 (d, J = 12.0 Hz, 1H), 3.26 (s, 1H), 2.75 (s, 1H), 1.37 (s, 3H), 1.21 (t, J = 7.0 Hz, 3H), 1.04 (dd, J = 27.6, 8.0 Hz, 2H), 0.78–0.72 (m, 2H). MS-ESI (*m/z*): 212.14 $(M + H)^+$.

4.2.28. 4-((Cyclopropylamino)methyl)pyrrolidin-3-one O-benzyl oxime dihydrochloride ***11a3***

The title compound **11a3** (89.3%) was obtained from **10a3** in a similar manner as for the preparation of **11a1** as an off-white solid, mp: 179.4–180.6 °C. ^{1}H NMR (500 MHz, DMSO-*d6*) δ (ppm): 7.40–7.12 (m, 5H), 5.15 (s, 2H), 3.98 (dd, J = 42.5, 16.9 Hz, 2H), 3.75 (dd, J = 10.9, 7.8 Hz, 1H), 3.41 (dd, J = 32.3, 21.5 Hz, 4H), 2.72 (s, 1H), 0.94 (dd, J = 34.9, 11.2 Hz, 2H), 0.77–0.68 (m, 2H). MS-ESI (*m/z*): 260.70 $(M + H)^+$.

*4.2.29. 4-((Cyclopropylamino)methyl)-4-methylpyrrolidin-3-one O-benzyl oxime dihydrochloride **11b3***

The title compound **11b3** (36.3%) was obtained from **10b3** in a similar manner as for the preparation of **11a1** as a yellow solid easily absorbing moisture. ^{1}H NMR (500 MHz, DMSO-*d6*) δ (ppm): 7.36–7.16 (m, 5H), 5.14–5.14 (m, 2H), 3.91–3.80 (m, 2H), 3.68 (dd, J = 11.1, 8.8 Hz, 1H), 3.23–3.18 (m, 1H), 3.11–2.96 (m, 2H), 2.72 (s, 1H), 1.48–1.25 (m, 3H), 1.08–0.95 (m, 2H), 0.76–0.67 (m, 2H). MS-ESI (*m/z*): 274.18 $(M + H)^+$.

*4.2.30. General procedure for the synthesis of target compounds **19–21***

To a suspension of **11a**,**b** (1.6 mmol) in dry acetonitrile (20 mL) was added triethylamine (7.9 mmol), and then stirred for 0.5 h at room temperature. To the reaction mixture was added **12–14** (0.8 mmol), and then stirred at 50 °C for another 3 h under an atmosphere of nitrogen and then concentrated under reduced pressure. The residue was dissolved with 5% aqueous acetic acid solution (6.5 mL), and then stirred for 0.5 h at 70 °C. After cooling to room temperature, ammonia water (12%) was added dropwise to adjust pH 6.5–7.0 and then filtered. The precipitate was washed with water (20 mL), and dried in vacuo at 60 °C for 10 h to yield the title compounds **19–21**.

*4.2.30.1. 1-Cyclopropyl-7-(3-((cyclopropylamino)methyl)-4-(methoxyimino)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19a1***. The title compound **19a1** was obtained from **11a1** and **12** as an off-white solid (35.6%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.60 (s, 1H), 8.04 (d, J = 12.7 Hz, 1H), 4.57 (s, 2H), 4.17 (s, 1H), 3.92 (s, 1H), 3.84 (s, 3H), 3.73 (d, J = 3.9 Hz, 1H), 3.19 (s, 1H), 2.92 (dd, J = 12.0, 5.2 Hz, 1H), 2.73 (dd, J = 11.9, 8.8 Hz, 1H), 2.12–2.09 (m, 1H), 1.19 (d, J = 6.8 Hz, 2H), 1.10 (d, J = 3.8 Hz, 2H), 0.39–0.37 (m, 2H), 0.22 (dd, J = 9.9, 3.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ (ppm): 177.04, 166.24, 149.12 (d, J = 12.2 Hz), 147.54, 147.29, 146.47, 145.82, 118.96 (d, J = 20.1 Hz), 112.74, 108.93, 62.24, 51.34, 50.37, 48.85, 35.40, 30.44, 29.85, 7.34 × 2, 6.92, 6.48. MS-ESI (*m/z*): 430.29 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{25}O_4N_5F$ $(M + H)^+$: 430.18851; Found: 430.18799.

*4.2.30.2. 1-Cyclopropyl-7-(3-((cyclopropylamino)methyl)-4-(methoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19b1***. The title compound **19b1** was obtained from **11b1** and **12** as an off-white solid (23.8%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.70 (s, 1H), 8.03 (d, J = 12.4 Hz, 1H), 4.64–4.55 (m, 2H), 4.14 (d, J = 10.6 Hz, 1H), 3.93 (s, 3H), 3.75 (d, J = 8.2 Hz, 1H), 3.66 (td, J = 7.4, 3.7 Hz, 1H), 2.98 (d, J = 12.0 Hz, 1H), 2.83 (d, J = 12.0 Hz, 1H), 2.19–2.16 (m, 1H), 1.32 (s, 3H), 1.29 (td, J = 6.9, 3.6 Hz, 2H), 1.09–1.07 (m, 2H), 0.47–0.41 (m, 2H), 0.30 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.21, 167.04, 149.40 (d, J = 11.7 Hz), 147.61, 147.41, 146.38, 145.89, 118.88 (d, J = 20.7 Hz), 112.60, 108.96, 62.42, 58.08, 55.91, 49.00, 48.96, 34.86, 31.13, 23.03, 7.61, 7.53, 6.83 × 2. MS-ESI (*m/z*): 444.50 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{27}O_4N_5F$ $(M + H)^+$: 444.20416; Found: 444.20435.

*4.2.30.3. 1-cyclopropyl-7-(3-((cyclopropylamino)methyl)-4-(ethoxyimino)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19a2***. The title compound **19a2** was obtained from **11a2** and **12** as a yellow solid (11.5%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.67 (s, 1H), 7.99 (d, J = 11.7 Hz, 1H), 4.59 (s, 2H), 4.35 (s, 1H), 4.18 (q, J = 7.0 Hz, 2H), 3.84–3.12 (m, 5H), 2.36 (s, 1H), 1.29 (dd, J = 21.1, 14.1 Hz, 7H), 1.05 (s, 2H), 0.62 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.14, 166.97, 149.12 (d, J = 12.2 Hz), 147.54, 147.29, 146.47, 145.82, 118.96 (d, J = 20.1 Hz), 112.74, 108.93, 70.47, 51.40, 50.37, 48.85, 34.93, 30.64, 29.85, 14.82, 7.62, 7.55, 5.85, 5.72. MS-ESI (*m/z*): 444.20 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{22}H_{27}O_4N_5F$ $(M + H)^+$: 444.20416; Found: 444.20380.

*4.2.30.4. 1-Cyclopropyl-7-(3-((cyclopropylamino)methyl)-4-(ethoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19b2***. The title compound **19b2** was obtained from **11b2** and **12** as a yellow solid (31.0%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.68 (s, 1H), 8.00 (d, J = 12.5 Hz, 1H), 4.64–4.55 (m, 2H), 4.18–4.12 (m, 3H), 3.75 (d, J = 11.0 Hz, 1H), 3.65 (td, J = 7.3, 3.7 Hz, 1H), 2.98 (d, J = 12.0 Hz, 1H), 2.83 (d, J = 12.0 Hz, 1H), 2.20–2.16 (m, 1H), 1.32 (s, 3H), 1.28 (dd, J = 7.1, 2.4 Hz, 5H), 1.08–1.06 (m, 2H), 0.46–0.41 (m, 2H), 0.31 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.20, 167.04, 149.46 (d, J = 12.5 Hz), 147.81, 147.43, 146.37, 145.75, 118.80 (d, J = 21.3 Hz), 112.55, 108.94, 70.13, 58.13, 55.90, 49.05, 49.00, 34.86, 31.15, 23.06,14.82, 7.59, 7.52, 6.76 × 2. MS-ESI (*m/z*): 458.37 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{23}H_{29}O_4N_5F$ $(M + H)^+$: 458.21981; Found: 458.22025.

*4.2.30.5. 7-(3-((Benzyloxy)imino)-4-((cyclopropylamino)methyl)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19a3***. The title compound **19a3** was obtained from **11a3** and **12** as a yellow solid (11.2%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.68 (s, 1H), 8.02 (d, J = 12.3 Hz, 1H), 7.39–7.36 (m, 4H), 7.35–7.26 (m, 1H), 5.16 (s, 2H), 4.63 (s, 2H), 4.24 (s, 1H), 3.86 (s, 1H), 3.66–3.62 (m, 1H), 3.27 (s, 1H), 3.00–2.94 (m, 2H), 2.14 (dt, J = 9.8, 3.2 Hz, 1H), 1.27 (q, J = 6.5 Hz, 2H), 1.06 (q, J = 6.1 Hz, 2H), 0.46–0.45 (m, 2H), 0.34 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.04, 166.80, 149.09 (d, J = 12.2 Hz), 147.43, 147.20, 146.31, 145.71, 137.55, 128.47, 128.19 × 2, 128.08 × 2, 118.78 (d, J = 20.7 Hz), 112.55, 108.83, 76.53, 51.35, 50.62, 49.05, 49.01, 34.73, 30.18, 7.44,7.41, 6.41, 6.3. MS-ESI (*m/z*): 506.19 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{27}H_{29}O_4N_5F$ $(M + H)^+$: 506.21981; Found: 506.21951.

*4.2.30.6. 7-(4-((Benzyloxy)imino)-3-((cyclopropylamino)methyl)-3-methylpyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **19b3***. The title compound **19b3** was obtained from **11b3** and **12** as a yellow solid (15.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.62 (s, 1H), 8.03 (d, J = 12.3 Hz, 1H), 7.41–7.25 (m, 5H), 5.17 (s, 2H), 4.67 (s, 2H), 4.21 (s, 1H), 3.87 (s, 1H), 3.67–3.63 (m, 1H), 3.01–2.92 (m, 2H), 2.18–2.15 (m, 1H), 1.32 (s, 3H), 1.27 (q, J = 6.5 Hz, 2H), 1.06 (q, J = 6.1 Hz, 2H), 0.46–0.45 (m, 2H), 0.34 (s, 2H). MS-ESI (*m/z*): 520.40 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{28}H_{31}O_4N_5F$ $(M + H)^+$: 520.23546; Found: 520.23592.

*4.2.30.7. 7-(3-((Cyclopropylamino)methyl)-4-(methoxyimino)pyrrolidin-1-yl)-6- fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20a1***. The title compound **20a1** was obtained from **11a1** and **13** as a yellow solid (33.8%). ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.70 (s, 1H), 8.04 (d, J = 12.6 Hz, 1H), 5.15 (d, J = 64.9 Hz, 1H), 4.58 (s, 2H), 4.17 (s, 1H), 3.86 (s, 3H), 3.84 (d, J = 1.1 Hz, 1H), 3.75 (s, 1H), 3.19 (s, 1H), 2.94–2.91 (m, 1H), 2.75 (dd, J = 12.0, 8.7 Hz, 1H), 2.11 (dt, J = 9.8, 3.3 Hz, 1H), 1.91–1.84 (m, 1H), 1.64 (qd, J = 15.0, 9.0 Hz, 1H), 0.42–0.35 (m, 2H), 0.25–0.19 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.45, 165.48, 148.87 (d, J = 12.3 Hz), 147.75, 147.15, 146.96, 145.44, 117.77 (d, J = 20.6 Hz), 110.97, 108.09, 70.59, 69.13, 61.76, 51.35, 50.81, 48.60, 36.09 (d, J = 8.5 Hz), 29.93, 11.91 (d, J = 10.6 Hz), 6.39, 5.97. MS-ESI (*m/z*): 448.40 $(M + H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{24}O_4N_5F_2$ $(M + H)^+$: 448.17909; Found: 448.17903.

4.2.30.8. 7-(3-((Cyclopropylamino)methyl)-4-(methoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-

*oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20b1***. The title compound **20b1** was obtained from **11b1** and **13** as a yellow solid (58.3%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.72 (s, 1H), 8.02 (d, J = 12.6 Hz, 1H), 4.97 (dd, J = 63.2, 2.5 Hz, 1H), 4.59–4.57 (m, 2H), 4.14 (s, 1H), 3.92 (s, 3H), 3.74 (s, 1H), 3.61 (dd, J = 12.8, 6.3 Hz, 1H), 2.96 (dd, J = 12.2, 7.1 Hz, 1H), 2.82 (d, J = 12.0 Hz, 1H), 2.16–2.15 (m, 1H), 1.71–1.63 (m,2H), 1.31 (d, J = 2.4 Hz, 3H), 0.44–0.40 (m, 2H), 0.28 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 176.39, 165.82, 148.56 (d, J = 12.1 Hz), 146.71, 146.31, 146.08, 144.99, 117.87 (d, J = 21.1 Hz), 111.38, 108.56, 68.87(d, J = 6.0 Hz), 67.38(d, J = 4.5 Hz), 61.42, 57.09, 55.01, 47.98, 35.27 (dd, J = 9.2, 4.0 Hz), 30.13, 30.12, 21.97 (d, J = 12.1 Hz), 11.48 (d, J = 10.6 Hz), 5.87, 5.82. MS-ESI (m/z): 462.35 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{26}O_4N_5F_2$ $(M + H)^+$: 462.19474; Found: 462.19476.

*4.2.30.9. 7-(3-((Cyclopropylamino)methyl)-4-(ethoxyimino)pyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20a2***. The title compound **20a2** was obtained from **11a2** and **13** as a yellow solid (33.3%). ^{1}H NMR (600 MHz, DMSO-*d6*) δ (ppm): 8.71 (s, 1H), 8.03 (d, J = 12.5 Hz, 1H), 5.14 (d, J = 65.4 Hz, 1H), 4.58 (s, 2H), 4.17 (s, 1H), 4.11 (q, J = 7.0 Hz, 2H), 3.89 (s, 1H), 3.74 (s, 1H), 3.18 (s, 1H), 2.92 (ddd, J = 11.9, 5.1, 3.1 Hz, 1H), 2.77–2.73 (m, 1H), 2.12 (dt, J = 9.4, 3.0 Hz, 1H), 1.91–1.84 (m, 1H), 1.64 (td, J = 15.2, 8.9 Hz, 1H), 1.23 (t, J = 7.0 Hz, 3H), 0.42–0.35 (m, 2H), 0.27–0.18 (m, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ (ppm): 176.46, 165.49, 148.85 (d, J = 12.2 Hz), 147.79, 147.16, 146.96, 145.44, 117.76 (d, J = 20.9 Hz), 110.97, 108.09, 70.60, 69.10, 51.35, 50.91, 50.85, 48.59, 36.11, 29.95, 14.62, 11.93 (d, J = 10.1 Hz), 6.42 (d, J = 3.4 Hz), 6.01 (d, J = 5.8 Hz). MS-ESI (m/z): 462.41 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{26}O_4N_5F_2$ $(M + H)^+$: 462.19474; Found: 462.19468.

*4.2.30.10. 7-(3-((Cyclopropylamino)methyl)-4-(ethoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20b2***. The title compound **20b2** was obtained from **11b2** and **13** as a yellow solid (8.9%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.73 (s, 1H), 8.02 (d, J = 12.5 Hz, 1H), 5.03–4.89 (m, 1H), 4.60 (s, 2H), 4.19–4.14 (m, 3H), 3.74 (d, J = 9.5 Hz, 1H), 3.61 (dd, J = 12.7, 5.8 Hz, 1H), 2.98 (t, J = 12.0 Hz, 1H), 2.83 (d, J = 12.0 Hz, 1H), 2.17–2.16 (m, 1H), 1.73–1.61 (m, 2H), 1.32–1.27 (m, 6H), 0.44–0.40 (m, 2H), 0.30 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.43, 167.84, 149.70 (d, J = 12.5 Hz), 147.91, 147.33, 147.13, 145.85, 118.88 (d, J = 20.0 Hz), 112.41, 109.62, 70.10 (dd, J = 14.1, 4.2 Hz), 68.26 (d, J = 6.3 Hz), 58.11, 56.01, 55.86, 49.01, 36.30 (d, J = 10.0 Hz), 31.16 (d, J = 5.0 Hz), 23.06 (d, J = 23.8 Hz), 14.85, 12.51 (d, J = 7.7 Hz), 6.80, 6.73. MS-ESI (m/z): 476.34 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{28}O_4N_5F_2$ $(M + H)^+$: 476.21039; Found: 476.21055.

*4.2.30.11. 7-(3-((Benzyloxy)imino)-4-((cyclopropylamino)methyl) pyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20a3***. The title compound **20a3** was obtained from **11a3** and **13** as a yellow solid (27.0%). ^{1}H NMR (600 MHz, DMSO-*d6*) δ (ppm): 8.71 (s, 1H), 8.03 (d, J = 12.5 Hz, 1H), 7.39–7.36 (m, 4H), 7.32 (td, J = 5.7, 2.7 Hz, 1H), 5.18–5.07 (m, 3H), 4.65 (s, 2H), 4.17 (s, 1H), 3.88 (s, 1H), 3.74 (s, 1H), 3.19 (s, 1H), 2.89–2.87 (m, 1H), 2.74 (t, J = 8.9 Hz, 1H), 2.08 (d, J = 3.3 Hz, 1H), 1.91–1.84 (m, 1H), 1.64–1.62 (m, 1H), 0.36–0.32 (m, 2H), 0.21–0.17 (m, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ (ppm): 176.46, 165.48, 148.86 (d, J = 12.2 Hz), 147.78, 147.16, 146.95, 145.44, 137.90, 128.29 × 2, 127.77, 127.69 × 2, 117.75 (d, J = 20.7 Hz), 110.99, 108.10, 75.32, 70.58, 69.12, 51.30, 50.74 (d, J = 8.7 Hz), 48.78, 36.11, 29.91, 11.92 (d, J = 10.3 Hz), 6.36 (d, J = 4.1 Hz), 6.01 (d, J = 5.5 Hz). MS-ESI (m/z): 524.11 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{27}H_{28}O_4N_5F_2$ $(M + H)^+$: 524.21039; Found: 524.21069.

*4.2.30.12. 7-(4-((Benzyloxy)imino)-3-((cyclopropylamino)methyl)-3-methylpyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **20b3***. The title compound **20b3** was obtained from **11b3** and **13** as a yellow solid (18.9%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.73 (s, 1H), 8.02 (d, J = 12.5 Hz, 1H), 7.39–7.33 (m, 5H), 5.18–5.07 (m, 3H), 4.65 (s, 2H), 4.17 (s, 1H), 3.74 (s, 1H), 3.61(dd, J = 12.7, 6.0 Hz, 1H), 2.91–2.89 (m, 1H), 2.82 (t, J = 12.0 Hz, 1H), 2.13 (d, J = 3.3 Hz, 1H), 1.71–1.63 (m, 2H), 1.31 (s, 3H), 0.44–0.40 (m, 2H), 0.23–0.16 (m, 2H). MS-ESI (m/z): 538.31 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{28}H_{30}O_4N_5F_2$ $(M + H)^+$: 538.22604; Found: 538.22656.

*4.2.30.13. 1-(6-Amino-3,5-difluoropyridin-2-yl)-7-(3-((cyclopropylamino)methyl)-4-(methoxyimino)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **21a1***. The title compound **21a1** was obtained from **11a1** and **14** as an off-white solid (20.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.78 (s, 1H), 8.08 (d, J = 12.3 Hz, 1H), 7.35 (t, J = 8.2 Hz, 1H), 4.77 (s, 2H), 4.38 (s, 1H), 3.91 (s, 3H), 3.90 (s, 1H), 3.59 (s, 1H), 3.15 (s, 1H), 2.99 (dd, J = 12.2, 6.7 Hz, 1H), 2.86 (dd, J = 12.1, 7.2 Hz, 1H), 2.18 (d, J = 3.3 Hz, 1H), 1.28–1.24 (m, 1H), 0.49–0.29 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 177.80, 166.36, 149.24 (d, J = 13.1 Hz), 147.64, 147.11, 146.29 × 2, 145.57, 145.51, 144.52, 144.41, 118.94 (d, J = 21.3 Hz), 113.07 (t, J = 21.4 Hz), 111.81, 110.11, 62.36, 51.17, 51.13, 50.79, 48.58, 30.18, 6.52, 6.30. MS-ESI (m/z): 518.29 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{23}O_4N_7F_3$ $(M + H)^+$: 518.17581; Found: 518.17606.

*4.2.30.14. 1-(6-Amino-3,5-difluoropyridin-2-yl)-7-(3-((cyclopropylamino)methyl)-4-(methoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **21b1***. The title compound **21b1** was obtained from **11b1** and **14** as a yellow solid (29.8%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.77 (s, 1H), 8.05 (d, J = 12.3 Hz, 1H), 7.33 (s, 1H), 4.81 (s, 2H), 4.34 (s, 2H), 3.88 (s, 4H), 2.87 (d, J = 12.1 Hz, 1H), 2.74 (d, J = 12.2 Hz, 1H), 2.11 (s, 1H), 1.22 (s, 4H), 0.40 (s, 2H), 0.22 (d, J = 14.3 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.92, 166.55, 149.49 (d, J = 12.1 Hz), 147.63, 147.05, 146.35, 145.91, 145.65, 145.31, 144.56 (d, J = 13.6 Hz), 131.84 (d, J = 10.6 Hz), 118.95 (d, J = 19.6 Hz), 113.17 (t, J = 21.1 Hz),111.81, 110.19, 62.39, 57.85, 55.82, 48.78, 48.74, 31.01, 22.78, 6.87, 6.76. MS-ESI (m/z): 532.39 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{25}O_4N_7F_3$ $(M + H)^+$: 532.19146; Found: 532.19207.

*4.2.30.15. 1-(6-Amino-3,5-difluoropyridin-2-yl)-7-(3-((cyclopropylamino)methyl)-4-(ethoxyimino)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **21a2***. The title compound **21a2** was obtained from **11a2** and **14** as a yellow solid (43.3%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.79 (s, 1H), 8.08 (d, J = 12.0 Hz, 1H), 7.34 (s, 1H), 4.77 (s, 2H), 4.33 (s, 1H), 4.15 (d, J = 5.4 Hz, 2H), 3.94 (s, 1H), 3.51 (s, 1H), 3.15–2.86 (m, 3H), 2.16 (s, 1H), 1.27 (s, 4H), 0.47–0.30 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.98, 166.49, 149.12 (d, J = 12.2 Hz), 147.54, 147.29, 146.47, 146.36, 145.95 145.63, 144.85, 144.45, 119.11 (d, J = 19.4 Hz), 113.21, 111.95, 110.29, 70.20, 51.25, 50.99, 50.67, 48.83, 30.32, 14.65, 6.57, 6.41. MS-ESI (m/z): 532.23 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{25}O_4N_7F_3$ $(M + H)^+$: 532.19146; Found: 532.19238.

*4.2.30.16. 1-(6-Amino-3,5-difluoropyridin-2-yl)-7-(3-((cyclopropylamino)methyl)-4-(ethoxyimino)-3-methylpyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid **21b2***. The title compound **21b2** was obtained from **11b2** and **14** as a grey solid (27.9%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.77 (s, 1H), 8.04 (d, J = 12.3 Hz, 1H), 7.32 (t, J = 8.3 Hz, 1H), 4.82 (s, 2H), 4.12 (q, J = 7.0 Hz, 1H), 3.71 (q, J = 7.0 Hz, 2H), 2.87 (d, J = 12.0 Hz, 1H), 2.74 (d, J = 12.2 Hz, 1H), 2.11 (s, 1H), 1.27–1.21 (m, 7H), 0.40 (s, 2H), 0.24 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.96, 166.55, 149.56

(d, J = 12.1 Hz), 147.83, 147.22, 146.29, 145.76, 145.67, 145.16 (d, J = 2.5 Hz), 144.52 (t, J = 12.5 Hz), 131.89 (d, J = 18.8 Hz), 118.91 (d, J = 20.0 Hz), 113.17 (t, J = 21.1 Hz), 111.79, 110.22, 69.99, 57.90, 55.74, 48.97, 48.92, 31.02, 22.84, 14.58, 6.83 × 2. MS-ESI (m/z): 546.30 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{25}H_{27}O_4N_7F_3$ $(M + H)^+$: 546.20711; Found: 546.20826.

4.2.30.17. 1-(6-Amino-3,5-difluoropyridin-2-yl)-7-(3-((benzyloxy) imino)-4-((cyclopropylamino)methyl)pyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***21a3****.* The title compound **21a3** was obtained from **11a3** and **14** as a yellow solid (18.8%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.77 (s, 1H), 8.07 (d, J = 12.2 Hz, 1H), 7.40 (d, J = 7.0 Hz, 2H), 7.35 (d, J = 7.3 Hz, 3H), 7.13 (s, 1H), 5.12 (s, 2H), 4.69 (s, 2H), 4.35 (s, 1H), 3.14 (s, 1H), 2.91 (s, 1H), 2.84 (s, 1H), 2.10 (s, 1H), 1.25 (s, 1H), 0.43 (s, 2H), 0.27 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 177.98, 166.45, 149.34 (d, J = 12.2 Hz), 147.57, 147.47, 146.97, 146.39, 145.85, 145.57, 144.48, 144.39, 137.37, 128.66 × 2, 128.50, 128.34 × 2, 119.12 (d, J = 20.7 Hz), 113.15 (t, J = 21.4 Hz), 111.95, 110.32, 76.79, 51.27, 50.98, 50.67, 49.04, 30.24, 6.70, 6.56. MS-ESI (m/z): 594.47 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{29}H_{27}O_4N_7F_3$ $(M + H)^+$: 594.20711; Found: 594.20740.

4.2.30.18. (6-Amino-3,5-difluoropyridin-2-yl)-7-(4-((benzyloxy) imino)-3-((cyclopropylamino)methyl)-3-methylpyrrolidin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid ***21b3****.* The title compound **21b3** was obtained from **11b3** and **14** as a yellow solid (16.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.77 (s, 1H), 8.04 (d, J = 12.3 Hz, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.33 (d, J = 7.0 Hz, 3H), 7.11 (s, 1H), 5.11 (s, 2H), 4.73 (s, 2H), 4.31 (s, 1H), 2.91 (s, 1H), 2.85 (s, 1H), 2.74 (d, J = 12.2 Hz, 1H), 2.05 (s, 1H), 1.22 (s, 4H), 0.38 (s, 2H), 0.22 (s, 2H). MS-ESI (m/z): 608.64 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{30}H_{29}O_4N_7F_3$ $(M + H)^+$: 608.22277; Found: 608.22328.

4.2.31. General procedure for the synthesis of target compounds ***22–25***

To a suspension of **11a**,**b** (0.5 mmol) in dry acetonitrile (7.0 mL) was added triethylamine (2.2 mmol), and then stirred for 0.5 h at room temperature. To the reaction mixture was added **15–18** (0.5 mmol), and then stirred at 35 °C for another 40 h under an atmosphere of nitrogen and then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) eluting with dichloromethane and acetone (v: v = 2: 1) to get the chelates. The chelates (0.3 mmol) was dissolved with 5% aqueous acetic acid (4.5 mL), and then stirred for 2 h at 80 °C. After cooling to room temperature, the mixture was extracted with ethyl acetate (3 mL × 2), and then 12% ammonia water was added dropwise to adjust pH 6.5–7.0 and then filtered. The precipitate was washed with water (3 mL), and dried in vacuo at 60 °C for 10 h to yield the title compounds **22–25**.

4.2.31.1. (3S)-10-(3-((Cyclopropylamino)methyl)-4-(methoxyimino) pyrrolidin-1-yl)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H- [1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid ***22a1****.* The title compound **22a1** was obtained from **11a1** and **15** as a yellow solid (6.5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.72 (s, 1H), 7.90 (s, 1H), 4.56–4.47 (m, 4H), 3.91 (s, 1H), 3.80–3.73 (m, 1H), 3.60 (s, 1H), 3.51–3.34 (m, 1H), 2.96 (s, 1H), 2.88 (s, 1H), 2.56 (s, 1H), 2.48 (s, 1H), 1.66 (s, 7H), 1.26 (s, 1H), 0.87 (s, 1H). MS-ESI (m/z): 445.34 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{26}O_5N_4F$ $(M + H)^+$: 445.18817; Found: 445.18790.

4.2.31.2. (3S)-10-(3-((Cyclopropylamino)methyl)-4-(methoxyimino)-3-methylpyrrolidin-1-yl)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid ***22b1****.* The title compound **22b1** was obtained from **11b1** and **15** as a yellow solid (15.9%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.60 (s, 1H), 7.68 (d, J = 13.3 Hz, 1H), 4.52–4.47 (m, 2H), 4.42 (t, J = 14.3 Hz, 2H), 4.36–4.34 (m, 1H), 3.89–3.82 (m, 4H), 3.53 (ddd, J = 25.6, 10.0, 2.1 Hz, 1H), 2.93 (dd, J = 12.0, 5.0 Hz, 1H), 2.83 (d, J = 12.1 Hz, 1H), 2.15 (s, 1H), 1.61 (d, J = 6.5 Hz, 3H), 1.29 (s, 3H), 0.42 (d, J = 6.4 Hz, 2H), 0.31 (t, J = 9.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.13, 167.32, 163.79, 155.68, 144.83, 137.42 (d, J = 7.5 Hz), 130.55 (d, J = 7.5 Hz), 124.97, 119.04 (d, J = 10.0 Hz), 107.87, 105.61 (d, J = 23.8 Hz), 68.27, 62.14, 61.22(t, J = 7.5 Hz), 55.66, 55.58, 50.96, 45.58, 31.20, 21.98 (d, J = 18.8 Hz), 18.47 (d, J = 2.5 Hz), 6.74. MS-ESI (m/z): 459.72 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{28}O_5N_4F$ $(M + H)^+$: 459.20382; Found: 459.20369.

4.2.31.3. (3S)-10-(3-((Cyclopropylamino)methyl)-4-(ethoxyimino)-3-methylpyrrolidin-1-yl)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid ***22b2****.* The title compound **22b2** was obtained from **11b2** and **15** as a yellow solid (19.6%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.61 (s, 1H), 7.68 (d, J = 13.3 Hz, 1H), 4.55–4.35 (m, 5H), 4.13 (q, J = 7.0 Hz, 2H), 3.86 (ddd, J = 22.3, 10.0, 2.3 Hz, 1H), 3.54 (ddd, J = 24.5, 10.0, 2.3 Hz, 1H), 2.93 (dd, J = 12.0, 4.8 Hz, 1H), 2.84 (dd, J = 12.0, 1.7 Hz, 1H), 2.16–2.15 (m, 1H), 1.62 (d, J = 6.8 Hz, 3H), 1.27 (dd, J = 13.6, 6.5 Hz, 6H), 0.41 (t, J = 7.0 Hz, 2H), 0.30 (dd, J = 12.7, 6.1 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.13, 167.33, 163.36, 155.72, 144.82, 137.45 (d, J = 8.3 Hz), 130.63 (d, J = 13.8 Hz), 124.98, 119.01 (d, J = 10.0 Hz), 107.86, 105.58 (d, J = 25.0 Hz), 69.83, 68.27, 61.26(t, J = 6.3 Hz), 55.65, 55.59, 51.00 (d, J = 5.0 Hz), 45.58, 31.21, 22.02 (d, J = 16.3 Hz), 18.47 (d, J = 2.5 Hz), 14.87, 6.71. MS-ESI (m/z): 473.35 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{30}O_5N_4F$ $(M + H)^+$: 473.21947; Found: 473.21965.

4.2.31.4. (3S)-10-(3-((Benzyloxy)imino)-4-((cyclopropylamino) methyl)pyrrolidin-1-yl)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid 22a3. The title compound **22a3** was obtained from **11a3** and **15** as a yellow solid (4.5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.63 (s, 1H), 7.73 (dd, J = 13.1, 2.2 Hz, 1H), 7.40 (d, J = 4.3 Hz, 4H), 7.36 (td, J = 8.4, 4.5 Hz, 1H), 5.16 (s, 2H), 4.50 (dt, J = 22.6, 14.5 Hz, 3H), 4.38 (t, J = 12.6 Hz, 2H), 4.00 (dt, J = 17.4, 7.8 Hz, 1H), 3.69–3.63 (m, 1H), 3.15 (d, J = 6.0 Hz, 1H), 3.03–2.99 (m, 1H), 2.95 (dd, J = 11.9, 6.9 Hz, 1H), 2.19 (s, 1H), 1.64 (d, J = 6.4 Hz, 3H), 0.49 (d, J = 4.8 Hz, 2H), 0.38 (s, 2H). MS-ESI (m/z): 521.42 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{28}H_{30}O_5N_4F$ $(M + H)^+$: 521.21947; Found: 521.21956.

4.2.31.5. (3R)-10-(3-((Cyclopropylamino)methyl)-4-(methoxyimino)-3-methylpyrrolidin-1-yl)-9-fluoro-3-(fluoromethyl)-7-oxo-3,7-dihydro-2H- [1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid ***23b1****.* The title compound **23b1** was obtained from **11b1** and **16** as a yellow solid (9.8%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.63 (s, 1H), 7.67 (d, J = 13.3 Hz, 1H), 4.86–4.60 (m, 4H), 4.42 (dd, J = 28.3, 17.7 Hz, 3H), 3.89–3.83 (m, 4H), 3.57–3.48 (m, 1H), 2.93 (dd, J = 12.1, 4.8 Hz, 1H), 2.84–2.82 (m, 1H), 2.15 (d, J = 3.2 Hz, 1H), 1.28 (s, 3H), 0.42 (d, J = 6.5 Hz, 2H), 0.31 (t, J = 9.6 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.38, 167.14, 163.61, 155.59, 146.78, 136.74 (d, J = 7.5 Hz), 131.12 (d, J = 12.5 Hz), 124.89, 118.79 (d, J = 8.8 Hz), 108.09, 106.17 (d, J = 2.5 Hz), 81.62, 80.20, 63.91, 62.26, 61.32(d, J = 7.5 Hz), 58.79(d, J = 21.3 Hz), 55.65 (d, J = 10.0 Hz), 51.09 (d, J = 6.3 Hz), 45.63, 31.31, 22.10 (d, J = 20.0 Hz), 6.84. MS-ESI (m/z): 477.48 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{27}O_5N_4F_2$ $(M + H)^+$: 477.19440; Found: 477.19406.

4.2.31.6. (3R)-10-(3-((Benzyloxy)imino)-4-((cyclopropylamino) methyl)pyrrolidin-1-yl)-9-fluoro-3-(fluoromethyl)-7-oxo-3,7-dihydro-2H- [1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid ***23a3****.*

The title compound **23a3** was obtained from **11a3** and **16** as a yellow solid (4.7%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.63 (s, 1H), 7.69 (d, J = 13.2 Hz, 1H), 4.86–4.60 (m, 4H), 4.49–4.28 (m, 3H), 3.99–3.95 (m, 1H), 3.90 (s, 3H), 3.65–3.63 (m, 1H), 3.12–3.07 (m, 1H), 3.04–3.00 (m, 1H), 2.92–2.88 (m, 1H), 2.22–2.16 (m, 1H), 0.49–0.43 (m, 2H), 0.38–0.33 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm): 177.30, 166.96, 161.05, 155.60, 153.61, 146.70, 130.78 (d, J = 13.7 Hz), 124.73, 119.06 (d, J = 9.4 Hz), 108.09, 106.11 (d, J = 24.9 Hz), 81.50, 80.08, 63.87, 62.25, 58.67 (d, J = 21.5 Hz), 54.80, 50.48, 41.59, 30.36, 6.63, 6.44. MS-ESI (m/z): 463.18 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{22}H_{25}O_5N_4F_2$ $(M + H)^+$: 463.17875; Found: 463.17849.

4.2.31.7. 1-Cyclopropyl-7-(3-((cyclopropylamino)methyl)-4-(methoxyimino)pyrrolidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***24a1****.* The title compound **24a1** was obtained from **11a1** and **17** as a yellow solid (9.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.81 (s, 1H), 7.87 (d, J = 13.1 Hz, 1H), 4.33 (dd, J = 39.2, 17.8 Hz, 2H), 4.00–3.97 (m, 2H), 3.93 (s, 3H), 3.64 (s, 3H), 3.62–3.61 (m, 1H), 3.15 (d, J = 6.8 Hz, 1H), 3.06 (d, J = 12.0 Hz, 1H), 2.94 (dd, J = 11.7, 7.4 Hz, 1H), 2.21 (s, 1H), 1.22 (d, J = 6.8 Hz, 2H), 1.00 (s, 2H), 0.48 (d, J = 6.3 Hz, 2H), 0.39 (s, 2H). MS-ESI (m/z): 459.37 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{23}H_{28}O_5N_4F$ $(M + H)^+$: 459.20382; Found: 459.20360.

4.2.31.8. 7-(3-((Cyclopropylamino)methyl)-4-(ethoxyimino)pyrrolidin-1-yl)-6-fluoro-1-((1R,2S)-2-fluorocyclopropyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***25a2****.* The title compound **25a2** was obtained from **11a2** and **18** as a yellow solid (3.0%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.75 (s, 1H), 7.86 (d, J = 11.7 Hz, 1H), 4.92–4.60 (m, 1H), 4.46–4.22 (m, 1H), 4.16 (q, J = 7.0 Hz, 2H), 3.91 (s, 2H), 3.67 (s, 3H), 3.57 (s, 1H), 3.25–2.98 (m, 2H), 2.23 (dd, J = 24.5, 17.0 Hz, 1H), 2.02–1.99 (m, 1H), 1.66–1.61 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H), 1.26 (d, J = 5.6 Hz, 2H), 0.88 (t, J = 6.9 Hz, 1H), 0.54 (s, 2H). MS-ESI (m/z): 491.57 $(M + H)^+$. HRMS-ESI (m/z): Calcd. for $C_{24}H_{29}O_5N_4F_2$ $(M + H)^+$: 491.21005; Found: 491.20977.

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 [26]. 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.

Acknowledgments

This work was supported by the National S&T Major Special Project on Major New Drug Innovations (2012ZX09301002-001-017/023, 2014ZX09507009-003) and NSFC 81373267-003.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2015.09.030. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] Y.B. Zhang, G.Q. Li, M.L. Liu, X.F. You, L.S. Feng, K. Lv, J. Cao, H.Y. Guo, Bioorg. Med. Chem. Lett. 21 (2011) 928–931.
[2] Y. Chai, J. Wang, M.L. Liu, H. Yi, L.Y. Sun, X.F. You, H.Y. Guo, Bioorg. Med. Chem. Lett. 21 (2011) 3377–3380.
[3] A. Zumla, P. Nahid, S.T. Cole, Nat. Rev. Drug Discov. 12 (2013) 388–404.
[4] Z. Ma, C. Lienhardt, H. McIlleron, A.J. Nunn, X.X. Wang, Lancet 375 (2010) 2100–2109.
[5] B.J. Bradbury, M.J. Pucci, Curr. Opin. Pharmacol. 8 (2008) 574–581.
[6] A.S. Ginsburg, J.H. Grosset, W.R. Bishai, Lancet. Infect. Dis. 3 (2003) 432–442.
[7] H.M. Liu, J. Huang, J.Y. Wang, M.H. Wang, M.L. Liu, B. Wang, H.Y. Guo, Y. Lu, Eur. J. Med. Chem. 57 (2014) 628–638.
[8] A. Dalhoff, Infection 40 (2012) 239–262.
[9] J.X. Wang, Y.B. Zhang, M.L. Liu, B. Wang, Y. Chai, S.J. Li, H.Y. Guo, Eur. J. Med. Chem. 46 (2011) 2421–2426.
[10] L.S. Feng, M.L. Liu, S. Wang, K. Lv, J. Cao, S.J. Li, H.Y. Guo, Tetrahedron 67 (2011) 8264–8270.
[11] Y. Chai, M.L. Liu, K. Lv, L.S. Feng, S.J. Li, L.Y. Sun, S. Wang, H.Y. Guo, Eur. J. Med. Chem. 46 (2011) 4267–4273.
[12] C.Y. Hong, Y.K. Kim, J.H. Chang, S.H. Kim, H. Choi, D.H. Nam, Y.Z. Kim, J.H. Kwak, J. Med. Chem. 40 (1997) 3584–3593.
[13] D.R. Choi, J.H. Shin, J. Yang, S.H. Yoon, Y.H. Jung, Bioorg. Med.Chem. Lett. 14 (2004) 1273–1277.
[14] A.R. Kwon, Y.H. Min, J.M. Ryu, D.R. Choi, M.J. Shim, E.C. Choi, J. Antimicrob. Chemother. 58 (2006) 684–688.
[15] K. Lv, Y.X. Sun, L.Y. Sun, H.Y. Guo, J.W. Wu, M.L. Liu, Chem. Med. Chem. 7 (2012) 1230–1236.
[16] X.Y. Wang, Q. Guo, Y.C. Wang, B.Q. Liu, M.L. Liu, L.Y. Sun, H.Y. Guo, Acta. Pharm. Sin. 43 (2008) 819–827.
[17] L.S. Feng, K. Lv, M.L. Liu, S. Wang, J. Zhao, X.F. You, S.J. Li, J. Cao, H.Y. Guo, Eur. J. Med. Chem. 55 (2012) 125–136.
[18] K. Lv, M.L. Liu, L.S. Feng, L.Y. Sun, Y.X. Sun, H.Y. Guo, Eur. J. Med. Chem. 47 (2012) 619–625.
[19] Y. Asahina, M. Takei, T. Kimura, Y. Fukuda, J. Med. Chem. 51 (2008) 3238–3249.
[20] L.S. Feng, M.L. Liu, K. Lv, S. Wang, H.Y. Guo, Asian. J. Chem. 25 (2013) 2327–2328.
[21] Z.L. Wan, Y. Chai, M.L. Liu, H.Y. Guo, Chin. J. Med. Chem. 19 (2009) 109–111.
[22] Y. Chai, Z.L. Wan, B. Wang, H.Y. Guo, M.L. Liu, Eur. J. Med. Chem. 44 (2009) 4063–4069.
[23] L. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[24] Y. Lu, M.Q. Zheng, B. Wang, L. Fu, W.J. Zhao, P. Li, J. Xu, H. Zhu, H.X. Jin, D.L. Yin, H.H. Huang, A.M. Upton, Z.K. Ma, Antimicrob. Agents Chemother. 55 (2011) 5185–5193.
[25] D. Sriram, A. Aubry, P. Yogeeswaria, L.M. Fisher, Bioorg. Med. Chem. Lett. 16 (2006) 2982–2985.
[26] MIC values were determined as described by the NCCLS (see: National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement, vol. 21; NCCLS: Wayne, PA, USA, 2001, M100–S11). MIC was Defined as the Lowest Concentration of Each Compound that Inhibits Visible Growth of Bacteria after Incubation at 35°C for 18–24 h.
[27] H.J. Yun, Y.H. Min, J.A. Lim, J.W. Kang, S.Y. Kim, M.J. Kim, J.H. Jeong, Y.J. Choi, H.J. Kwon, Y.H. Jung, M.J. Shim, E.C. Choi, Antimicrob. Agents. Chemother. 46 (2002) 3071–3074.